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05/04/2022

RELATIVE SUSCEPTIBILITY OF CORN HYBRIDS TO EUROPEAN CORN-BORER DAMAGE (LEPIDOPTERA, PYRALIDAE)

LYNCH, RE; GUTHRIE, WD. 1980

PHENOTYPIC DIFFERENTIATION AND HYBRID BREAKDOWN WITHIN PHYCIODES-THAROS (LEPIDOPTERA, NYMPHALIDAE) IN THE NORTHEASTERN UNITED-STATES

OLIVER, CG. 1980

EUROPEAN CORN-BORER - LEPIDOPTERA, PYRALIDAE YIELD LOSSES IN RELATION TO HYBRID AND STAGE OF CORN DEVELOPMENT

LYNCH, RE. 1980

PERSISTENCE OF ABNORMAL SPERMIOGENESIS IN THE BACKCROSS GENERATIONS OF INTERSPECIFIC HYBRIDS BETWEEN HELIOTHIS-VIRESCENS X HELIOTHIS-SUBFLEXA (LEPIDOPTERA, NOCTUIDAE)

GOODPASTURE, C; LACHANCE, LE; RICHARD, RD. 1980

HYBRIDIZATION STUDIES BETWEEN OSTRINIA-NUBILALIS, OSTRINIA-OBUMBRATALIS, AND OSTRINIA-PENITALIS (LEPIDOPTERA, PYRALIDAE)

GUTHRIE, WD; BARRY, D; HUGGANS, JL. 1981

FALL DIAPAUSE AND SPRING EMERGENCE OF HELIOTHIS-VIRESCENS, (LEPIDOPTERA, NOCTUIDAE) HELIOTHIS-SUBFLEXA, (LEPIDOPTERA, NOCTUIDAE) AND BACKCROSSES OF THEIR HYBRID

STADELBACHER, EA; MARTIN, DF. 1981

REARING OF THE HELIOTHIS (LEPIDOPTERA, NOCTUIDAE) STERILE HYBRID WITH A MULTICELLULAR LARVAL REARING CONTAINER AND PUPAL HARVESTING

HARTLEY, GG; KING, EG; BREWER, FD; GANTT, CW. 1982

ACCEPTANCE AND DEVELOPMENT OF HELIOTHIS-SUBFLEXA AND HELIOTHIS-VIRESCENS (LEPIDOPTERA, NOCTUIDAE), AND THEIR HYBRID AND BACKCROSS PROGENY ON SEVERAL PLANT-SPECIES

LASTER, ML; PAIR, SD; MARTIN, DF. 1982

COMPARATIVE-STUDY OF THE GROWTH-RATE AND THE PHOTOPERIOD SENSITIVITY OF 2 STRAINS OF THE EUROPEAN CORN-BORER (OSTRINIA-NUBILALIS HUBN, LEPIDOPTERA, PYRALIDAE) AND THEIR HYBRIDS

STENGEL, M; SCHUBERT, G. 1982

DISTRIBUTION AND HYBRIDIZATION OF HYALOPHORA-COLUMBIA (LEPIDOPTERA, SATURNIIDAE) IN WISCONSIN

FERGE, LA. 1983

DOMINANT BLACK-BODY MUTANT IN HELIOTHIS-VIRESCENS (LEPIDOPTERA, NOCTUIDAE) AND TRANSFER TO HELIOTHIS-SUBFLEXA (LEPIDOPTERA, NOCTUIDAE) THROUGH HYBRIDIZATION

PROSHOLD, FI; KARPENKO, CP; RAULSTON, JR. 1983

DISTURBANCE OF ECLOSION SEQUENCE IN HYBRID LEPIDOPTERA

OLIVER, CG. 1983

HYBRID STERILITY IN HELIOTHIS-SUBFLEXA X H-VIRESCENS (LEPIDOPTERA, NOCTUIDAE) CROSSES - EXPRESSION AFTER INJECTION WITH ANTIVIRAL AGENTS, HEAT SHOCKS, AND REARING AT EXTREME TEMPERATURES

LACHANCE, LE; KARPENKO, CP. 1983

TIME AND LEVEL OF INFESTATION BY 2ND-GENERATION EUROPEAN CORN BORERS (LEPIDOPTERA, PYRALIDAE) ON A RESISTANT AND A SUSCEPTIBLE MAIZE HYBRID IN RELATION TO YIELD LOSSES

JARVIS, JL; GUTHRIE, WD; BERRY, EC. 1983

HYBRID STERILITY - EUPYRENE SPERM PRODUCTION AND ABNORMALITIES IN THE BACKCROSS GENERATIONS OF INTERSPECIFIC HYBRIDS BETWEEN HELIOTHIS-SUBFLEXA AND H-VIRESCENS (LEPIDOPTERA, NOCTUIDAE)

LACHANCE, LE. 1984

EUROPEAN CORN-BORER (LEPIDOPTERA, PYRALIDAE) - RATE OF 1ST-GENERATION LARVAL MORTALITY IN SORGHUM HYBRIDS COMPARED WITH INBRED LINES OF MAIZE DURING THE WHORL STAGE OF PLANT DEVELOPMENT

DHARMALINGAM, S; GUTHRIE, WD; JARVIS, JL; KINDLER, D; ATKINS, RE; TSENG, CT; ZHOU, D. 1984

THE EFFECT OF HYBRIDIZATION ON THE PERIODICITY OF SEX-PHEROMONE RELEASE OF EUXOA SPECIES (LEPIDOPTERA, NOCTUIDAE)

TEAL, PEA. 1985

POSTDIAPAUSE DEVELOPMENT OF SYMPATRIC CHORISTONEURA-OCCIDENTALIS AND CHORISTONEURA-RETINIANA (LEPIDOPTERA, TORTRICIDAE) AND THEIR HYBRIDS

VOLNEY, WJA; LIEBHOLD, AM. 1985

LARVAL GROWTH AND BEHAVIOR OF THE FALL ARMYWORM (LEPIDOPTERA, NOCTUIDAE) ON CALLUS INITIATED FROM SUSCEPTIBLE AND RESISTANT CORN HYBRIDS

WILLIAMS, WP; BUCKLEY, PM; DAVIS, FM. 1985

LARVAL COLORATION OF HYBRIDS BETWEEN CHORISTONEURA-OCCIDENTALIS AND C RETINIANA (LEPIDOPTERA, TORTRICIDAE)

LIEBHOLD, AM. 1986

SURVIVAL, GROWTH, AND DEVELOPMENT OF SOUTHWESTERN CORN-BORER (LEPIDOPTERA, PYRALIDAE) ON RESISTANT AND SUSCEPTIBLE MAIZE HYBRIDS

DAVIS, FM; WILLIAMS, WP. 1986

YIELD LOSSES FROM 2ND-GENERATION EUROPEAN CORN BORERS (LEPIDOPTERA, PYRALIDAE) IN LONG-SEASON MAIZE HYBRIDS PLANTED EARLY COMPARED WITH SHORT-SEASON HYBRIDS PLANTED LATE

JARVIS, JL; GUTHRIE, WD; ROBBINS, JC. 1986

EUROPEAN CORN-BORER (LEPIDOPTERA, PYRALIDAE) - YIELD LOSSES AND DAMAGE IN POPCORN IN RELATION TO HYBRID AND STAGE OF PLANT DEVELOPMENT

JARVIS, JL; ZIEGLER, KE; GUTHRIE, WD. 1986

NATURAL HYBRID BETWEEN 2 DIFFERENT KARYOTYPES OF PIERIS-RAPAE IN JAPAN (LEPIDOPTERA, PIERIDAE)

MAEKI, K; MIYAWAKI, R. 1987

INTERACTION BETWEEN TILLAGE SYSTEMS, MAIZE HYBRIDS, EUROPEAN CORN BORERS (LEPIDOPTERA, PYRALIDAE), AND STALK ROT PATHOGENS

JARVIS, JL; CLARK, RL; BLOCK, CC; BERRY, EC; GUTHRIE, WD. 1987

INTERSPECIFIC HYBRIDIZATION OF HELIOTHIS-SUBFLEXA AND HELIOTHIS-VIRESCENS (LEPIDOPTERA, NOCTUIDAE) FROM ARGENTINA

LASTER, ML; KING, EG; FURR, RE. 1988

ELECTROPHORETIC DETECTION OF INTERSPECIFIC HYBRIDS IN PARNASSIUS (LEPIDOPTERA, PAPILIONIDAE)

DESCIMON, H; GEIGER, H. 1988

DIAPAUSE OF THE INTER-SPECIFIC F1 HYBRIDS BETWEEN ANTHERAEA-YAMAMAI (GUERIN-MENEVILLE) AND ANTHERAEA-PERNYI (G-M) (LEPIDOPTERA, SATURNIIDAE)

SHIMADA, T; YAMAUCHI, H; KOBAYASHI, M. 1988

NATURAL HYBRIDIZATION BETWEEN PARNASSIUS-APOLLO (L) AND PARNASSIUS-PHOEBUS (F) IN SOUTHERN ALPS (LEPIDOPTERA, PAPILIONIDAE)

DESCIMON, H; GENTY, F; VESCO, JP. 1989

POPULATION-STRUCTURE AND HOST USE IN HYBRIDIZING SUBSPECIES OF PAPILIO-GLAUCUS (LEPIDOPTERA, PAPILIONIDAE)

HAGEN, RH. 1990

Two parapatric subspecies of the eastern tiger swallowtail butterfly, *Papilio glaucus glaucus* and *P. g. canadensis*, differ greatly in larval survival and growth on host plants in the Magnoliaceae, Salicaceae, and Betulaceae. In the first part of this study, butterflies were collected from 17 sites along a transect from Georgia to Quebec and used for allozyme electrophoresis. Two X- (or Z-)linked enzyme loci (Ldh and Pgd) showed allele frequency differences that delineated a **hybrid** zone between the subspecies in northern Pennsylvania and south-central New York. No significant linkage disequilibrium could be detected among allozyme loci within the **hybrid** zone samples. Genetic differentiation at Ldh and Pgd coincides with subspecies differences in diapause control and female mimicry, which are also sex-linked. Larval offspring of butterflies from 13 of the sites were then tested in the laboratory for survival and growth on *Liriodendron tulipifera* (Magnoliaceae), *Populus deltoides* (Salicaceae) and *Betula lutea* (Betulaceae). Steep clines in survival rates matched the position of the **hybrid** zone. Hybrid zone larvae showed intermediate survival rates and significant heterogeneity among families on all three plants, indicating presence of substantial genetic variation. The results suggest that differential host use by *P. g. glaucus* and *P. g. canadensis* is maintained primarily by independent clines in selection intensity for ability to use allopatric sets of host plants, coupled with restricted gene flow across the **hybrid** zone.

OVIPOSITIONAL RESPONSE OF SOUTHWESTERN CORN-BORER (LEPIDOPTERA, PYRALIDAE) AND FALL ARMYWORM (LEPIDOPTERA, NOCTUIDAE) TO SELECTED MAIZE HYBRIDS

NG, SS; DAVIS, FM; WILLIAMS, WP. 1990

NATURAL HYBRIDS OF PAPILIO (INSECTA, LEPIDOPTERA) - POOR TAXONOMY OR INTERESTING EVOLUTIONARY PROBLEM

SPERLING, FAH. 1990

LOCK-AND-KEY HYPOTHESIS - LACK OF MECHANICAL ISOLATION IN A BUTTERFLY (LEPIDOPTERA, PIERIDAE) HYBRID ZONE

PORTER, AH; SHAPIRO, AM. 1990

EFFECT OF 2ND-GENERATION EUROPEAN CORN BORERS (LEPIDOPTERA, PYRALIDAE) AND STALK ROT FUNGI ON POPCORN HYBRIDS

JARVIS, JL; ZIEGLER, KE; WEBBER, DF; GUTHRIE, WD. 1990

2 NEW ABERRANT FORMS OF TIGER SWALLOWTAIL BUTTERFLY FROM THE GREAT-LAKES HYBRID TRANSITION ZONE (LEPIDOPTERA, PAPILIONIDAE)

SCRIBER, JM. 1990

POTENTIAL FOR THE USE OF GENETIC METHODS FOR THE CONTROL OF CHILO SPP

OKOTH, VAO. 1990

The control of pest populations through the manipulation of their genetic component or mechanism of inheritance is becoming increasingly important. Two of the most promising genetic methods for the control of epidopteran insects are inherited or F1 and **hybrid** sterility. To induce F1 sterility in *C. partellus*, male pupae were irradiated with various doses of gamma radiation (0-15 krad) and emerging adults of normal appearance were used in crosses with normal females. The F1 progeny were reared on artificial diet and the resulting adult males were crossed with unirradiated females to give F2, the process being repeated until F5 progeny were obtained. For each generation lifespan of males, oviposition, egg hatchability and sex ratio were carefully recorded. The results showed that females mated with irradiated males oviposited significantly fewer eggs than normal and that this phenomenon was perpetuated over several generations. Hatchability of eggs laid by females mated to irradiated males or male progeny was significantly lower than that of the controls and sex ratio was distorted slightly in favour of males in all generations observed. The lower oviposition, lower egg hatch, distortion of sex ratio in favour of males and the apparent persistence of sterility over several

generations offer the potential of inherited sterility for the control of *C. partellus*.

FIELD-EVALUATION OF A DNA HYBRIDIZATION ASSAY FOR NUCLEAR POLYHEDROSIS-VIRUS IN GYPSY-MOTH (LEPIDOPTERA, LYMANTRIIDAE) LARVAE

KEATING, ST; ELKINTON, JS; BURAND, JP; PODGWAITE, JD; FERGUSON, CS. 1991

DNA **hybridization** assays were used to detect the presence of viral DNA in gypsy moth (*Lymantria dispar* L.) larvae collected weekly from high density populations or reared from field-collected egg masses. DNA was extracted from larvae, bound to nitrocellulose filters, and **hybridized** with digoxigenin-labeled *L. dispar* NPV (LdNPV) DNA probes. The virus incidence determined from DNA **hybridization** assays was compared with that determined with conventional microscopic examination of larvae for polyhedral inclusion bodies. Among neonates reared from field-collected egg masses, average mortality from LdNPV (15.4%) within 10 d after hatch was not significantly different from the percentage of extracts containing LdNPV DNA (14.8%) found among larvae frozen 5 d after hatch before any mortality occurred. Field-collected larvae were split into two groups: half were frozen immediately and probed for LdNPV DNA and the other half were reared on artificial diet. The proportion containing LdNPV DNA closely approximated the proportion that died within 6 d of collection, but the proportion that died within 13 d of collection was underestimated.

GENE FLOW AMONG 3 RACES OF EUROPEAN CORN BORERS (LEPIDOPTERA, PYRALIDAE) IN NEW-YORK-STATE

GLOVER, T.J; KNODEL, J.J; ROBBINS, PS; ECKENRODE, C.J; ROELOFS, WL. 1991

In New York state, there are three races of European corn borer moths, which are characterized principally by differences in voltinism and the sex pheromone communication system. One race is bivoltine, with females producing and males responding to a 99:1 E/Z isomeric ratio of DELTA-11-14:OAc's as the sex pheromone. Two races, one univoltine and one bivoltine, produce and respond to a 3:97 E/Z blend of DELTA-11-14:OAc's. The races are referred to as BE, UZ, and BZ to denote these differences. Analyses by gas chromatography of field-collected females indicate that there is significant **hybridization** between the Z and E races when in sympatry, with **hybrid** females producing a 68:32 ratio of E/Z11-14:OAc. Gene flow among the races was investigated using differences in triose phosphate isomerase (TPI) allozyme frequencies of moths from sites sampled across the state. The fixed nature of the Tpi-1 allele in the BE race and increases in the Tpi-1 allele frequencies in UZ populations in sympatry with the BE race support the hypothesis that gene flow following **hybridization** is unidirectional from BE populations into the Z populations. In addition, the TPI genotypes of the 10 **hybrid** females analyzed were consistent with **hybridization** occurring by E males mating with Z females.

COURTSHIP BEHAVIOR OF HELIOTHIS-SUBFLEXA (GN) (LEPIDOPTERA, NOCTUIDAE) AND ASSOCIATED BACKCROSS INSECTS OBTAINED FROM HYBRIDIZATION WITH H-VIRESCENS (F)

CIBRIANTOVAR, J; MITCHELL, ER. 1991

This study describes the courtship behavior sequence under wind tunnel conditions of *Heliothis subflexa* (Guenée) and selected backcross insects (generations 5 and 6) obtained from **hybridization** with *Heliothis virescens* (F.). The rhythmic release of pheromone by *H. subflexa*, *H. virescens* and backcross females was correlated with the response rhythm of males. The behavioral courtship sequence of *H. subflexa* males ($P < 0.05$) included oriented flight, landing, antennation, attempted copulation, and mating behaviors. Backcross insects did not antennate, but they fully displayed their hairpencils as did males of *H. virescens*. Factors that increased the probability of successful matings were identified as an acquiescent female, antennating by *H. subflexa* males, full hairpencil display (backcross males) and the male's ability to clasp the female genitalia. Overall, 63% of *H. subflexa* and 33% of backcross pairs culminated the courtship sequence with successful mating. The tendency of backcross males to settle down after limited flight activity contributed greatly to the increased proportion of unsuccessful males.

CAUSES OF SEX-RATIO BIAS MAY ACCOUNT FOR UNISEXUAL STERILITY IN HYBRIDS - A NEW EXPLANATION OF HALDANES RULE AND RELATED PHENOMENA

HURST, LD; POMIANKOWSKI, A. 1991

Unisexual **hybrid** disruption can be accounted for by interactions between sex ratio distorters which have diverged in the species of the **hybrid** cross. One class of unisexual **hybrid** disruption is described by Haldane's rule, namely that the sex which is absent, inviable or sterile is the heterogametic sex. This effect is mainly due to incompatibility between X and Y chromosomes. We propose that this incompatibility is due to a mutual imbalance between meiotic drive genes, which are more likely to evolve on sex chromosomes than autosomes. The incidences of taxa with sex chromosome drive closely matches those where Haldane's rule applies: Aves, Mammalia, Lepidoptera and Diptera. We predict that Haldane's rule is not universal but is correct for taxa with sex chromosome meiotic drive. A second class of **hybrid** disruption affects the male of the species regardless of which sex is heterogametic. Typically the genes responsible for this form of disruption are cytoplasmic. These instances are accounted for by the release from suppression of cytoplasmic sex ratio distorters when in a novel nuclear cytotyp. Due to the exclusively maternal transmission of cytoplasm, cytoplasmic sex ratio distorters cause only female-biased sex ratios. This asymmetry explains why **hybrid** disruption is limited to the male.

ANALYSIS OF THE TROPHIC RELATIONS OF EUROPEAN CORN-BORER AND BRUSHLEG STEM BORER (LEPIDOPTERA, PYRAUSTIDAE) ON A CORN FIELD, OVERGROWN WITH MUGWORT

FROLOV, AN. 1991

A study was conducted on the larval distribution of European corn borer *Ostrinia nubilalis* (Hbn.) and brushleg borer *O. scapularis* (Wlk.) on corn and mugwort in Charkassy Region. In the area of direct contact between host plants, at the edges of a corn field, overgrown with mugwort, our studies recorded an average 11.9% brushleg borer larvae on corn, and 8.1% corn borer larvae on mugwort over a four-year period. Outside these areas corn borer larvae were encountered exclusively on corn, and brushleg borer on mugwort. It transpires that feeding by brushleg borer larvae on corn at the field edge is not connected with the effects of **hybridization** with its close relative, the European corn borer, but may serve as a model of the prevailing conditions, when the corn borer initially altered its food preference to cereal crops.

A NEW MEASURE OF GENETIC IDENTITY BETWEEN POPULATIONS OF SEXUAL AND ASEXUAL SPECIES

TOMIUK, J; LOESCHCKE, V. 1991

We define a new genetic identity measure that is especially well suited for asexual polyploid species as it circumvents errors in the estimation of gene frequencies. It also can be applied to sexuals allowing the study of phylogenetic relationships in species complexes consisting of sexuals and asexuals of different ploidy levels. The measure groups genotypes into classes dependent on homozygosity vs heterozygosity and the number of ancestral allele types vs the number of presumed new mutations. Its value is related to evolutionary time since divergence. The application of the method is illustrated by using electrophoretic data on the species group

Solenobia triquetrella (Lepidoptera: Psychidae). A high similarity of estimated relationships among the proposed as well as other genetic identity measures is shown in the case of diploid sexual and asexual races of this species group. The phylogenetic relationships within the group are reanalyzed and monophyletic vs polyphyletic origin of parthenogenesis in this species complex is discussed. The genetic identity values found by the proposed procedure are explained by a polyphyletic origin of parthenogenesis, though a monophyletic origin of parthenogenesis in a broader sense cannot be excluded. The explanation of the phylogenetic relationships is based on the assumption of **hybridization** between related species and the extinction of one ancestral species. Furthermore, the genetic diversity is compared among sexual and parthenogenetic races of the species.

PHYLOGENETIC-RELATIONSHIPS OF 3 AFRICAN SWALLOWTAIL BUTTERFLIES, PAPILIO-DARDANUS, P-PHORCAS AND P-CONSTANTINUS - NEW DATA FROM HYBRIDS (LEPIDOPTERA, PAPILIONIDAE)

CLARKE, CA; GORDON, IJ; SMITH, CR; VANEWRIGHT, RI. 1991

Various laboratory-bred **hybrids** between *Papilio dardanus*, *P. phorcas* and *P. constantinus* are reported. The principal object of the research was to obtain evidence regarding the evolutionary relationships of the three species, with particular reference to understanding the evolution of the mimetic patterns of *P. dardanus*. With regard to the latter, little progress was made but other findings were interesting, notably those concerning sex ratio, pupal coloration, and differences in male genitalia.

COLONIZATION, SURVIVAL, AND CAUSES OF MORTALITY OF CAMERARIA-HAMADRYADELLA (LEPIDOPTERA, GRACILLARIIDAE) ON 4 SPECIES OF HOST PLANTS

CONNOR, EF. 1991

1. The abundance, survival, and causes of mortality of *Cameraria hamadryadella* (Clemens) (Lepidoptera: Gracillariidae) were examined on four host plant species in Virginia, U.S.A. *Quercus alba* L. and *Q. rubra* L. are native within the geographic range of *C. hamadryadella*, and *Q. robur* L. and *Q. benderi* Baenitz are exotic. *Q. robur* is native to Europe, North Africa, and Asia and was probably introduced prior to 1850, and *Q. benderi* is of **hybrid** origin and introduced to cultivation before 1900. *Q. alba* and *Q. robur* are in the subgenus *Lepidobalanus* (white oaks), and *Q. rubra* and *Q. benderi* are in the subgenus *Erythrobalanus* (red oaks). 2. Larval mines of *C. hamadryadella* were abundant on both white oak species, including the exotic *Q. robur*, but were rare on host plants in the red oak subgenus. Un-hatched eggs of *C. hamadryadella* were not observed on red oaks. Other observations on host distribution indicate that *C. hamadryadella* is rarely found on red oaks. These observations are interpreted as circumstantial evidence that *C. hamadryadella* generally avoids ovipositing on red oaks. 3. Survival of *C. hamadryadella* to the adult stage was similar among all host species, but larvae tended to survive longer on hosts in the red oak subgenus. The observation of higher survival rates of early instar larvae on red oaks suggests that no nutritional or secondary chemical barrier reinforces the observed pattern of oviposition. 4. Significant differences in the distribution of the causes of mortality were detected between native and exotic host plant species. Larvae and pupae on native hosts were more likely to die because of predation, while those on exotic host plants were more likely to die because of parasitism and host feeding by adult female parasitoids. This pattern could arise because parasitoids prefer to forage on exotic host plants or because predators avoid foraging on exotic plants. 5. This study shows for *C. hamadryadella* that the only barriers to colonization and use of exotic hosts, in the white and red oak subgenera, are the presence of cues sufficient to stimulate oviposition and/or the absence of cues to deter oviposition. It also suggests that the presence of closely related native host plants in the region of introduction will increase the probability that exotic plants will be colonized by phytophagous insects.
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PHYLOGENETIC-RELATIONSHIPS OF 3 AFRICAN SWALLOWTAIL BUTTERFLIES, PAPILIO-DARDANUS, P-PHORCAS AND P-CONSTANTINUS - A CLADISTIC-ANALYSIS (LEPIDOPTERA, PAPILIONIDAE)

VANEWRIGHT, RI; SMITH, CR. 1991

The well-known polymorphic mimetic African papilionid butterfly, *Papilio dardanus* Brown, is demonstrated by cladistic analysis to form a monophyletic group (the phorcas-group) with two other African swallowtails, *P. phorcas* Cramer and *P. constantinus* Ward. Within this group, *dardanus* and *phorcas* are shown to be sister-species, with *constantinus* as the stem. The supposed relationship of this group of three Rutaceae-feeding species to the five members of the Canellaceae/Lauraceae-feeding *Papilio hesperus*-group (including *P. nobilis* Rogenhofer) is questioned. The significance of this analysis is discussed with special reference to Trimmen's original hypothesis on the evolution of mimetic patterns in female *Papilio dardanus*, the unique male patterns of *phorcas* and *dardanus*, and natural and laboratory-produced **hybrids**. A pathway modelling technique is developed to represent the possible course of evolution of the phorcas-group, in terms of speciation events and the accumulation and decay of the pattern polymorphisms.

COMPARISON OF AN ADULT AND LARVAL CONTROL STRATEGY FOR THE EUROPEAN CORN-BORER (LEPIDOPTERA, PYRALIDAE) IN SEED CORN

DERRICK, ME; SHOWERS, WB. 1991

Efficacy of adult-directed insecticide applications were compared with standard larval control treatments in an effort to reduce second generation larval infestations of the European corn borer, *Ostrinia nubilalis* (Hubner) in seed corn. The treatments included methomyl applied to grassy adult aggregation areas, or "action sites," for adult suppression, fenvalerate applied to the field for larval control, a combination of methomyl applied to the action site with fenvalerate applied to the field, and an untreated check. Two applications were made in and around fields of **hybrid** seed production during the second flight (mid-July to late-August) in Iowa. The male, or tasselled inbred, sustained higher levels of larval infestation, resulting in a greater response to treatment. Larval tunnelling damage and infestation level were reduced with applications of fenvalerate to the field. Although applications of methomyl resulted in fewer adults in the grass following action site treatment, the subsequent effect on infestation and damage was not significant. Adult sampling data during high population levels in 1986 documented a temporary reduction of adults in the action site after treatment, suggesting a lack of residual activity by methomyl. Blacklight trap data indicated that the timing of initial insecticide applications in the action site corresponded with a majority of newly mated and actively ovipositing females and second applications targeted ovipositing and post-oviposition females.

STIMULATION OF OVIPOSITION AND HETEROSIS IN INTERSTRAIN CROSSES OF EUROPEAN CORN-BORER (LEPIDOPTERA, PYRALIDAE)

KEIL, CB; TIPPING, C. 1992

Hybrid matings of two *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) colonies (N and O), established in the laboratory a year apart, demonstrated positive heterosis in the F1 generation as indicated by increases in pupal weight (6%), number of egg masses per female (65%) and number of eggs per female (77%) as compared to intra-colony crosses in mass matings. In contrast, pupation date, an indicator of development time, was intermediate in the **hybrids** relative to the parental colonies. There was also a 37% increase in the numbers of eggs/female and a 45% increase in egg masses/female in the **hybrid** parental cross. We investigated this stimulation of oviposition in single female, reciprocal crosses. Oviposition stimulation was directional with the cross of N colony males x O colony females exhibiting significantly higher numbers of total eggs/female and egg masses/female on a daily basis. Colony O females in this cross laid more large egg masses than females in other crosses. Data from individual female crosses demonstrated that egg production in **hybrid** mass mating experiments was consistent with random mating of males and females from both colonies.

OCCURRENCE AND SIGNIFICANCE OF NATURAL HYBRIDS BETWEEN PAPILIO-DARDANUS AND P-PHORCAS (LEPIDOPTERA, PAPILIONIDAE)

VANEWRIGHT, RI; SMITH, CR. 1992

Species can exchange genes through occasional **hybridization**. As a result, and contrary to some expectations, closely related species may be genetically more compatible in sympatry than in allopatry. This could be so for two well-known African swallowtails, *Papilio dardanus* Brown and *P. phorcas* Cramer. Five natural **hybrids** between the two species are documented, their significance is discussed, and further information requested.

PRIMARY STRUCTURE OF V-ATPASE SUBUNIT-B FROM MANDUCA-SEXTA MIDGUT

NOVAK, FJS; GRAF, R; WARING, RB; WOLFERSBERGER, MG; WIECZOREK, H; HARVEY, WR. 1992

The amino acid sequence of a vacuolar-type ATPase (V-ATPase) subunit B has been deduced from a cDNA clone isolated from a *Manduca sexta* larval midgut library. The library was screened by **hybridization** with a labeled cDNA encoding subunit B of *Arabidopsis thaliana* tonoplast V-ATPase. The *M. sexta* V-ATPase subunit B consists of 494 amino acids with a calculated M(r) of 54902. The amino acid sequence deduced for V-ATPase subunit B of *M. sexta* is between 98% and 76% identical with that of seven other V-ATPase subunits B and > 52% identical with three archaeobacterial ATPase subunits B.

DIAGNOSTIC ALLELES FROM ELECTROPHORESIS DISTINGUISH 2 NOCTUID PEST SPECIES, HYDRAECIA-IMMANIS AND H-MICACEA (LEPIDOPTERA, NOCTUIDAE)

SCRIBER, JM; BOSSART, JL; SNIDER, D. 1992

Native hop vine borer (*Hydraecia immanis*) and introduced potato stem borer (*H. micacea*) populations in Midwest corn have reached noticeable levels near the Great Lakes plant community ecotone between boreal forests and temperate deciduous forests. The hop vine borer is more specialized in its diet and occurs in corn generally south of the plant community ecotone, whereas the potato stem borer is polyphagous and occurs in corn mostly north of the Great Lakes plant transition zone. We analyzed the genetic composition of each species using cellulose acetate electrophoresis and resolved 19 loci of which 6 exhibited fixed or nearly fixed allelic differences. We expect that this will be useful in determining the degree of **hybridization** where the two species become sympatric due to expected continued range expansions in Michigan, Wisconsin, and New York State.

PARTIAL LIFE-TABLES FOR CORN-EARWORM (LEPIDOPTERA, NOCTUIDAE) ON COMPACT-PANICLE AND LOOSE-PANICLE SORGHUM HYBRIDS

TEETES, GL; SCULLY, MJ; PETERSON, GC. 1992

Population dynamics were assessed and partial life tables constructed for corn earworm, *Helicoverpa zea* (Boddie), infesting panicles of two sorghum, *Sorghum bicolor* (L.) Moench, **hybrids** in field experiments in central Texas in 1985 and 1986. The **hybrids** were commercially acceptable types, similar agronomically and developmentally but differing in degree of panicle compactness at maturity. Almost all oviposition on panicles occurred just before and during flowering. Subsequent larval stages were associated with progressive stages of panicle development. Significantly more corn earworm eggs and larvae were recovered from panicles of the compact-panicle **hybrid** than the loose-panicle **hybrid**. However, life table construction revealed that the distribution and level of egg and larval mortality were similar for corn earworm on either panicle type. Data from all experiments revealed that real mortality ($r(x)$) was >98% by the end of the last larval stage, and was >90% during the egg and small larval stages. Parasitism by *Trichogramma* spp. and failure to hatch caused the greatest amount of egg mortality. Few parasitized larvae were found. Disease-induced larval mortality was low but was highest among large larvae. The greater number of larvae recovered from sorghum with compact panicles than those with loose panicles was a function of the greater number of eggs deposited on those panicles. Significant relationships existed between abundance of large larvae (>24 mm) and that of small and medium larvae. Equations were derived to predict densities of large corn earworm larvae on sorghum panicles based upon densities of earlier corn earworm life stages.

ISOLATION AND SEQUENCE OF A 2-KBP MINIPLASMID FROM BACILLUS-THURINGIENSIS VAR KURSTAKI HD-3A3B - RELATIONSHIP WITH MINIPLASMIDS OF OTHER BACILLUS-THURINGIENSIS STRAINS

MARIN, R; TANGUAY, RM; VALERO, J; LETARTE, R; BELLEMARE, G. 1992

The miniplasmid profiles of 18 *Bacillus thuringiensis* strains belonging to 8 different serotypes were determined using an alkaline hydrolysis method for isolation of low molecular mass plasmids. Nearly all the strains contained covalently closed circular (CCC) DNA species ranging from 2 to 5 species per strain and from 1.5 to 10.5 kbp in size (values corresponding to CCC forms). A 2-kbp plasmid from *B. thuringiensis* var. *kurstaki* HD-3a3b futura strain was used in Southern **hybridization** experiments to analyse relationships among the low molecular mass plasmids of different *B. thuringiensis* strains. This 2-kbp miniplasmid was present in most strains which show toxicity against lepidoptera. It was not present in those strains toxic against diptera (*B. thuringiensis* var. *israelensis*) or coleoptera (*B. thuringiensis* var. *tenebrionis*). The 2-kbp miniplasmid from *B. thuringiensis* var. *kurstaki* HD-3a3b futura was cloned and fully sequenced. Sequence analysis of the 2058 bp of the miniplasmid revealed the presence of an ORF (630 bp, 210 amino acids in size) that is preceded by a consensus sequence of *B. thuringiensis* crystal protein gene transcription promoters. No significant homology was observed with known *B. thuringiensis* toxin nucleic acid sequences or with other known sequences.

PATTERNS OF HERBIVORY IN THE QUERCUS-GRISEA X QUERCUS-GAMBELII SPECIES COMPLEX

AGUILAR, JM; BOECKLEN, WJ. 1992

In a recent paper, Whitham proposed the **hybrids-as-sinks** hypothesis, which posits that **hybrid** hosts are more susceptible to phytophagous insects than are parental hosts. We test the **hybrids-as-sinks** hypothesis in the *Quercus grisea* Liebm. X *Quercus gambelii* Nutt. **hybrid** complex. We measure densities of leaf-mining moths (Lepidoptera: Nepticulidae and Gracillariidae) and gall wasps (Hymenoptera: Cynipidae) on parental and **hybrid** host trees. We find no support for the **hybrids-as-sinks** hypothesis in the present **hybrid** complex. Hosts in this complex exhibited a unidirectional herbivore gradient, where **hybrid** hosts supported intermediate levels of herbivores when compared to parental species. We discuss possible causes affecting patterns of herbivore densities and distribution in this species complex, including host architecture, leaf morphology, and geographic range.

EVOLUTIONARY PATTERNS IN CHROMOSOME-NUMBERS IN NEOTROPICAL LEPIDOPTERA. 1. CHROMOSOMES OF THE HELICONIINI (FAMILY NYMPHALIDAE, SUBFAMILY NYMPHALINAE)

BROWN, KS; EMMEL, TC; ELIAZAR, PJ; SUOMALAINEN, E. 1992

Chromosome counts in meiotic metaphase plates in the gonads of 67 of the probable 68 species of mimetic neotropical heliconian butterflies (Nymphalidae), representing 1524 individuals in 617 subspecies and geographically separate populations from southern Texas to northern Argentina, revealed a consistent haploid number of $n = 21$ in the genus *Heliconius* (except for the most advanced species with $n = 33, 37, 56$, and 60) and $n = 31$ in the more primitive genera (*Eueides*, *Dryas*, *Dryadula*, *Agraulis*, and *Dione*), with a transitional genus (*Neruda*) showing three species with $n = 28-32, 21-22 + 5-10$ "microchromosomes", and $20-22 + 1-5$ "microchromosomes". The genus *Laparus*, with a single polymorphic species *doris*, probably an offshoot of early *Heliconius*, shows wide karyotypic variation ($n = 20-30, 38$) sometimes even within a single individual. The two most primitive genera also show much variation: *Podotricha* has two species with $n = 9$ and $n = 26-29$; and *Philaethria* shows many phenotypically similar species, two with $n = 29$ and a still uncertain number (at least 3) with $n = 88$ (most common), $67-72$ (most widespread), 62 (very restricted geographically), $52, 21$, and 12 . Several interspecific **hybrids** (*Heliconius cydno* x *H. melpomene*) showed normal chromosome pairing, while deficient pairing was seen in intersubspecific **hybrids** in *Eueides* tales and *Heliconius sara*. The importance of these results in the evolutionary study of polytypic tropical species is discussed.

INTERSPECIFIC HYBRIDIZATION BETWEEN HELIOTHIS-VIRESCENS AND HELIOTHIS-SUBFLEXA (LEPIDOPTERA, NOCTUIDAE) AFFECTS THE PRESENCE AND STRUCTURE OF HAIR-PENCIL GLANDS OF MALES

TEAL, PEA; OOSTENDORP, A. 1993

The effect of interspecific **hybridization** on the inheritance of hair-pencil glands was studied by crossing *Heliothis virescens* (F.), whose males have hair-pencil glands, with *H. subflexa* (Guenée), whose males lack hair pencils. Males from reciprocal interspecies crosses had hair-pencil glands the structure of which resembled those of *H. virescens* males. Backcross males from crosses between (*H. virescens* female x *H. subflexa* male) F1 x *H. subflexa* male lacked hair-pencil glands and were morphologically similar to *H. subflexa* males. The hair-pencil glands of first-generation backcross males from all other combinations resembled those of *H. virescens*. Second-generation backcross males from crosses, in which both Z chromosomes were inherited from *H. subflexa*, lacked hair pencils. However, second-generation backcross males that inherited at least one of the Z chromosomes from *H. virescens* were phenotypically similar to *H. virescens*. These results indicated that the presence of hair-pencil glands is regulated by a dominant allele of a locus or a group of genes located on the Z chromosome of *H. virescens*.

A REVISION OF THE NORTH-AMERICAN OPEROPHTERA (LEPIDOPTERA, GEOMETRIDAE)

TROUBRIDGE, JT; FITZPATRICK, SM. 1993

The North American members of the genus *Operophtera* Hubner (Lepidoptera: Geometridae) are revised to include three species: *O. brumata* (L.), *O. bruceata* (Hulst), and *O. danbyi* (Hulst). *Operophtera occidentalis* (Hulst) is now treated as a subspecies of a *bruceata*. All species are illustrated and characters to distinguish the species are given. The most useful characters for separating *O. brumata* from *O. bruceata* include the following: (1) hindwing dorsal surface with discal dot, which is almost always visible on *O. bruceata* but absent from *O. brumata*; (2) forewing colour, which is grey-brown or light brown in *O. bruceata* but reddish-brown in *O. brumata*; (3) abdomen colour, which is golden-brown to brown in western *O. bruceata* but brown in *O. brumata*; and (4) the costa of the forewing ventral surface, which is usually golden-brown in *O. bruceata* but brown in *O. brumata*. The structure of the genitalia of these two species allows **hybridization** between *O. bruceata* males and *O. brumata* females, but not vice versa. Hypothesized species interrelationships between palaearctic and nearctic *Operophtera* are illustrated in a cladogram. Maps for the distribution of each species are also included.

POLYMORPHISM AND EVOLUTION IN THE BUTTERFLY DANAUS-CHRYSIPPUS (L) (LEPIDOPTERA, DANAINAE)

SMITH, DAS; OWEN, DF; GORDON, IJ; OWINY, AM. 1993

Analysis of the genetic structure of a sample of the polymorphic butterfly *Danaus chrysippus* from Kampala, Uganda shows that the population is undergoing substantial evolutionary change. Comparison with samples from the same area going back to before 1900, indicate that the frequency of form *alcipus* has increased from 16 per cent to 71 per cent (1909-91) while *f. dorippus* has decreased from 14 per cent to 2 per cent, *f. aegyptius* from 66 per cent to 24 per cent and *f. albinus* from 4 per cent to 3 per cent. Genotype frequency differences between the sexes at two of the three loci examined suggest that a balanced polymorphism is maintained by opposing selective forces acting on males and females. Non-gametic (genotypic) disequilibrium between two pairs of unlinked loci indicates that natural selection is involved, again with sex differences. It is suggested that the polymorphism originated after **hybridization** of allopatric races which evolved during the Pleistocene but are now maintained sympatrically. The selective agents have not been identified but mimetic relationships, both Batesian and Mullerian, are almost certainly involved.

OVIPOSITION PATTERN IN SAMIA SILKMOTHS (LEPIDOPTERA, SATURNIIDAE)

SAITO, H. 1993

Oviposition patterns in the silkmotths, *Samia cynthia ricini*, *Samia cynthia pryeri* and their reciprocal F1 **hybrids** were investigated under a 16L-8D photoperiod at 25+/-1-degrees-C. Female moths of *S. c. ricini* mated with males of the same subspecies deposited eggs. About 75% of the total number of eggs were laid during the dark phases of days 1 and 2 after copulation. In virgin females, oviposition occurred mainly on days 3 to 8 after emergence. The average number of eggs in each egg mass of *S. c. ricini* and *S. c. Pryeri* was about 10 and 25, respectively. The number of eggs per egg mass of *S. c. ricini* was large, and sometimes more than 100 were laid in concentrated large masses (L-type). On the other hand, female *S. c. pryeri* laid widely-scattered egg masses in small groups of 1 to 10 eggs (S-type). Inter-subspecific reciprocal crossing produced F1 progenies with differential oviposition patterns. The average number of eggs per egg mass from *S. c. ricini* (female) x *S. c. pryeri* (male) was about 28. (Each mass was of the S-type.) However, the number from *S. c. pryeri* (female) x *S. c. ricini* (male) was about 14. (Each mass was of the L-type.) These results suggest that the gene(s) controlling oviposition behavior is located on the sex chromosome.

MITOCHONDRIAL-DNA VARIATION AND HALDANE RULE IN THE PAPILIO-GLAUCUS AND P-TROILUS SPECIES GROUPS

SPERLING, FAH. 1993

Variation in mitochondrial DNA (mtDNA) was surveyed, using restriction endonucleases, in all species of the *Papilio glaucus* and *P. troilus* groups (Lepidoptera: Papilionidae). Phylogenetic and distance relationships of mtDNA generally confirmed traditional species limits in the two species groups and compared favourably with a prior survey of their allozymes. The most notable exceptions were *P. rutulus* and *P. eurymedon*, which share their mtDNA, yet maintain numerous morphological and ecological distinctions in sympatry. In organisms with heterogametic females, like the Lepidoptera, the deficiencies of **hybrid** females that are expected on the basis of Haldane's rule should lead to congruence of mtDNA variants with species boundaries. In the *P. glaucus* group, the extent of mtDNA differences between species pairs generally coincided with the extent of the Haldane effect, as well as with the presence of major species differences on sex chromosomes (X effect).

MITOCHONDRIAL-DNA PHYLOGENY OF THE PAPILIO-MACHAON SPECIES GROUP (LEPIDOPTERA, PAPILIONIDAE)

SPERLING, FAH. 1993

In swallowtail butterflies of the *Papilio machaon* species group, mitochondrial (mt) DNA divergence has allowed speciation and adaptation to be understood more precisely. The reconstructed phylogeny of mtDNA of the *P. machaon* group is largely congruent with prior systematic hypotheses based on allozymes and color pattern. Genetic divergences of mtDNA support use of broad, character-based species concepts for the *P. machaon* group, and allow inferences regarding the origin of **hybrid** populations. The mtDNA phylogeny provides a guide for evolutionarily appropriate comparisons in studies of the chemical and genetic basis of hostplant use. Finally, mtDNA demonstrates the phylogenetically distinct status of an endangered species, *P. hospiton*.

SIZE DIFFERENCE AMONG 16S RIBOSOMAL-RNA GENES FROM ENDOSYMBIONTIC BACTERIA FOUND IN TESTES OF HELIOTHIS-VIRESCENS, HELIOTHIS-SUBFLEXA, (LEPIDOPTERA, NOCTUIDAE) AND BACKCROSS STERILE MALE MOTHS

KRUEGER, CM; DEGRUGILLIER, ME; NARANG, SK. 1993

Experimentally induced interspecific matings between *Heliothis virescens* male and *H. subflexa* female moths result in sterile male and fertile female offspring. The fertile female progeny can be backcrossed with *H. virescens* males to produce more sterile male and fertile female offspring. Successive generations of backcross female progeny continue to produce sterile male and fertile female offspring when backcrossed to *H. virescens* males. Backcross sterile males readily mate with wild-type *H. virescens* females under field conditions. Backcross male sterility is of interest for its potential to suppress populations of the pest moth *Heliothis virescens*. Rickettsia-like organisms have been observed in the testes of backcross sterile males and the two parental species of *Heliothis*. Prokaryotic endosymbionts have been associated with **hybrid** sterility in other insects. Therefore, we investigated *Heliothis* endosymbionts via an analysis of their PCR-amplified rRNA genes (rDNA). Prokaryotic-specific primers were used to amplify 16S rDNA from aseptically removed testes. PCR reactions without testes extract were negative. Endosymbionts from *H. virescens* and *H. subflexa* were genetically different. The size of PCR-amplified 16S rDNA indicates that endosymbionts in backcross sterile males originate from *H. subflexa*. Sterile males have essentially a *H. virescens* nuclear genome as a result of backcrossing. We propose that backcross male sterility results from an incompatibility between the maternally inherited endosymbiont type found in the cytoplasm and the predominantly paternal genetic material found in the nucleus.

DIAPAUSE DYNAMICS AND HOST-PLANT UTILIZATION OF COLIAS-PHILODICE, COLIAS-INTERIOR AND THEIR HYBRIDS (LEPIDOPTERA, PIERIDAE)

KAROWE, DN. 1994

Abnormal diapause dynamics and, to a lesser extent, reduced efficiency of host utilization by **hybrid** larvae constitute potential post-zygotic barriers to gene flow between a multivoltine legume-feeder, *Colias philodice* (Lepidoptera: Pieridae) and a univoltine *Vaccinium*-feeder, *C. interior*. At the time when *C. interior* larvae enter diapause, approximately 50% of **hybrid** larvae ceased feeding but did not enter diapause, and subsequently starved. Hybrid larvae readily accepted the host plants of both parental species. However, relative to *C. philodice*, **hybrid** larvae displayed a significantly reduced fifth instar relative growth rate (RGR) when fed the primary legume host, *Medicago sativa*. Reduced growth of **hybrid** larvae was attributable to both reduced relative consumption rate (RCR) and reduced efficiency of conversion of digested food (ECD), but not to reduced digestive efficiency (AD). Relative to *C. interior*, **hybrid** larvae fed *Vaccinium myrtilloides* displayed reduced pupal weight and reduced ECD. These results may explain in part why all known naturally-occurring **hybrids** among North American *Colias* arise from parental species with similar host plant ranges and diapause strategies.

COMPARATIVE-STUDIES OF IRIDOVIRUSES - FURTHER SUPPORT FOR A NEW CLASSIFICATION

WILLIAMS, T. 1994

Changes in the classification of invertebrate iridoviruses (IVs) (Iridoviridae) have recently been proposed (Williams and Cory, 1994). The previous system of naming isolates according to the host and sequence of discovery (IV type 1, IV2, IV3, etc.) is not adequate for the purposes of taxonomy, since iridovirus isolates may infect many species, including hosts from diverse invertebrate orders. The new system of invertebrate iridovirus nomenclature, as with several other virus families, is based on geographical origin. Proposals have been made, based on DNA **hybridization** and other characteristics, by which invertebrate iridovirus isolates can be assigned to one of four recognized complexes, or considered as candidates for alternative assignments. This study reports comparative data on the DNA of 14 invertebrate iridovirus isolates used in the Williams and Cory study plus the two type vertebrate iridoviruses, frog virus 3 and flounder lymphocystis disease virus. DNA studies support the validity of assigning several isolates a common name and of grouping the known isolates into four complexes. The detection of such complexes is in broad agreement with previous serological studies. A previously undescribed isolate (San Miguel IV) obtained from the lepidopteran pest *Anticarsia gemmatilis* (Lep.: Noctuidae) has been initially characterized following the procedures recommended by Williams and Cory. DNA **hybridization** and Southern blot analysis identified this isolate as a new member of the Polyiridovirus complex. The San Miguel IV MSP gene was identified and a central fragment of ca. 719 bp was recovered by PCR amplification. The restriction endonuclease profiles (5 enzymes) of this isolate were distinct from others previously described.

1ST RECORD OF AN HETEROTIC, ADULT FEMALE HYBRID LIMENITIS (BASILARCHIA) RUBIDUS (LEPIDOPTERA, NYMPHALIDAE)

PLATT, AP; HARRISON, SJ. 1994

The interspecific **hybrid** cross *Limenitis arthemis astyanax* x *L. archippus* yields the intermediate **hybrid** morph *L. "rubidus"* (Strecker). Such F1 **hybrids** occur in nature but they are quite rare; those reported to date have all been males. Laboratory crosses generally are lethal to females (which are heterogametic), with most dying during development; only a few have eclosed as malformed adults. This paper reports a laboratory cross that produced the first known heterotic female specimen of this remarkable F1 morph. It is intermediate between two related species of mimetic butterflies which are completely unlike in phenotype.

STEINERNEMA-RIOBRAVIS N-SP (RHABDITIDA, STEINERNEMATIDAE) FROM TEXAS

CABANILLAS, HE; POINAR, GO; RAULSTON, JR. 1994

Steinernema riobravus n. sp. is a new entomopathogenic nematode species discovered in the Lower Rio Grande Valley of Texas. Morphological, **hybridization**, and DNA examinations indicated the distinctness of *S. riobravus* n. sp. from *S. carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. intermedia*. Diagnostic characters include the length of the infective-stage juveniles, the color and shape of the spicules and gubernaculum, and lack of a tail projection in the male. The ratio E (distance from anterior end to excretory pore divided by tail length) of the infective juveniles separates this new species from previously described steinernematids. *S. riobravus* n. sp. did not **hybridize** with other *Steinernema* species. DNA analysis showed that the 304 base pair region of the 26 S. ribosomal subunit examined in *S. riobravus* n. sp. is significantly divergent from the same region in *S. carpocapsae*, *S. feltiae*, *S. glaseri* and *S. serratum*. It appears to be naturally selected for the subtropical semi-arid

environment where it serves as a biological control agent for corn earworm, *Helicoverpa zea*, and fall armyworm, *Spodoptera frugiperda*, (Lepidoptera : Noctuidae), at high temperatures.

CLONING, CHARACTERIZATION, AND GENETICS OF THE JUVENILE-HORMONE ESTERASE GENE FROM *HELIOTHIS-VIRESCENS*

HARSHMAN, LG; WARD, VK; BEETHAM, JK; GRANT, DF; GRAHAN, LJ; ZRAKET, CA; HECKEL, DG; HAMMOCK, BD. 1994

The gene for juvenile hormone esterase (JHE) was cloned from *Heliothis virescens* (Lepidoptera: Noctuidae). A genomic library was constructed from embryonic DNA and screened with a homologous N-terminal probe from the JHE cDNA. Five genomic clones were isolated and analyzed by dot blot **hybridization** using regions of the JHE cDNA as probes. Clone C **hybridized** to both 5' and 3' probes from the JHE cDNA, suggesting that clone C contains both ends of JHE gene. This was verified by sequencing the ends of the JHE gene from clone C using primers from both the 5' and 3' ends of the JHE cDNA. Additional sequencing and restriction mapping were used to characterize the gene. The gene is c. 8 kb long and contains four introns with consensus intron-exon junctions. One of the introns is relatively large (4 kb) and is situated near the extreme 5' end of the gene. Genetic analysis of RFLP variation in interspecific and intraspecific crosses shows that the JHE locus is single-copy with no closely related paralogs and is autosomally encoded in *Heliothis*. Therefore the developmental pattern of expression of this gene and the previously documented sequence variation in cDNA clones is not explainable by reference to a JHE gene family with distinct structural loci for the different forms.

POLYMORPHIC MULLERIAN MIMICRY IN A GROUP OF AFRICAN BUTTERFLIES - A REASSESSMENT OF THE RELATIONSHIP BETWEEN *DANAUS-CHRYSIPPUS*, *ACRAEA-ENCEDON* AND *ACRAEA-ENCEDANA* (LEPIDOPTERA, NYMPHALIDAE)

OWEN, DF; SMITH, DAS; GORDON, IJ; OWINY, AM. 1994

Mullerian mimicry, in which there is convergence in coloration between unrelated unpalatable species, should lead to uniformity in appearance, not polymorphism, and so the occurrence in tropical Africa of unrelated species of unpalatable butterflies with corresponding polymorphic colour forms suggested a problem of special evolutionary interest. Field work in Uganda and Sierra Leone in 1964-72 demonstrated a statistical association between the occurrence and relative frequencies of polymorphic forms in *Danaus chrysippus* (Danainae) and *Acraea encendon* (Acraeinae) which was deemed as confirmation of a Mullerian relationship between them. There were, however, certain anomalies which at the time remained unresolved. Later, in 1976, it was discovered that what had been called *A. encendon* is in reality two sibling species *A. encendon* and a new one, named as *A. encedana*. The two differ in the structure of both male and female genitalia and in the coloration and the food-plants of the larvae. The recognition of the additional species has enabled a re-assessment of the polymorphic Mullerian association with *D. chrysippus*. It emerges that, although there is a close qualitative and quantitative reciprocal mimetic relationship between *A. encedana* and *D. chrysippus*, the relationship between *A. encendon* and *D. chrysippus* is much weaker, and in places non-existent. The possible origin of the mimetic polymorphism is discussed in terms of **hybridization** of previously allopatric and monomorphic populations which have met as a consequence of recent expansions of geographical range in all three species resulting from forest clearance and the spread of savanna-like conditions in previously forested areas.

DIAPAUSE RESPONSE IN *SAMIA-CYNTHIA* SUBSPECIES AND THEIR HYBRIDS (LEPIDOPTERA, SATURNIIDAE)

SAITO, H. 1994

CORRELATION BETWEEN DENDRITE DIAMETER AND ACTION-POTENTIAL AMPLITUDE IN SEX-PHEROMONE SPECIFIC RECEPTOR NEURONS IN MALE *OSTRINIA-NUBILALIS* (LEPIDOPTERA, PYRALIDAE)

HANSSON, BS; HALLBERG, E; LOFSTEDT, C; STEINBRECHT, RA. 1994

Outer dendritic segments of olfactory receptor neurons tuned to sex pheromone components were measured morphometrically on the antenna of male European corn borers, *Ostrinia nubilalis*, to determine if a correlation exists between the diameter of the outer dendritic segment and the spike amplitude. The olfactory sensilla investigated each contained three receptor cells. Two cells were each specific for one of the two pheromone components, (Z)-11-tetradecenyl acetate (Z11-14:OAc) and (E)-11-tetradecenyl acetate (E11-14:OAc). Two strains of cornborers (Z and E) differ as to which of the two pheromone components is the main one. In both strains a large difference could be observed between the spike amplitudes elicited in the receptor cells by the two pheromone components, the main component always eliciting the large spike. In F1-**hybrids** (EZ) of these two strains, producing both pheromone components in similar quantities, the spike amplitudes were equal in the two pheromone-specific receptor cells. The third cell responded specifically to a behavioural antagonist, (Z)-9-tetradecenyl acetate (Z9-14:OAc) in both the parental and **hybrid** strains, and always showed the smallest spike amplitude. In a morphometric study, the outer dendritic segments were shown to differ more in diameter between the largest and second largest cell in the two parental strains than in the **hybrid** strain, while the smallest diameter cell did not differ between the different strains. These results imply that receptor cells with larger diameter produce spikes with greater amplitude. The data also show that all three types of receptor neurons display outer dendritic segments with strong variation in the diameter along the length of the segment, and with a pronounced taper towards the tip.

HOST-RANGE OF THE MESQUITE CUTWORM, *MELIPOTIS-INDOMITA* (LEPIDOPTERA, NOCTUIDAE), A POTENTIAL BIOCONTROL AGENT FOR MESQUITE (*PROSOPIS* SPP)

DELOACH, CJ; CUDA, JP. 1994

The mesquite cutworm, *Melipotis indomita* (Walker), is the major defoliator of mesquite in central Texas. In the laboratory, the cutworm larvae developed normally on honey mesquite (*P. glandulosa* Torrey var. *glandulosa*) and the Argentine vinal (*P. ruscifolia* Grisebach). Velvet mesquite (*P. velutina* Wootton), western honey mesquite (*P. g.* var. *torreyana* (Benson) Johnston), the Mexican *P. laevigata* (H. and B. ex. Willdenow) Johnston and *P. juliflora* (Swartz) DC, and the Argentine *P. flexuosa* DC were slightly less suitable hosts. Larvae developed occasionally on a natural **hybrid** of the Argentine *P. alba* Grisebach from Tucson, Arizona, but not on a commercial *P. alba* **hybrid** developed in southern Texas. Larvae did not develop on the two screw-bean mesquites tested (*P. pubescens* Benth and *P. reptans* Benth var. *cinerascens* (A. Gray) Burkart) or on 12 other species of woody legumes from central Texas, although an occasional larva fed for a few days on some of them. Among 11 species of mostly tropical leguminous trees tested, larvae developed and produced adults normally on manila tamarind (*Pithecellobium dulce* (Roxburgh) Benth) and one adult was produced on monkeypod (*Samanea saman* (Jacquin) Merrill). In a biological control program, the mesquite cutworm would complement the damage from other foliage feeders that could be introduced from South America. It could be a candidate for introduction into other countries where mesquite has become a pest, provided that a foliage feeder were the type of control agent wanted.

MONITORING ADULT EUROPEAN CORN-BORER (LEPIDOPTERA, PYRALIDAE) IN POTATOES ON PRINCE-EDWARD-ISLAND

STEWART, JG. 1994

Experiments to determine the effectiveness and time required to sample for males of the European corn borer, *Ostrinia nubilalis* (Hubner), with blacklight, water-pan,

Heliothis-cone, and delta-wing traps, along with sweep-net samples of grassy areas near potato fields were conducted at four sites in 1990 and 1991 on Prince Edward Island. Compared with the blacklight trap, the water-pan trap caught 1.72 times more males in 1990 and 1.97 times more males in 1991. The Heliothis-cone and delta-wing traps, and sweep-net samples were ineffective for male European corn borer. Blacklight traps required significantly more time to sample than any of the pheromone-based traps. A water-pan trap is an effective tool to monitor adult males of the European corn borer in potatoes on Prince Edward Island. In 1992, the mean number of males per pan trap per week was 7.4 for the Iowa strain, 1.1 for the New York strain, 0.6 for the **hybrid** strain, and 1.1 for an unbaited trap. This suggests that the Iowa strain predominates in the population of European corn borer on Prince Edward Island.

SEX-PHEROMONE CHARACTERIZATION AND FIELD TRAPPING OF THE EUROPEAN CORN-BORER, OSTRINIA-NUBILALIS (LEPIDOPTERA, PYRALIDAE), IN SOUTH MORAVIA AND SLOVAKIA

KALINOVA, B; MINAIF, A; KOTERA, L. 1994

The analysis of pheromone glands from individual females of the European corn borer, *Ostrinia nubilalis* (Hubner), originating in South Moravia and Slovakia showed that this population utilizes the "Z" pheromone system. The ratio of (Z)- and (E)-11-tetradecenyl acetates was found in the range of 98.5:1.5-99.5:0.5. Field experiments confirmed the identity of the local population as being predominantly of the Z strain. Individuals responding to E and **hybrid** blends were detected.

EFFECT OF INTERSPECIFIC HYBRIDIZATION BETWEEN HELIOTHIS-VIRESCENS AND HELIOTHIS-SUBFLEXA (LEPIDOPTERA, NOCTUIDAE) ON SEX-PHEROMONE PRODUCTION BY FEMALES

TEAL, PEA; OOSTENDORP, A. 1995

The effect of interspecific **hybridization** between *Heliothis virescens* and *H. subflexa* on the production of sex pheromone by females was studied. The F-1 **hybrid** females from crosses between *H. virescens* male and *H. subflexa* female did not produce sex pheromone during a distinct period in the scotophase. The mean amounts of pheromone present in extracts obtained from insects sampled at 1 h intervals remained relatively constant from the second to ninth hours of the scotophase. Hybrid females from the reciprocal cross (*H. subflexa* male x *H. virescens* female) had peak pheromone production at the sixth hour of the scotophase. Homogenates of cephalic ganglia obtained from either of the **hybrid** lines stimulated pheromone production when injected into either of the parent species or either of the F-1 **hybrid** lines during the photophase. Thus, F-1 females had the capacity to produce pheromone and pheromonotropic substances were present in the cephalic ganglia. We conclude that **hybridization** between *H. virescens* male and *H. subflexa* female affected the endogenous release of pheromonotropic substances from the cephalic ganglia.

PRODUCTION OF PHEROMONE BY HAIRPENCIL GLANDS OF MALES OBTAINED FROM INTERSPECIFIC HYBRIDIZATION BETWEEN HELIOTHIS-VIRESCENS AND HELIOTHIS-SUBFLEXA (LEPIDOPTERA, NOCTUIDAE)

TEAL, PEA; OOSTENDORP, A. 1995

Pheromone produced by the hairpencil glands of interspecific **hybrid**- and backcross-generation males from crosses between *Heliothis virescens* (F.) with *H. subflexa* (Gn.) was studied. Males of reciprocal F-1 **hybrids**, all of which had hairpencil glands morphologically similar to those of *H. virescens*, produced neither the same pheromone blend nor amounts of pheromone that were produced by males of *H. virescens*. Instead, these **hybrid** males produced pheromone that was quantitatively and qualitatively similar to that produced by *H. subflexa*. Hairpencil gland extracts from males obtained from backcrossing F-1 females of either cross to males of *H. subflexa* were the same as those of *H. subflexa*. However, extracts from backcross males of crosses between F-1 females and *H. virescens* were variable. Some extracts from these backcross males were like those of *H. virescens* while others were either like *H. subflexa* or were intermediate between those of the parent species. These results showed that the production of pheromone by the hairpencil glands of **hybrid** and backcross males is under the dominant regulation of autosomal genes of the *H. subflexa* genome.

GENETIC-ANALYSIS OF DIFFERENCES IN OVIPOSITION PREFERENCES OF HELIOTHIS-VIRESCENS AND HELIOTHIS-SUBFLEXA (LEPIDOPTERA, NOCTUIDAE)

SHECK, AL; GOULD, F. 1995

Heliothis virescens (F.) is a polyphagous pest on cotton soybean, anti tobacco and *H. subflexa* (Guenée) is a monophagous nonpest on *Physalis* spp. Oviposition choices of the reciprocal F-1 **hybrids** of these two species were compared with those of the parental strains to determine sex linkage, quantify niche breadth, and rank host preferences. Strains of *H. virescens*, *H. subflexa*, and the reciprocal F-1 **hybrids** (SV0 and VS0) were each offered a choice of four plants on which to oviposit (cotton, soybean, tobacco, and *Physalis angulata*). Host preferences were ranked based on the proportion of eggs laid on each host. *H. virescens* overwhelmingly preferred tobacco, *H. subflexa* preferred *Physalis* and SV0 and VS0 preferred tobacco. There were no significant differences among strains in their responses to cotton and soybean. Because SV0 and VS0 had similar patterns of preference across hosts, there was no indication of sex linkage. Niche breadth, a measure of the degree of specialization, was calculated for each strain. Under greenhouse conditions, *H. virescens* had the narrowest ovipositional niche and *H. subflexa* and the **hybrids** had broader niches and were similar to each other. When a novel host (*Oenothera* sp.) was offered to the strains, there was no difference in the responses among strains nor were there changes in preference ranks or diet breadth. The **hybrid**, despite a major genetic reorganization relative to the parents, did not behave in an unpredictable way.

EFFECTIVENESS OF 4 BLENDS OF EUROPEAN CORN-BORER (LEPIDOPTERA, PYRALIDAE) SEX-PHEROMONE ISOMERS AT 3 LOCATIONS IN SOUTH-CAROLINA

DURANT, JA; FESCEMYER, HW; MASON, CE; UDAYAGIRI, S. 1995

A field study was conducted at Florence, Newberry, and Clemson, South Carolina, to determine the relative attractiveness of four isomeric blends of 11-tetradecenyl acetate to male moths of the European corn borer (ECB), *Ostrinia nubilalis* (Hubner). The E and Z isomers were formulated in E:Z ratios of 99:1, 97:3, 65:35, and 3:97. Trap capture data indicated that the E pheromone strain of the ECB was predominant at Florence and the Z strain was predominant at Clemson. Both strains appeared to occur sympatrically at Newberry. Pheromone analysis of 46 ECB female moths at Florence indicated that 43 were the E strain and 3 were **hybrids**. The 99E:1Z pheromone blend captured over four times as many male moths as the 97E:3Z blend at Florence. The 65E:35Z blend captured 5%, 11%, and 12% of the moths at Florence, Newberry, and Clemson, respectively, indicating the possible presence of **hybrid** moths. The superior performance of the 99E:1Z blend of 11-tetradecenyl acetate should enhance the development of a more effective lure for the E strain of the ECB.

BACTERIAL ENTOMOPATHOGENS FROM THE DROSOPHILA-PAULISTORUM SEMISPECIES COMPLEX

MILLER, SG; CAMPBELL, BC; BECNEL, J; EHRMAN, L. 1995

Bacteria which are infectious by inoculation in lepidoptera have been isolated and characterized from semispecies comprising the *Drosophila paulistorum* complex. These microorganisms are pathogenic toward lepidopteran hosts such as *Heliothis virescens* when introduced by injection of *Drosophila* tissue extracts and have been given the trivial name DpLE (*D. paulistorum* lepidopteran entomopathogen). The DpLE from two of the semispecies, Transitional and Andean, were determined to be related to *Proteus vulgaris* based upon nucleotide sequence comparisons of 16S rDNA genes. Infectivity and 16S rDNA-based PCR assays showed the bacterium to be localized in a number of drosophilid tissues except adult heads and thoraces. Based upon similar experiments, the DpLE in transinfected *Heliothis* larvae were found in all tissues assayed prior to the onset of mortality. Stocks of *Drosophila* which had spontaneously lost DpLE continued to produce sterile sons when crossed with incompatible semispecies' females, confirming that the bacilliform DpLE is not the causative agent of the *Drosophila paulistorum* intersemispecific **hybrid** male sterility. Acquisition of the sequences of the 16S rDNA molecules of DpLE from all six semispecies permitted the construction of a phylogenetic tree in which the groupings were found not to be congruent with the phylogenies of their insect hosts.

LIMITATIONS TO THE INFERENCE OF GENE FLOW AT REGIONAL GEOGRAPHIC SCALES - AN EXAMPLE FROM THE PIERIS-NAPI GROUP (LEPIDOPTERA, PIERIDAE) IN EUROPE

PORTER, AH; GEIGER, H. 1995

We used hierarchical and pairwise F-statistics to describe generic differentiation and infer gene flow (M) on local and regional scales within and among parapatric European butterfly taxa in the *Pieris napi* (L.) group. Within-population allozyme variability is consistently high, and local effective population sizes are inferred to be in the thousands of individuals. The pairwise analysis yields an average neighbourhood area of radius 3.5 km. Among populations within most regions, differentiation is low and $M > 2$ effective individuals population(-1) generation(-1). Pairwise comparisons within the britannica group show a disjunction indicating that it is out of equilibrium, perhaps as a result of secondary contact between highland and lowland groups. Comparison between meridionalis groups on mainland Italy and Corsica yields $M > 12$; this is surely too high and lack of equilibrium resulting from initial colonization is suspected. The hierarchical analysis indicates that 23 less than or equal to M less than or equal to 88 among the taxa napi, bryoniae and meridionalis that meet in **hybrid** zones; no effective gene flow barrier exists among them. This high estimate could also result from recent primary contact, but such a genetic barrier should produce the 'edge effects' seen in population genetic simulations, and no evidence of this was found among geographically close samples of napi and bryoniae populations from Switzerland. Studies of gene flow among geographic regions are greatly limited by the equilibrium assumption, though studies of local differentiation are much less so. Population studies of gene flow on local scales at regional boundaries provide limited means of testing the equilibrium assumption, and both regional and local analyses provide testable predictions about local population structure. When the equilibrium assumption is not upheld, local patterns at regional boundaries can provide historical information about primary vs. secondary contact.

What can hybrid zones tell us about speciation? The case of *Heliconius erato* and *H. himera* (Lepidoptera: Nymphalidae)

Jiggins, CD; McMillan, WO; Neukirchen, W; Mallet, J. 1996

To understand speciation we need to study the genetics and ecology of intermediate cases where interspecific **hybridization** still occurs. Two closely related species of *Heliconius* butterflies meet this criterion: *Heliconius himera* is endemic to dry forest and thorn scrub in southern Ecuador and northern Peru, while its sister species, *H. erato*, is ubiquitous in wet forest throughout south and central America. In three known zones of contact, the two species remain distinct, while **hybrids** are found at low frequency. Collections in southern Ecuador show that the contact zone is about 5 km wide, half the width of the narrowest dines between colour pattern races of *H. erato*. The narrowness of this dine argues that very strong selection (s approximate to 1) is maintaining the parapatric distributions of these two species. The zone is closely related with a habitat transition from wet to dry forest, which suggests that the narrow zone of parapatry is maintained primarily by ecological adaptation. Selection on colour pattern loci, assortative mating and **hybrid** inviability may also be important. The genetics of **hybrids** between the two species shows that the major gene control of pattern elements is similar to that found in previous studies of *H. erato* races, and some of the loci are homologous. This suggests that similar generic processes are involved in the morphological divergence of species and races. Evidence from related *Heliconius* supports a hypothesis that ecological adaptation is the driving force for speciation in the group. (C) 1996 The Linnean Society of London

Infection *Enterococcus* from *Heliothis virescens* x *H. subflexa* backcross hybrids (Lepidoptera: Noctuidae)

Miller, SG; Miller, RD. 1996

Plating assays have revealed the presence of a bacterium in extracts of tissues from backcross **hybrid** insects, derived from crosses between *Heliothis virescens* and *H. subflexa*, a bacterium found rarely and in much lower abundance in either of the 2 parent species. The bacterium is primarily localized within the gut but is also consistently recovered in small numbers from other somatic and germinal tissues. Fatty acid profile analyses and the sequence of its 16s ribosomal RNA indicate that the bacterium is an *Enterococcus* related to *Enterococcus sulfureus* and *E. casseliflavus*, species that previously have been encountered in insects and on plant tissues. Injection of the *Enterococcus* into *H. virescens* hosts results in the establishment of a bacterial infection similar to that of naturally infected backcross **hybrids** in terms of tissue distribution and relative magnitude among tissues. Like backcross **hybrid** males, almost 90% of *H. virescens* male moths infected as larvae were sterile in single pair test matings. The inability to either rescue male fertility in backcross lines cured of the *Enterococcus* or to observe characteristic abnormalities in sperm ultrastructure, however, casts doubt on the likelihood that this microorganism is the proximate cause of male sterility in backcross **hybrid** insects.

Genetics of mimicry in the tiger swallowtail butterflies, *Papilio glaucus* and *P. canadensis* (Lepidoptera: Papilionidae)

Scriber, JM; Hagen, RH; Lederhouse, RC. 1996

The tiger swallowtail butterfly, *Papilio glaucus*, exhibits a female-limited polymorphism for Batesian mimicry; the Canadian tiger swallowtail, *Papilio canadensis*, lacks the mimetic (dark) form entirely. The species **hybridize** to a limited extent where their ranges overlap. Field collections and censuses indicate that mimetic females occur throughout the range of *P. glaucus* but at lowest frequencies in populations at the latitudinal edges of its geographic range such as the southernmost part of Florida and along the entire northern edge of its distribution from Massachusetts to Minnesota. Frequencies of mimetic females have remained relatively stable over time. Inheritance of the mimetic form is controlled primarily by two interacting sex-linked loci. The typical matrilineal pattern of inheritance in *P. glaucus* can be explained by polymorphism at a Y-linked locus, b. Analysis of *P. glaucus* X *P. canadensis* crosses has also revealed an X-linked locus, s, which controls the expression of the mimetic phenotype. The *P. canadensis* allele, s(can), suppresses the mimetic phenotype in **hybrid** and backcross females. Results from more than 12 yr of rearing tiger swallowtails, including interspecies **hybrids**, indicate that the absence of mimetic *P. canadensis* females is due to both a high frequency of the "suppressing" allele s(can) and low frequency of the black-pigment-determining b+ allele. The frequency of s(can) (or other suppressing alleles of s) in *P. glaucus* populations outside the **hybrid** zone is low. Some males heterozygous at the s locus and some suppressed mimetic females occur within the **hybrid** zone. A simple genetic model predicts the frequency of daughters that differ in phenotype from their mothers.

Chloroclystis v-ata relictata ssp n (Lepidoptera, Geometridae), a new taxon from SW Finland, doomed to go extinct?

Krogerus, H. 1996

An isolated population of *Chloclystis v-ata* (Haworth, 1809), occurring at Karkali Peninsula, a nature reserve in southwestern Finland, is described as a new subspecies, ssp. *relicta* ssp.n., on the basis of differences in appearance and phenology compared to the nominate subspecies. The Central European population of *Ch. v-ata* has been expanding to the north, reaching the Baltic Sea area in recent decades and Finland in 1985. Thus, the new subspecies which is actually protected by law is now threatened by **hybridization** with the nominate subspecies. In contrast to the bivoltine nominate subspecies, the Karkali population is univoltine. The biology of the new subspecies is discussed.

Field evaluation of and modeling the impact of three control methods on the larval dynamics of *Ostrinia nubilalis* (Lepidoptera: Pyralidae)

Labatte, JM; Meusnier, S; Migeon, A; Chaufaux, J; Couteaudier, Y; Riba, G; Got, B. 1996

Impact of 3 control methods on larval European corn borer, *Ostrinia nubilalis* Hubner, dynamics on corn, Zee mays (L.), was evaluated under field conditions at Versailles, France. The control methods studied were a chemical insecticide, *Beauveria bassiana* Vuillemin (Deuteromycotina: Hyphomycete), and a transgenic corn **hybrid**. The experimental study showed that *B. bassiana* control was similar to chemical control. The transgenic **hybrid** control was always very high throughout the corn cycle studied. A substantial decrease of *B. bassiana* and chemical control efficacy was observed with an increase in the delay between treatment and infestation. The complementary studies of *B. bassiana* persistence, control impact, and pathogen contact showed control-larval behavior interactions, which could explain this decrease in efficacy. To take into account the main factors that condition control efficacy, a modular and mechanistic model was proposed to describe larval dynamic and control impact. The proposed control model made it possible to integrate *O. nubilalis* dynamics, and thus to describe the time response of control.

Sex-specific and hormone-controlled expression of a vitellogenin-encoding gene in the gypsy moth

Adamczyk, JJ; Fescemyer, HW; Heckel, DG; Gahan, LJ; Davis, RE; Kelly, TJ. 1996

Microvitellogenin and vitellogenin cDNA from *Manduca sexta* (tobacco hornworm) were tested for use as molecular probes to investigate the expression of genes coding for vitellogenins in *Spodoptera frugiperda* (fall armyworm) and *Lymantria dispar* (gypsy moth). Cross-**hybridization** was not observed between the *M. sexta* cDNAs and *S. frugiperda* DNA and mRNA. Vitellogenin cDNA from *M. sexta* did not **hybridize** to *L. dispar* DNA or mRNA. However, the 834 bp microvitellogenin cDNA from *M. sexta* **hybridized** to an approximately 850 bp transcript in *L. dispar* mRNA. A 2.5 kb cDNA clone, pz64, was isolated from late last instar larvae of female *L. dispar* by differential screening. This clone has 38% amino acid sequence (deduced) and 55% nucleic acid sequence similarities with the 3'-end of high molecular weight vitellogenin in *Bombyx mori* (silkworm). When used as a probe in northern analysis of *L. dispar* mRNA, this cDNA **hybridized** to a 5.3 kb transcript in female last instar larvae, pupae, and adults, but not to male last instar larvae and adults. This cDNA did not **hybridize** to mRNA from *M. sexta* or *S. frugiperda*. Expression of the 5.3 kb vitellogenin transcript **hybridizing** to the 2.5 kb cDNA clone was suppressed in 5-day-old last instar larvae of female *L. dispar* treated on day 2 with doses of the juvenile hormone analog, methoprene, greater than 10 nmol. Apparently, the high in vivo titer of juvenile hormone during the first 2 days of the last instar represses the transcription of vitellogenin mRNA. (C) 1996 Wiley-Liss, Inc.

Effects of hybrid poplar (Salicaceae) clone and phenology on gypsy moth (Lepidoptera: Lymantriidae) performance in Wisconsin

Kruse, JJ; Raffa, KF. 1996

Gypsy moth (*Lymantria dispar*) developmental interactions with two **hybrid** *Populus* species clones were studied in laboratory trials. Significant differences in larval performance were found between clones and within the same clone at different phenological states. No larvae were able to complete development on clone NM6. All gypsy moth larvae feeding on clone NC5271 survived when leaf flush was synchronized with gypsy moth eclosion in early May. However, neonates feeding on NC5271 foliage in July experienced increased mortality. Weights of surviving gypsy moth larvae feeding on NC5271 foliage in May versus July were not significantly different.

Pheromone-mediated diel activity rhythms of male Asian gypsy moths (Lepidoptera: Lymantriidae) in relation to female eclosion and temperature

Carde, RT; Charlton, RE; Wallner, WE; Baranchikov, YN. 1996

Male Asian gypsy moths, *Lymantria dispar* (L.), were attracted to synthetic pheromone (cis-7R,8S-epoxy-2-methyloctadecane) in the Russian Far East, Central Siberia, and Germany, where this strain has recently been detected. A bimodal pattern of attraction was evident at all 3 sites. One peak of attraction was in early to midafternoon, with a 2nd, usually smaller peak, following sunset. Temperature modulated attraction: warm daytime temperatures increased catch, whereas low nighttime temperatures generally suppressed or eliminated attraction. However, the surge of male attraction to pheromone in the hour after sunset seemed unaffected by falling temperatures. As average temperatures at the 3 sites decreased, the daytime peak of male activity progressively shifted to later in the afternoon. Female eclosion in Germany also followed a bimodal pattern, with a major midmorning to midday peak of emergence and a 2nd, smaller peak, in the afternoon. The timing of male attraction allowed coordination of the ranging flight of the male with the availability of emerging and pheromone-emitting females. The congruence in daily activity patterns suggest that the timing of mating would not be a barrier to the **hybridization** of the Asian, European, and North American strains of this lymantriid in new areas of sympatry.

Mitochondrial DNA sequence variation among pheromotypes of the dingy cutworm, *Feltia jaculifera* (Gn) (Lepidoptera:Noctuidae)

Sperling, F; Byers, R; Hickey, D. 1996

The dingy cutworm, *Feltia jaculifera*, is a complex of at least four pheromonal races (pheromotypes). We examined mitochondrial DNA (mtDNA) sequence and restriction-site variation in the cytochrome oxidase subunit I and II genes. Among 74 specimens representing the four pheromotypes, we found three discrete mitochondrial lineages. The most divergent mtDNA within *F. jaculifera*, the gamma lineage, was associated with pheromotype C. This result confirms an earlier allozyme survey, which also found that pheromotype C was the most genetically distinct race. Thus, pheromotype C probably represents a classic sibling species. Of the two most similar mitochondrial lineages, the alpha lineage was disproportionately associated with pheromotype A and the beta lineage was disproportionately associated with pheromotypes B and D. Although the correspondence between mitochondrial lineages and pheromotypes was incomplete, mtDNA variation provides strong nonbehavioral evidence for genetic divergence between pheromotypes A and B + D. Allozymes revealed more minor differentiation among the A, B, and D races. For pheromotypes A and B, the presence of distinct mtDNA lineages within populations without perfect correspondence to pheromone polymorphism has several possible explanations. It may be due to retained ancestral mtDNA polymorphism, whether initial divergence of pheromone races occurred in allopatry or sympatry, or to divergence in isolation with introgression following secondary contact. The greater differences in allele frequencies of mtDNA than of allozymes may be due either to the susceptibility of mtDNA to bottleneck effects or to asymmetric selection against **hybrid** females.

Reductase specificity and the ratio regulation of E/Z isomers in pheromone biosynthesis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae)

Zhu, JW; Zhao, CH; Lu, F; Bengtsson, M; Lofstedt, C. 1996

Species specificity of moth sex pheromones is in many cases achieved by means of specific blends rather than by specific components, Two pheromone strains of the European corn borer, *Ostrinia nubilalis*, use (E)- and (Z)-11-tetradecenyl acetate in different ratios as their pheromone, but show the same ratio of the pheromone precursors (70:30 E/Z-11-tetradecenoic acid), The hypothesis that the ratio of the pheromone components in the two strains and their **hybrids** is controlled by the specificity of the reductase system, responsible for conversion of acid to the corresponding alcohol precursors, was tested. Deuterium-labeled alcohols, aldehydes and fatty acids corresponding to the two pheromone components were topically applied to the pheromone glands in different ratios and their selective incorporation into pheromone components was determined by gas chromatography with mass: selective detection, Acetylation of the (E)- and (Z)-11-tetradecenols was unselective, whereas the corresponding aldehydes and acids were selectively incorporated into the pheromone components, Z strain females selectively metabolized the Z-isomers whereas E strain females converted the E-isomers, The E strain being the most selective of the two strains, Hybrids converted both geometric isomers, The relative conversion rate of both E- and Z-isomers of all tetradecenoic acids with the double bond in positions from 7-12, was also determined, In addition to the Delta 11-isomers, the E strain females converted (E)-8-tetradecenoic acid into acetate and the Z strain females converted (E)-12-tetradecenoic acid. None of these substrates occur naturally in the pheromone gland, but (E)-12-tetradecenyl acetate is a pheromone component of the Asian corn borer *O. furnacalis*, Thus the possibility for conversion of (E)-12-tetradecenoic acid to acetate in the Z strain, as well as the earlier reported conversion of (Z)-11-tetradecenoic acid to acetate in *O. furnacalis*, suggests that *O. furnacalis* is closest related to the Z strain of *O. nubilalis*.

Abundance of *Cardiochiles nigriceps* (Hymenoptera: Braconidae) on *Nicotiana* species (Solanaceae)

Jackson, DM; Nottingham, SF; Schlotzhauer, WS; Horvat, RJ; Sisson, VA; Stephenson, MG; Foard, T; McPherson, RM. 1996

The presence of adult *Cardiochiles nigriceps* Viereck, a nearly obligatory parasitoid of tobacco budworm larvae, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), was monitored in fields with up to 117 accessions of *Nicotiana* species (Solanaceae) during 1985-1994 at Oxford, NC, Tifton, GA, and Athens, GA. Large numbers of wasps were observed flying over or resting on several *Nicotiana* species, especially *N. noctiflora* Hooker and *N. sanderae* Hort. ex Watson (**hybrid** between *N. alata* Link & Otto and *N. forgetiana* Hort, ex Hemsley). During the IO-vr sampling period, an average of 60 times as many *C. nigriceps* adults were counted in *N. noctiflora* plots and 19 times as many wasps were counted in *N. sanderae* plots than were found in commercial tobacco, *N. tabacum* L. ('NC 2326'), even though *N. noctiflora* and *N. sanderae* are poor host plants for tobacco budworm larvae. Male wasps predominated in plots of *N. noctiflora*; whereas, female wasps predominated in cultivated tobacco fields. There were no significant differences in the sex ratios of wasps collected from *N. alata* or *N. sanderae*. *C. nigriceps* adults were associated primarily with the flowers of, *N. sanderae*, *N. alata*, and *N. forgetiana*, but they were found equally on the leaves and flowers of *N. noctiflora*. *C. nigriceps* adults appeared to be attracted to volatile components from *N. noctiflora* leaves and flowers. Several kilograms of field-grown *N. noctiflora* flowers and leaves were extracted with methylene chloride, and the volatile components were isolated. Gas chromatography showed only 4 volatile peaks of interest in *N. noctiflora*, and they were identified as nitrogen-sulfur heterocycles.

A new mimetic species of *Heliconius* (Lepidoptera:Nymphalidae), from southeastern Colombia, revealed by cladistic analysis of mitochondrial DNA sequences

Brower, AVZ. 1996

A new species of *Heliconius* and a new geographical race of *Heliconius melpomene* are described from the vicinity of Mocoa, Dpto. Putumayo, Colombia, based on molecular and morphological characters. The new species, *H. tristero*, is a close relative of *H. cydno*, a geographically differentiated species which lacks red coloration and engages in Mullerian mimicry with other blue and yellow *Heliconius* species in Central and northwestern South America. *H. tristero* has switched mimetic associations, instead mimicking the local, sympatric forms of two widespread mimetic species, *H. erato* and *H. melpomene*. This discovery provides evidence that the splinter species *H. heurippa*, *H. tristero* and *H. timareta* represent phenotypically divergent members of the *H. cydno* group that are endemic to successive river valleys on the eastern slope of the northern Andean Cordillera. The nominal taxon *Heliconius amaryllis bellula* Stichel, currently misapplied to both *H. tristero* and *H. melpomene* populations from the Mocoa region of Colombia, is considered here to represent a **hybrid** between *H. heurippa* and *H. tristero*. The Mocoa *melpomene* race is formally named *Heliconius melpomene mocoa*, new subspecies. (C) 1996 The Linnean Society of London.

Ecology and genetics of interspecific hybridization in the swallowtails, *Papilio hospiton* Gene and *P. machaon* L, in Corsica (Lepidoptera: Papilionidae)

Aubert, J; Barascud, B; Descimon, H; Michel, F. 1997

The Corsican swallowtail butterfly, *Papilio hospiton*, is endemic to Corsica and Sardinia (France and Italy) and included in the list of endangered species by the Washington Convention. It is spread all over Corsica in scattered populations linked to diverse habitats. A study by enzyme electrophoresis showed that the genetic diversity of the species is of the same order of magnitude as that of *Papilio machaon* from continental France and Corsica. The differentiation between populations is rather low, which is consistent with the high vagility of the adults. Natural **hybridization** between *P. hospiton* and *P. machaon* is frequent, and laboratory crosses show that the **hybrids** are not sterile. However, developmental perturbations impair the viability of further **hybrid** progenies. Although limited introgression between the two species is likely to take place, enzyme electrophoresis and PCR-RFLP analysis of mitochondrial DNA show that their gene pools remain distinct. Genetic assimilation by *P. machaon* therefore does not seem to be a threat for *P. hospiton*. (C) 1997 The Linnean Society of London.

Hybridization and speciation in *Hyalophora* (Insecta: Lepidoptera: Saturniidae): A reappraisal of W.R. Sweadner's classic study of a hybrid zone

Collins, MM. 1997

The historical significance of W. R. Sweadner's (1937) classic study of natural **hybridization** in *Hyalophora* is discussed in terms of contemporary and modern species concepts. Sweadner proposed that *Hyalophora* "kasloensis," from the Bitterroot Mountains of the Pacific Northwest, represents a natural **hybrid** population. In this monograph I re-examine his thesis using experimental **hybridization** and multivariate analysis, with laboratory **hybrids** serving as standards of reference. The ecology and immature stages of "kasloensis" are described. Biogeographical data, morphology of immature stages, morphometric data, and genetic compatibility in crosses with congeners all independently support the thesis that "kasloensis" populations are of **hybrid** origin. Two other newly discovered **hybrid** *Hyalophora* populations from the Pacific Northwest are described. Natural **hybridization** in *Hyalophora* is discussed in terms of various **hybrid** zone models. The genus *Hyalophora* represents a hierarchy of species, lacking effective prezygotic isolation, but exhibiting varying degrees of postzygotic barriers.

Development and pheromone communication systems in hybrids of *Agrotis ipsilon* and *Agrotis segetum* (Lepidoptera: Noctuidae)

Gadenne, C; Picimbon, JF; Becard, JM; LalanneCassou, B; Renou, M. 1997

Hybrids were obtained by crossing males of the turnip moth *Agrotis segetum*, which has a period of arrested development in the larval stage, with females of the migrant and nondiapausing black cutworm, *Agrotis ipsilon*. No progeny were obtained by crossing females of *A. segetum* with males of *A. ipsilon*. Backcrosses were successful only by crossing **hybrids** with both sexes of *A. ipsilon*. No larval oligopause occurred when **hybrid** larvae were reared under conditions inducing arrest in *A. segetum* (12L:12D, 21 degrees C). Wind tunnel tests showed interattractiveness of F-1 **hybrids** with *A. ipsilon* but not with *A. segetum*. Single sensillum recordings of pheromone-sensitive hairs on the antennae of males of the parent species revealed differences in the distribution of sensilla. F-1 and F-2 **hybrids** exhibited the same receptor cell types and distribution as *A. ipsilon*. Gas chromatographic analysis of female pheromone gland extracts revealed that F-1 **hybrids** were also very close to

A. ipsilon.

Prevalence and persistence of nuclear polyhedrosis virus in fluctuating populations of forest tent caterpillars (Lepidoptera: Lasiocampidae) in the area of Prince George, British Columbia

Kukan, B; Myers, JH. 1997

Infection by nuclear polyhedrosis virus commonly occurs in outbreak populations of forest Lepidoptera. However, nothing is known about viral infection in populations of forest caterpillars that declined to low density. We used DNA dot-blot **hybridization** to identify viral infection in caterpillars collected from 6 field populations of forest tent caterpillars in the vicinity of Prince George, BC. Over the 5 yr of observations, populations at 4 sites declined from high to low densities. Viral infection was generally highest among caterpillars from dense and declining populations but persisted at low levels in sparse populations. Populations at 2 sites which erupted in the last 2 yr of the study had levels of infection comparable to populations which were at high density at the beginning of the study.

A genetic analysis of some components of reproductive isolation between two closely related species, *Spodoptera latifascia* (Walker) and *S-descoinsi* (Lalanne-Cassou and Silvain) (Lepidoptera: Noctuidae)

Monti, L; Genermont, J; Malosse, C; LalanneCassou, B. 1997

The two closely related noctuid moths *Spodoptera latifascia* and *S. descoinsi* have been found in sympatry in some parts of French Guiana. They differ by i) ecological preferences? ii) some anatomical features of the genital tract, iii) the relative amounts of the two main components of the female pheromonal blend, and iv) the temporal pattern of sexual behaviour. Viable and fertile interspecific **hybrids** can be obtained in the laboratory, thus permitting a genetic study of some of these traits. The anatomical differences cause perturbations in mating efficiency. Experimental results show that these differences are hereditarily transmitted, but their exact mode of inheritance remains unknown. The timing of initiation of female calling activity is very likely to be under polygenic control. Conclusive evidence showed that a pair of major autosomal alleles determined the pheromone composition. Moreover, some of the data suggest that the *descoinsi* allele would become lethal in certain mosaic *latifascia/descoinsi* genomic environments. The ecological factor is likely to be the major component of reproductive isolation between the two species in natural conditions. However in contact zones, the differences in pheromone composition and in timing of sexual activity are probably involved in preventing interspecific **hybridization**. Two speciation scenarios are proposed, both sympatric. In one of them, the primary event would be a fragmentation of the tropical forest followed by disruptive selection associated with habitat diversification. In the other, the speciation process might have been initiated by the occurrence of mutations acting on the pheromone mediated mate recognition system.

A nuclear polyhedrosis virus from the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae)

Lucarotti, CJ; Morin, B. 1997

A nuclear polyhedrosis virus (CrMNPV) has been isolated from larvae of the obliquebanded leafroller *Choristoneura rosaceana* (Harris) collected near St.-Quentin in northwest New Brunswick, Canada. Restriction endonuclease digestion-gel electrophoresis of this virus, using a number of different endonucleases and whole CrMNPV genome Southern **hybridization**, have shown it to be different but similar to the equivalent virus from the spruce budworm *Choristoneura fumiferana* (Clem.) (CfMNPV). The polyhedrin gene of CrMNPV has been isolated, cloned, and sequenced and shown to have a predicted amino acid sequence only two amino acids different from the CfMNPV polyhedrin. In bioassays against spruce budworm larvae, CrMNPV had little pathogenicity to that host. (C) 1997 Academic Press.

Characterization of nuclear polyhedrosis viruses from three subspecies of *Lambdina fiscellaria*

Levin, DB; Laitinen, AM; Clarke, T; Lucarotti, CJ; Morin, B; Otvos, IS. 1997

The eastern hemlock looper (EHL), *Lambdina fiscellaria fiscellaria*, the western hemlock looper (WHL), *L. fiscellaria lugubrosa*, and the western oak looper (WOL), *L. fiscellaria somnaria*, are closely related insects that are pests in Canadian forests. We have used restriction endonuclease (REN) and Southern blot analyses to characterize DNA of nuclear polyhedrosis viruses (NPVs) isolated from each of the three subspecies of *L. fiscellaria*. REN analysis of EHL-NPV, WHL-NPV, and WOL-NPV DNAs revealed that they have nonidentical REN-banding patterns. NPV DNA extracted from WOL larvae collected from Douglas fir trees had a different REN pattern than NPV DNA extracted from WOL larvae collected from Carry oak trees. Hybridization of radiolabeled EHL-NPV genomic DNA revealed that the EHL- and WHL-NPVs share considerable DNA sequence similarity, as radiolabeled EHL-NPV DNA **hybridized** strongly with all WHL-NPV DNA bands. The EHL-NPV polyhedrin gene **hybridized** strongly with WHL-NPV DNA, but the **hybridization** patterns of the two NPVs were different. Very weak **hybridization** was detected between the EHL-NPV polyhedrin gene and DNAs of the two WOL-NPVs. Nucleotide sequence analysis revealed that the polyhedrin genes of EHL-NPV and WHL-NPV differ in sequence by only eight nucleotide substitutions (five third position and three second position changes, resulting in four amino acid substitutions). Phylogenetic analysis using the polyhedrin genes of EHL-NPV and WHL-NPV indicate that these two viruses cluster with the Group II NPVs as described by Zanotto, Kessing, and Maruniak. (C) 1997 Academic Press.

Interspecific crosses and fertile hybrids among the coniferophagous *Choristoneura* (Lepidoptera: Tortricidae)

Harvey, GT. 1997

Laboratory studies of seven North American *Choristoneura* species and two subspecies from widely distributed locations demonstrate the low level of genetic separation among them. No differences were found in numbers (n = 30) or morphology of chromosomes among members of the group or in any of the **hybrids** examined. High levels of mating success were obtained in all inter se matings as well as in crosses and back-crosses. However, mating success was greater for crosses within host type and within pheromone type than for crosses between types. Viability and fertility were similar in all the **hybrids** and close to those of the inter se progenies. Mean weights of initial eggs varied by a factor of 2 from the lowest (*C. pinus* Freeman) to the highest (*C. lambertiana ponderosana* Obraztsov). Mean weights of initial egg produced by **hybrids** were generally close to those of the parental species. However, when one parent was *C. fumiferana* (Clem.), mean weights of initial eggs were either much larger (male *C. fumiferana*) or much smaller (female *C. fumiferana*) than either parental type. These differences could affect survival of some progeny under harsh conditions. This evidence supports other studies in indicating that *C. fumiferana* is genetically distinct from other species in this group.

The *Pontia daplidice-edusa* hybrid zone in northwestern Italy

Porter, AH; Wenger, R; Geiger, H; Scholl, A; Shapiro, AM. 1997

The pierid butterflies *Pontia daplidice* and *P. edusa*, parapatrically distributed in southern Europe, have very similar morphologies and life histories, but show fixed differences at four allozyme markers. We sampled these allozymes in a 28-population transect north of Genoa in Italy, through the **hybrid** zone where these taxa meet.

We used the numerical techniques developed for **hybrid** zone analysis to study the patterns of genetic differentiation and their underlying evolutionary causes. The **hybrid** zone is characterized by a very short and steep central region, flanked by broad tails of introgression extended up to 100 km in either direction. From mean two-locus disequilibrium of $D = 0.148$ (maximum-likelihood two-unit support limits 0.139-0.153), and after accounting for minor differences in the center locations of the single-locus dines, which act to bias the dispersal estimate, we estimated a dispersal rate of $\sigma = 4.4$ (3.7-5.5) km/gen(1/2). The effective selection needed to maintain the steep central portion is strong, 0.47 less than or equal to s^* less than or equal to 0.64, when combined over potential intrinsic (genetic background) and extrinsic (ecological) sources of selection. The dines in allozyme loci showed variation that was significantly different between the most divergent shapes, and the differences are attributable to different degrees of introgression on the edusa side of the zone. The average selection acting on individual allozyme loci was high at $s(e)$ approximate to 1.5%, but because of the narrowness of the central region of the dine, we suspect that this estimate is somewhat biased by selection on loci closely linked to the allozyme markers. A common question for taxa that show fixed allozyme differences in parapatry is whether or not they are genetically isolated. A fairly general measure of genetic isolation across **hybrid** zones is the time, T , that it takes a neutral allele to cross the **hybrid** zone and recombine into the opposite genetic background, given by $T = (\beta/\sigma)^2$, where β is the barrier strength of the **hybrid** zone. Genetic isolation in the Pontia zone is weak, with T approximate to 25 generations for most allozyme markers. By this measure, populations of daplidice and edusa on opposite sides of the **hybrid** zone share more identical-by-descent alleles than do populations of phenotypically pure daplidice in, say, France and Morocco. Accordingly, we think it best for systematists to consider edusa as a well-marked subspecies of *P. daplidice*.

Meiosis in North American and Asian races of gypsy moth (Lepidoptera: Lymantriidae) and their hybrids

Krider, HM; Shields, KS. 1997

We have examined the meiosis of North American and Asian races of *Lymantria dispar* (L.) and their **hybrids**. The 31 prophase bivalents differ in size but bear few physical characteristics that allow a karyotype. Gypsy moth females are the heterogametic sex and we suggest possible elements of the ZW pair. The meiotic prophases are achiasmatic, but the chromosomes are tightly synapsed throughout prophase. A diffuse stage occurs, during which some chromosomes bear chromomeres. Male prophases are chiasmatic. Meiosis of interracial **hybrid** females are normal and do not show evidence of heteromorphic bivalents, inversions, or translocations. Division anomalies are prevalent in the males, most anaphase figures representing unbalanced segregations. These observations are discussed in relation to efforts to characterize the gypsy moth genome.

A physical map of the Mamestra configurata nucleopolyhedrovirus genome and sequence analysis of the polyhedrin gene

Li, SP; Erlandson, M; Moody, D; Gillott, C. 1997

The genome structure of a nucleopolyhedrovirus (NPV) isolated from the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae) (MacoNPV) was analysed with six restriction endonucleases (REN): BamHI, EcoRI, HindIII, PstI, SmaI and XhoI. More than 70 MacoNPV REN fragments were cloned into plasmids pUC18 and pBluescript SK(+). The physical map with 112 restriction sites for the above REN was constructed using double digests and Southern blot **hybridization** analysis of the MacoNPV DNA clones. The size of the DNA genome of the MacoNPV-90/2 isolate used for this study was estimated at 156 kbp based on REN fragment sizes. The position of the polyhedrin gene, which has by convention been used as the zero point of the REN maps of NPV, was determined by **hybridizing** the *Autographa californica* multicapsid nucleopolyhedrovirus HindIII-V fragment clone, which contains most of the polyhedrin gene, with genomic blots of MacoNPV. The cloned MacoNPV fragments identified as containing the polyhedrin gene were sequenced and an ORF coding for a 246 amino acid polypeptide with 98.7% sequence identity with *Panolis flammea* nucleopolyhedrovirus (PafINPV) polyhedrin protein was identified. The putative polyhedrin gene sequence had 97.2% and 91.2% identity with the PafINPV and *Mamestra brassicae* multicapsid nucleopolyhedrovirus polyhedrin gene sequences, respectively, and also contained an upstream region identical to the highly conserved 12 bp consensus sequence TGTAAGT-AATTT typical of NPV very late genes.

Identification and characterization of the Cecropin antibacterial protein gene locus in *Drosophila virilis*

Zhou, XJ; Nguyen, T; Kimbrell, DA. 1997

Cecropin is a type of antibacterial peptide that is synthesized in response to infection and has been characterized in many insect species and one mammal. The Cecropin locus of *Drosophila melanogaster* also contains the gene Andropin, which has been identified only in this species and encodes a male-specific antibacterial peptide. As a first step in studying the molecular evolution of the cecropin and andropin genes among *Drosophila* species, we have isolated genomic clones that cover the Cecropin locus in *Drosophila virilis*. The cloned region totals approximately 25 kb, within which a 9-kb fragment contains four cecropin genes and one pseudogene. All four genes have a high level of sequence homology to *D. melanogaster* Cecropin, about 80% identity in the coding regions, and the intron positions are conserved. As in *D. melanogaster* and other insects, KB-related cis-regulatory elements are found upstream of these cecropin genes. An Andropin-related sequence was not identified in *D. virilis*; however, genome Southern **hybridizations** suggest that Andropin-related sequences are present in at least the *melanogaster* species subgroup. Analysis of 19 insect cecropin genes identifies a common ancestral Cecropin before the divergence of Diptera and Lepidoptera. In addition, *D. melanogaster* and *D. virilis* can be identified by monophyletic clades for Cecropin. In contrast, the Lepidopteran species show polyphyletic relationships for duplicated cecropin genes.

Characterization of *Bacillus thuringiensis* mutants and natural isolates by molecular methods

Jung, YC; Kim, SU; Bok, SH; Park, HY; Cote, JC; Chung, YS. 1997

Two *Bacillus thuringiensis* var. *kurstaki* HD-1 mutants, two *Bacillus thuringiensis* var. *israelensis* HD-500 mutants, and four rice grain dust isolates were characterized using microscopic examination and protein profiles of purified crystals on sodium dodecyl sulfate - polyacrylamide gel electrophoresis. Specific detection of cryI- and cryIV-type genes was performed in a polymerase chain reaction using cryI and cryIV-specific oligonucleotide primers. The cry-type genes under study consisted of cryIA(a), cryI(A)b, cryI(A)c, cryIB, and cryIV. Presence or absence of the cryI- and cryIV-type genes was further confirmed by Southern blotting followed by **hybridization** with specific cryI and cryIV gene fragments. A genetically modified strain of *B. thuringiensis* var. *kurstaki* HD-1, called OZK-13 and obtained following mutagenesis with ozone, was shown to contain cryIA(a), cryIA(b), and cryIA(c) genes. Another *kurstaki* HD-1 mutant, called NGK-13 and obtained following treatment with N-methyl-N-1-nitro-N-nitrosoguanidine (MNNG), was shown to have lost the cryIA(b) gene while retaining the cryIA(a) and cryIA(c) genes. NGI-23-1, an oligosporogenous multicrystalliferous mutant of *B. thuringiensis* var. *israelensis* (Bti) HD-500, obtained following treatment with MNNG contained cryIV-type genes. NGI-22, an oligosporogenous -acrystalliferous mutant of Bti HD-500, contained no cryI- nor cryIV-type genes. The rice grain dust isolate BT-285 contained the cryIA(a) and cryIA(c) genes. Isolate BT-14 contained only the cryIA(c) gene, whereas isolate BT-209 contained cryIA(a), cryIA(b), and cryIB genes. Isolate BT-205 contained no cryI- nor cryIV-type genes. *Bacillus thuringiensis* mutants and natural isolates shown to contain cryI-type genes were tested for their insecticidal activities in a series of bioassays against *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). All cryI-carrying strains were toxic against the insect larvae. BT-205 was also tested and exhibited no toxicity against the insect larvae.

Central nervous processing of sex pheromones in two strains of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae)

Anton, S; Lofstedt, C; Hansson, BS. 1997

Antennal lobe neurones were investigated in the pyralid moth *Ostrinia nubilalis* using intracellular recording and staining techniques. Response characteristics of antennal lobe neurones from males in the so-called E and Z strains, in FI **hybrids** and in parental backcrosses were studied. The antennal lobe of a male *O. nubilalis* comprises approximately 30 ordinary glomeruli and three enlarged glomeruli making up the macroglomerular complex (MGC). Receptor neurones enter the antennal lobe via the antennal nerve and arborize in single glomeruli. Intracellularly stained, pheromone-responding projection neurones in both parental strains arborized in different glomeruli within the MGC, irrespective of their response characteristics. Neurones were grouped according to their specificity to single pheromone components and to pheromone blends. Component-specific, blend-specific and generalist neurones were found. Specificity only occurred at low stimulus concentrations and disappeared as concentrations increased. Although all neuronal types were present in both pheromone strains and crossings, differences in abundance and sensitivity were found. In the parental strains, neurones responding to the major pheromone component and to the respective strain-specific blend were more abundant than neurones responding to the minor component and the blend produced by the other strain. Neurones investigated in ZxE **hybrids** responded similarly to those of E-strain males, whereas neurones in EZxZ paternal backcrosses responded similarly to those of Z males. In the **hybrids** and paternal backcrosses, **hybrid**-blend-specific neurones were present that were not found in parental-strain males.

Host-plant characteristics and environmental factors influencing flight activity of angoumois grain moth (Lepidoptera: Gelechiidae) in the field.

Weston, PA; Barney, RJ; Ge, XS. 1997

Field studies were conducted to examine the effect of time of day and several phenological characteristics of maize plants on flight activity of *Sitotroga cerealella* (Olivier), a common pre and postharvest pest of stored maize, *Zea mays* L., in the southeastern United States and many subtropical and temperate areas. A diurnal trapping study conducted in central Kentucky during late summer/early autumn revealed that flight activity of males is concentrated almost exclusively at dusk (between 1700 and 2200 hours), but the window of flight activity can be shifted by meteorological conditions. Pheromone traps placed in plots of maize varying with respect to planting date and **hybrid** and in plots of sunflower, *Helianthus annuus* L., revealed that mere presence of tall vegetation increases local activity of male *S. cerealella*, and that maize-specific characteristics, probably volatile chemicals, further localize flight activity. Implications of this knowledge of *S. cerealella* behaviour for management of the pest are discussed.

Effects of selected midwestern larval host plants on performance by two strains of the gypsy moth (Lepidoptera: Lymantriidae) parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae)

Kruse, JJ; Raffa, KF. 1997

The effects of gypsy moth, *Lymantria dispar* (L.), larval diet on parasitism rates and performance by 2 strains of the larval parasitoid *Cotesia melanoscela* Ratzeburg were studied in laboratory trials. Gypsy moth larvae that were parasitized by *C. melanoscela* strains from France and South Korea, and an unparasitized control group, were allowed to develop on foliage from 5 tree species (red oak, tamarack, sugar maple, **hybrid** poplar clone NC5271, and white pine) and synthetic diet. Food plant had a significant effect on gypsy moth relative growth, development time, and pupal weight. Gypsy moth food plant affected *C. melanoscela* development time and size. Host plant influenced percentage of parasitism, but this effect varied between blocks. Parasitoid strain was a major factor in development time, size, and host selection, but was of marginal influence on parasitism rates and sex ratio. Plant suitability to gypsy moth, both in host species and phenology, was the strongest underlying factor in *C. melanoscela* parasitoid success, development time, and size. Plant and strain effects on *C. melanoscela* may be important factors in guiding biological control efforts against gypsy moth.

Estimating the mating behavior of a pair of hybridizing *Heliconius* species in the wild

Mallet, J; McMillan, WO; Jiggins, CD. 1998

Premating isolation between incipient species is rarely studied in nature, even though mating tests in captivity may give an inaccurate picture of natural **hybridization**. We studied premating barriers between the warningly colored butterflies *Heliconius erato* and *H. himera* (Lepidoptera) in a narrow contact zone in Ecuador, where **hybrids** are found at low frequency. Eggs obtained from wild-mated females, supplemented with eggs and young larvae collected from the wild, were reared to adulthood. Adult color patterns of these progeny were then used to infer how their parents must have mated. Likelihood was used to estimate both the frequencies of potential parental genotypes from adult phenotypes collected in the wild, and the degree of assortative mating from the inferred parents. The frequencies of parental genotypes varied across the **hybrid** zone, but our statistical method allowed estimates of **hybrid** deficit and assortative mating to be integrated across all sites sampled. The best estimate of the frequency of F(1) and backcross **hybrid** adults in the center of the **hybrid** zone was 10%, with support limits (7.1%, 13.0%; support limits are asymptotically equivalent to 95% confidence limits). Mating was highly assortative: in the center of the **hybrid** zone the cross-mating probability between *H. erato* and *H. himera* was only 5% (0.3%, 21.4%). Wild **hybrids** themselves mated with both pure forms, and the probabilities that they mated in any direction were not significantly lower than those among conspecifics. These results are consistent with earlier laboratory studies on mate choice, and suggest that selection against **hybrids** must be strong to prevent formation of a **hybrid** swarm. Unfortunately, the wide support limits on mating behavior precluded a measure of the strength of selection from these data alone. Our statistical approach provides a useful general method for estimating mate choice in the wild.

Randomly amplified polymorphic DNA evidence of introgression in two closely related sympatric species of coniferophagous *Choristoneura* (Lepidoptera : Tortricidae) in Atlantic Canada

Deverno, LL; Smith, GA; Harrison, KJ. 1998

DNA was extracted from selected desiccated museum specimens of *Choristoneura fumiferana* (Clemens) and *C. pinus* Freeman and analyzed by using the randomly amplified polymorphic DNA (RAPD) technique. Seventeen species-specific RAPD markers were used to differentiate between these 2 closely related and morphologically similar budworm species. Several morphologically atypical specimens of each budworm species were selected for analysis based on deviations from conventional descriptions of uncus shape and size, wing bands, maculation, and background coloration. Analysis of the distribution of species-specific markers in selected specimens from Atlantic Canada, where the 2 species are sympatric and their flight periods occasionally overlap, suggests that reproductive isolation of these 2 *Choristoneura* species is incomplete, thus providing the first evidence that **hybridization** of *C. fumiferana* and *C. pinus* has occurred in nature. The significance of these results to **hybridization**, introgression and retained ancestral polymorphism is discussed.

Genetic evidence for a sibling species of *Heliconius charithonia* (Lepidoptera; Nymphalidae)

Jiggins, CD; Davies, N. 1998

Heliconius charithonia is a widespread species which, unlike many *Heliconius*, is non-mimetic and shows little racial differentiation. Only one form, 'peruvianus', which occurs in the dry forest habitats of western Ecuador and Peru: has a distinct and clearly mimetic colour pattern. Here it was shown that *H. peruvianus* was distinct from *H. charithonia bassleri* at allozyme loci ($D = 0.25$ over 22 loci). This differentiation was ten times greater than that between *H. charithonia* sampled from Ecuador and the Caribbean ($D = 0.027$) and was consistent with analysis of mitochondrial sequence data (3.4-4% sequence divergence between *H. peruvianus* and *H. charithonia*). One individual with a *H. peruvianus* colour pattern and allozyme genotype was collected in an area where *H. charithonia* was known to be common,

demonstrating that contact between the taxa occurs in western Ecuador Furthermore, the allozyme genotype of another individual was heterozygous for four of the diagnostic loci and was most likely an F1 **hybrid** between *H. charithonia* and *H. peruvianus*. These data imply that *H. charithonia* and *H. peruvianus* are distinct species which **hybridize** occasionally. This species pair show many similarities with *W. erato* and *H. himera*, which are similarly differentiated genetically and also show ecological and colour pattern differences. These species fulfil some of the predictions of both allopatric refugium and parapatric adaptationist models of speciation in the neotropics, suggesting that elements of both hypotheses may be true. (C) 1998 The Linnean Society of London.

Infection of lepidoptera with a pseudotyped retroviral vector

Franco, MD; Rogers, ME; Shimizu, C; Shike, H; Vogt, RG; Burns, JC. 1998

Studies requiring the introduction and expression of manipulated gene constructs have been technically difficult in non-drosophilid insects. Retroviruses can be engineered to be replication defective and to serve as vectors for gene constructs of interest. In this study, pseudotyped MoMLV(VSV-C) retroviral vectors are shown to successfully infect lepidopteran cells in vitro and in vivo. In *Spodoptera frugiperda* cells in vitro and in *Manduca sexta* in vivo, infection and conversion to proviral DNA were confirmed by PCR amplification and Southern blot **hybridization** of vector-specific sequences. Gene expression and integration of proviral DNA were also documented in vitro. This is the first report of retroviral infection in lepidoptera and suggests that pseudotyped retroviral vectors could be powerful tools in gene manipulation studies of non-drosophilid insects. (C) 1998 Elsevier Science Ltd. All rights reserved.

The inheritance of diagnostic larval traits for interspecific hybrids of *Papilio canadensis* and *P. glaucus* (Lepidoptera : Papilionidae)

Scriber, JM. 1998

Traits distinguishing the closely related tiger swallowtail butterfly species, *Papilio canadensis* and *P. glaucus*, include fixed differences in diagnostic sex-linked and autosomal allozymes as well as sex-linked diapause regulation, and sex-linked differences in oviposition behavior. Larval detoxification abilities for plants of the Salicaceae and Magnoliaceae families are dramatically different and basically diagnostic as well. The distinguishing morphological traits of the adults and larvae have not been genetically characterized. Here we describe the segregation of diagnostic larval banding traits in offspring from the 2 species in their **hybrid** and reciprocal backcross combinations. Elucidation of genetic basis, and linkage relationships of a suite of distinguishing biochemical, physiological, morphological, and ecological traits with regard to their geographic concordance across the **hybrid** zone is fundamental to understanding the pattern and process of speciation.

Prothoracic gland inactivation in *Heliothis virescens* (F.) (Lepidoptera : Noctuidae) larvae parasitized by *Cardiochiles nigriceps* Viereck (Hymenoptera : Braconidae)

Pennacchio, F; Falabella, P; Sordetti, R; Varricchio, P; Malva, C; Vinson, SB. 1998

Heliothis virescens (F.) last instar larvae parasitized by the endophagous braconid *Cardiochiles nigriceps* Viereck fail to attain the pupal stage, due to a parasitoid-induced alteration of ecdysteroid biosynthesis and metabolism. Currently available information on host prothoracic gland inactivation in this host-parasitoid system is reported here. Prothoracic glands of *H. virescens* mature larvae show a depressed biosynthetic activity, without undergoing gross morphological disruption. The ultrastructure of gland cells is characterized by minor parasitoid-induced changes, with the rough endoplasmic reticulum appearing more developed and electron-dense than in nonparasitized controls. Eventually, the cells of prothoracic glands of parasitized host last instar larvae die but maintain their structural integrity. The inactivation of pupally committed host prothoracic glands is achieved through the disruption of the PTTH signal transduction pathway. The second messenger cAMP appears to be normally produced in response to PTTH stimulation of glands explanted from parasitized host larvae, however the downstream activation of the cAMP-dependent protein kinase does not appear to occur. In fact, a marked underphosphorylation of regulatory target proteins is observed. This underphosphorylation is associated with a significant reduction in general protein synthesis, which appears to be blocked at the translational level, to a redirection of specific protein synthesis and to a drastic suppression of ecdysteroidogenesis. These parameters appeared to be correlated in a kinetic time-course study, confirming their functional link. *C. nigriceps* polydnavirus (CnPDV) plays a major role in the inactivation of pupally committed host prothoracic glands, while putative factors occurring in the host haemolymph do not seem to be of particular importance at that developmental stage. Southern blot **hybridization** indicates the occurrence of PKI(protein kinase inhibitor)-like genes in the *C. nigriceps* genome, which, in contrast, are undetectable in *H. virescens*. (C) 1998 Elsevier Science Ltd. All rights reserved.

Yield responses of corn to simulated black cutworm (Lepidoptera : Noctuidae) damage

Santos, L; Shields, EJ. 1998

A field trial was conducted to study the impact of 6 increasing levels of simulated black cutworm, *Agrotis ipsilon* (Hufnagel), damage on grain and silage yields. Plants were damaged at the V3 and V5 stages of corn development. The experiment was conducted on 2 corn **hybrids**. Pioneer 3921 and Pioneer 3733, planted at 6.0 and 8.0 plants per square meter, in 1993 and 1994. A significant year effect on both grain and silage yield was detected. In both years of study, **hybrid** had a significant effect on grain yield but not on silage yield, whereas plant density had no significant effect on grain and silage whole-crop yields. Significant linear and quadratic relationships between both grain and silage yields and damage level were detected. Grain yield losses, averaged across plant density, varied from 0 to 24% and 0 to 81% when plants were damaged at the V3 and V5 stages of corn development respectively, depending on **hybrid**, damage level, and year. For P3733, maximum grain loss exceeded silage loss by as much as 9 and 14% in 1993 and 1994, respectively. P3921 grain losses paralleled silage losses in both years. A phenological delay associated with the simulated damage was observed.

Breeding biology of the Golden Oriole *Oriolus oriolus* in the fenland basin of eastern Britain

Milwright, RDP. 1998

This paper describes, for the first time, aspects of the breeding biology of the British Golden Oriole population. The Golden Oriole population in the fenland basin of East Anglia became established in 1967 and since the early 1980s the annual breeding population has varied from about 20 to 35 pairs. Males arrived in mid-March, and females about a week later. Nest building starts about five days after female arrival. Mean date for first egg laying was around 1 June. The mean clutch size was 3.53. Observed incubation periods were 18 to 19 days. Mean hatching date was 18 June, and the mean brood size at seven days was 2.60. Nestling periods were from 13 to 15 days, and the mean fledging date was 3 July. Failed clutches may be replaced; no evidence was found for genuine second broods. Estimated mean productivity of all nests including failures was 1.4-2.0 fledglings per nest. The greatest cause of failure was bad weather in June, and nests produced three times as many fledged young in warm dry June as in cool wet ones. The breeding habitat is very strongly associated with plantations of early leafing **hybrid** Black Poplars *Populus nigra*. A total of 94.2% of all nests found were in these trees, and since 1987 this figure has risen to 100%. The mean height of the nests in poplars was about 7 m below the top of the tree, irrespective of the height of the tree. Most nests were in the north, northeast or east sectors of the tree, and 80% were within two trees of the edge of the woodland. Breeding densities were generally at or below 0.25 pairs/km(2), but up to 2.25 pairs/km(2). Nestling diet contains many Poplar-feeding Lepidoptera and a surprising number of Bumblebees. Conservation efforts should aim to produce more areas of Poplar woodland with plenty of woodland edges and with 30 ha/km(2) of Poplar plantation.

Rapid ecological survey, inventory and implementation: A case study from Waikaia ecological region, New Zealand

Dickinson, KJM; Mark, AF; Barratt, BIP; Patrick, BH. 1998

General biogeographic features of the two Ecological Districts - Umbrella c. 150 000 ha and Nokomai, c. 110 000 ha - of the Waikaia Ecological Region, south-central South Island, are described. Results of normal and inverse cluster analyses of plot samples of the full range of indigenous vegetation remaining in each District are presented. Up to 17 plant communities from each District are characterised in terms of physiognomy, flora, and physiography. These range from beech forests and lowland red tussock grassland through upland shrublands, shrub-tussocklands, tussocklands, sedgelands, and wetlands to high-alpine communities (snowbank, cushionfield, scree). The 649 indigenous and 97 adventive vascular plant taxa plus 21 **hybrids** are listed by District. Plant distributions, particularly altitudinal and geographic limits for many alpine and some threatened taxa, are described. Of the region's fauna, 61 birds, 268 Lepidoptera, 202 Coleoptera (some in both orders undescribed), and several other invertebrates including the rare land snail *Powelliphanta spedeni* *spedeni* are listed by District. Distributions of several rare and/or local taxa are described both within and beyond the Region. Aquatic fauna of unmodified upland lakes and ponds of Umbrella District are recorded. Within the Region, 31 areas ranging from 25 ha to 2620 ha are recommended for protection, 20 (6260 ha or 2.4%) from the Umbrella District and 11 (11 615 ha or 9.5%) from the Nokomai District. These were selected so as to adequately represent the full range of remaining indigenous ecosystems and their associated landforms. Details of progress (to July 1997) with the implementation phase of the programme are outlined. Of the 31 Recommended Areas for Protection (RAPs) identified in the field surveys, seven now have some degree of protection over their full extent and nine are partly protected. These protected areas represent 44% (c. 7985 ha) of the total area of the 31 RAPs (18 060 ha) identified. Tenure review of pastoral leasehold land continues to be an important means of protecting conservation values in these two Districts, as in other parts of the South Island high country.

Behavioral and physiological differences between two parapatric *Heliconius* species

Davison, A; McMillan, WO; Griffin, AS; Jiggins, CD; Mallet, JLB. 1999

The behavior and physiology of two parapatric sibling species, *Heliconius erato cyrbia* Godt. and *H. himera* Hew., were investigated to assess if environmental adaptation enabled stable morphological, generic, and ecological differences to exist in the face of **hybridization**. Morning and evening activity, egg production, and Larval development time of *H. himera* and *H. erato* in insectaries were recorded; individuals were collected in allopatry and in sympatry from a **hybrid** zone in which the species overlapped. Studies were performed at ambient conditions within the natural range of *H. himera*. *H. himera* was considerably more active than *H. erato*, flying earlier in the morning and later in the evening, even when both species were collected in sympatry. Similarly, *H. himera* laid more eggs, and the hatched larvae developed more rapidly. The results suggest that physiological constraints are an important selective force that may have been important in speciation and counteracts **hybridization** in the maintenance of the *H. himera*/*H. erato* contact zone. Ecological selection, arising from adaptation to low temperatures, may help explain the competitive exclusion of *H. erato* by *H. himera* in the drier, cooler montane habitat favored by the latter species.

The evolution of sex chromosomes in insects: Differentiation of sex chromosomes in flies and moths

Traut, W. 1999

Although a monophyletic group, male (XX/XY) and female heterogametic (WZ/ZZ) sex chromosome systems with a couple of variants like XX/X, Z/ZZ and multiple sex chromosome systems occur in insects. Molecular and morphological differences between X and Y or W and Z range from imperceptible to conspicuous. This article illustrates sex chromosome differentiation mainly in two fly species, *Drosophila melanogaster* and *Megaselia scalaris*, and in Lepidoptera. The earliest phases of XY evolution are present in the fly *M. scalaris*. Occasionally in this species, the male determining gene jumps to another chromosome, transforming the new host chromosome to a functional Y chromosome. Thus, in *M. scalaris* there are strains with virtually no XY differentiation (except for the sex determining function) and others with a moderate degree of differentiation. Base substitutions and alterations like sequence deletions, duplications, and insertions of mobile sequences mark the onset of molecular differentiation. Accumulation of molecular changes and coarser alterations are thought to lead to the morphological differences seen in WZ chromosome pairs of Lepidoptera. The W chromosome probably evolved in the most numerous clade of Lepidoptera, the Ditrysia, after it diverged from the common lepidopteran stem. Extant species display various degrees of molecular and morphological differentiation of the W chromosome, translocation or fusion with autosomes, and loss of the W.

Systematics of the *Argyrotaenia franciscana* (Lepidoptera : Tortricidae) species group: Evidence from mitochondrial DNA

Landry, B; Powell, JA; Sperling, FAH. 1999

Moths of the *Argyrotaenia franciscana* species group represent a challenging case of evolutionary lability and taxonomic complexity in California. We studied their evolutionary relationships using mitochondrial DNA (mtDNA) sequences from 49 specimens in 18 populations of the *A. franciscana* group, as well as 2 outgroup species. Most specimens were sequenced over a 799-bp segment of the cytochrome oxidase subunit I (COI) gene. Single specimens each of *A. franciscana insulana* Powell and *A. citrana* (Fernald) were sequenced over a 2.3-kb region including COI, tRNA leucine (UUR), and cytochrome oxidase subunit II (COII), mtDNA variation within and among *Argyrotaenia citrana*, *A. franciscana* (Walsingham), and *A. franciscana insulana* is most simply interpreted as DNA polymorphism within a single species for which the oldest name is *A. franciscana*. Maximal divergence among haplotypes was 3.8%, which is on the high end of the range for intraspecific mtDNA variation in Lepidoptera. *Argyrotaenia niscana* (Kearfott) is most closely related to a new species, and this pair forms the closest outgroup to the *A. franciscana-citrana* complex. The status of *A. isolatissima* Powell remains uncertain.

Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera : Noctuidae) host strains

McMichael, M; Prowell, DP. 1999

Amplified fragment-length polymorphic (AFLP) loci were examined in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), to assess their ability to distinguish 2 host-associated strains. Cluster analyses of variation at 10 AFLP loci amplified with 5 primer pairs revealed 2 groups. Each group contained a majority of individuals from 1 or the other host-associated strain as defined by habitat and mtDNA type. Discordance between habitat/mtDNA strain assignments and AFLPs occurred more frequently in the rice strain (8%) than in the corn strain (3%), and at a frequency of 5% overall. Results indicate that AFLPs exhibit strain-specific variation comparable to that of mtDNA and allozymes. In combination with other markers, AFLP loci will be useful for developing a system for identification of fall armyworm strains and **hybrids**.

Development of an economic injury level for European corn borer (Lepidoptera : Pyralidae) on corn grown for silage

Myers, SW; Wedberg, JL. 1999

Studies were conducted in 1996 and 1997 at 2 locations in Wisconsin to assess the impact of European corn borer, *Ostrinia nubilalis* (Hubner), on corn grown for silage. Relationships between silage yield losses and control costs were used to develop economic injury levels specific to corn grown for silage in Wisconsin.

Treatments were established as the percentage of plants infested with European corn borers. Treatment levels of 0, 12, 24, 40, and 84% of plants infested were used to separately examine simulations of 1st- and end-generation European corn borer infestations. Experiments were performed using Pioneer dual purpose **hybrids** 3394 and 3751. Results from experiments showed that overall whole plant dry matter yields for 3394 were 8.5% > 3751 across the 3 experiments. Increasing European corn borer infestation proved to be highly correlated with a decrease in both whole plant and stover silage yields throughout experiments, **hybrids**, and simulated European corn borer generations. Differences in the rates of yield reduction were not seen between the 2 **hybrids**, however simulations of 1st-generation European corn borer infestation caused greater reductions than did simulations of 2nd-generation European corn borers. Linear regression models proved to best fit the yield loss relationships and were developed to describe silage yield losses resulting from European corn borer infestations. Economic injury levels were determined by comparing silage yield reductions and associated monetary losses with control costs. Economic injury levels were calculated separately for both 1st- and 2nd-generation European corn borers.

Reproductive isolation mechanisms between members of the *Spodoptera latifascia* - *S. cosmioides* - *S. descoinsi* (Lepidoptera : Noctuidae) neotropical species complex.

Lalanne-Cassou, B; Silvain, JF; Monti, L; Malosse, C. 1999

Spodoptera latifascia Walker, *S. cosmioides* Walker and *S. descoinsi* Lalanne-Cassou & Silvain are members of a neotropical species complex. *S. latifascia* is found in Central America, Southern USA and West Indies, *S. cosmioides* in tropical South America and *S. descoinsi* in the Amazon basin where it is parapatric with *S. cosmioides*. In addition to differences in the habitus and some genitalic traits, the three species show a temporal shift in their female sexual activity. They also differ by the ratio of two main compounds in their pheromone glands. Under laboratory conditions, **hybrids** can be obtained but the mating rates depend on the crosses. We discuss here the role of the female calling periods and pheromone differences as factors of reproductive isolation among the species.

Variable selection and the coexistence of multiple mimetic forms of the butterfly *Heliconius numata*

Joron, M; Wynne, IR; Lamas, G; Mallet, J. 1999

Polymorphism in aposematic animals and coexistence of multiple mimicry rings within a habitat are not predicted by classical Mullerian mimicry. The butterfly *Heliconius numata* Cramer (Lepidoptera: Nymphalidae; Heliconiinae) is both polymorphic and aposematic. The polymorphism is due to variation at a single locus (or 'supergene') which determines colour patterns involved in Mullerian mimicry. We sampled 11 sites in a small area (approx. 60x30km) of North-eastern Peru for *H. numata* and its co-mimics in the genus *Melinaea* and *Athyrtis* (Ithomiinae), and examined the role of temporal and spatial heterogeneity in the maintenance of polymorphism. Colour-patterns of *Melinaea* communities, which constitute the likely 'mimetic environment' for *H. numata*, are differentiated on a more local scale than morphs of *H. numata*, but the latter do show a strong and significant response to local selection for colour-pattern. In contrast, analysis of enzyme polymorphism in *H. numata* across the region revealed no spatial structure, which is consistent with a high mobility of this species. Differences in spatial variability in the two taxa may have caused *H. numata* to become polymorphic, while temporal variability, not significant in this study, probably has a lesser effect. The mimetic polymorphism is therefore explained by means of multiple selection-migration clines at a single locus, a similar process to that which explains narrow **hybrid** zones between geographic races of other *Heliconius* butterflies.

Tomato cultivar susceptibility to Egyptian cotton leaf worm (Lepidoptera : Noctuidae) and Colorado potato beetle (Coleoptera : Chrysomelidae)

Antonious, GF; Snyder, JC; Dahlman, DL. 1999

The development and deployment of crop varieties that resist or tolerate insect infestation is one tactic for pest management that can eliminate one or more spray applications per season, a significant savings to the grower. Seven commercial tomato, *Lycopersicon esculentum* Mill, cultivars 'Marmand', 'Edkawy', VF-145, GS-27, 'Pakmor-B', 'Floradade', and UCX-281 grown under greenhouse conditions were evaluated for differences in mortality and feeding (leaf area ingested) of fourth-instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisduvar), and Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Plants of the cultivars Floradade, GS-27, and Pakmor-B sustained the most damage (more leaf area ingested) by cotton leafworm, while GS-27 and VF-145 sustained the most damage by Colorado potato beetle. Mortality of Colorado potato beetle was lowest on the F1 **hybrid** GS-27, indicating that GS-27 was the most susceptible cultivar to Colorado potato beetle. Variation in chemical and nutrient composition (reducing sugars, total sugars, ascorbic acid, total phenols and total protein content) of leaves explained some of the observed differences in mortality and feeding among the tomato cultivars and insect species. The correlation coefficient of reducing sugars with leaf consumption was positive (0.758) for *S. littoralis* and negative (-0.485) for *L. decemlineata*. The correlation coefficients of ascorbic acid with insect mortality were negative (-0.389 for *S. littoralis* and -0.459 for *L. decemlineata*) indicating that they may require ascorbic acid for growth. Total phenols in tomato leaflets were positively correlated with mortality of *S. littoralis*. Edkawy, VF-145, and UCX-281 were the most resistant cultivars.

Suitability of polymerase chain reaction-based approaches for identification of different gypsy moth (Lepidoptera : Lymantriidae) genotypes in central Europe

Reineke, A; Zebitz, CPW. 1999

Two different molecular marker approaches-random amplified polymorphic DNA (RAPD) and restriction site polymorphism analysis of a polymerase chain reaction (PCR) product of the internal transcribed spacer region (ITS-2) - have been used to assess whether Asian genotypes of the gypsy moth, *Lymantria dispar* L., have been introduced or migrated to central Europe. In previous studies, both marker systems have proved to be reliable for distinguishing Asian and North American genotypes of this insect and thus for detecting the geographical origin of pheromone-trapped specimens in North America. RAPD analysis of >1,000 samples from 18 different geographical origins in Europe revealed that with the exception of 2 locations-Asian, North American, and **hybrid** RAPD markers were present at varying proportions in all European populations. However, none of the European gypsy moth specimens was classified as Asian genotype after analysis of the ITS-8 region. The results are discussed in relation to the possible bottlenecks associated with the release of a small number of European moths in North America and the different genomic regions analyzed with both marker systems. These effects may have influenced the suitability of both diagnostics for distinguishing gypsy moth isolates from different geographical origins.

Bm kettin, homologue of the *Drosophila* kettin gene, is located on the Z chromosome in *Bombyx mori* and is not dosage compensated

Suzuki, MG; Shimada, T; Kobayashi, M. 1999

In *Bombyx mori*, the female is the heterogametic sex and the sex determining system is referred to as ZZ/ZW. In a previous study, we found that this insect does not show dosage compensation at the transcriptional level. To confirm the validity of our conclusion, we investigated whether or not another sex-linked gene is dosage compensated. To identify new Z-linked genes, total RNA from reciprocal **hybrid** females between the silkworm strains p50 and C108 was compared using the differential display technique. Nine cDNA fragments corresponding to several differentially expressed mRNAs were cloned and sequenced. The analysis of nucleotide sequence polymorphisms confirmed that one of these cDNAs, ZDD4, originated from the Z chromosome. The amino acid sequence deduced from ZDD4 has homology with kettin, a modular protein in the Z-disc of *Drosophila melanogaster* muscles. On immunoblots of *Bombyx* larval muscle proteins a polypeptide of 380 kDa was labelled with antibody to the ZDD4 peptide. We considered that the gene corresponding to ZDD4 encodes a kettin homologue in the silkworm, and denote it

as Bm kettin. By a three-point cross, Bm kettin was mapped at 40.0 CM on the Z chromosome. Southern blot analysis revealed that Bm kettin was present at one copy in the genome. Northern blot analysis showed that Bm kettin mRNA was 9.1kb in length, and that the level of the mRNA in males was two times greater than that of females. Taken together with our previous observations, the present data suggest that lack of dosage compensation is a general rule in *B. mori*. Moreover, the twofold difference in Bm kettin expression between males and females may help explain the sexual difference in the wing flapping activity observed in some groups of Lepidoptera.

Inducible P450s of the CYP9 family from larval *Manduca sexta* midgut

Stevens, JL; Snyder, MJ; Koener, JF; Feyereisen, R. 2000

Several related cytochrome P450 cDNAs belonging to the CYP9 family have been cloned from the midgut of larval tobacco hornworms, *Manduca sexta*. The first P450, CYP9A2, was obtained by RT-PCR using degenerate primers. Northern blot analysis of expression in the midgut using the CYP9A2 probe revealed a significant induction by a variety of chemicals. Diets supplemented with the wild tomato compound 2-undecanone caused a dose-dependent induction which peaked after 48 h. Induction was also observed after addition to the diet of indole-3-carbinol, phenobarbital, 2-tridecanone and xanthotoxin. Neither alpha-pinene, clofibrate nor nicotine were effective inducers. The CYP9A2 probe **hybridized** to two mRNA species, one of 2.0 kb and another of 4.2 kb, suggesting cross-**hybridization** to other P450 mRNAs. Additional P450 clones of the CYP9 family were then obtained and sequenced. Northern **hybridization** revealed that the 4.2 kb band also **hybridized** to CYP9A4 whereas the 2.0 kb **hybridized** to CYP9A5. Despite being 91% identical, CYP9A4 and CYP9A5 were induced differentially by clofibrate and xanthotoxin. Multiple P450 genes from various families are therefore induced in Lepidoptera in response to plant allelochemicals or xenobiotics. (C) 2000 Elsevier Science Ltd. All rights reserved.

Developmental stability and pollination

Moller, AP. 2000

A number of different insect species (ranging from Diptera, Lepidoptera and Coleoptera to Hymenoptera) have been shown to discriminate between small degrees of asymmetry when visiting flowers or flower-like models. Such preferences for symmetry may have an innate basis. Small degrees of bilateral or radial asymmetry of flowers are considered to represent a measure of developmental instability, since deviations from perfect symmetry reflect the inability to maintain developmental precision during ontogeny. Environmental factors causing increased asymmetry in leaves and flowers include radioactive radiation, ultraviolet radiation, excess artificial fertilizer, various pollutants, extreme saline conditions, herbivory and competition. Genetic factors that contribute to increased asymmetry in plants include homozygosity, **hybridization**, mutation and quantitative genetic differences among individuals. Insect preferences for symmetric flowers increase reproductive success of both pollen donors and recipients by affecting seed set and embryo abortion. The ability of insects to discriminate between flowers of superior and inferior quality is hypothesized to depend on the level of developmental instability of the perceptive apparatus of insects. Hence, asymmetry of insects may have consequences for plant reproductive success and mating patterns.

Comparison of Bt (*Bacillus thuringiensis* Berliner) maize and conventional measures for control of the European corn borer (Lepidoptera : Crambidae)

Clark, TL; Foster, JE; Kamble, ST; Heinrichs, EA. 2000

Field experiments were conducted in 1997 to compare the efficacy St (*Bacillus thuringiensis* Berliner) maize **hybrids** and two conventional measures for control of the European corn borer, *Ostrinia nubilalis* (Hubner). Treatments consisted of transgenic St maize **hybrids** and their non-St isolines, and isolines treated with a formulated St or permethrin insecticide. All control measures significantly reduced *O. nubilalis* damage in terms of tunnels per plant, length of tunneling, and larvae per plant. The following hierarchy in terms of *O. nubilalis* efficacy was observed: transgenic Bt > permethrin > formulated Bt > control. In most cases, transgenic St maize was most effective in preventing European corn borer damage to ear shanks and generally produced the highest grain yields.

Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera : Noctuidae)

Wang, CZ; Dong, JF. 2001

Reciprocal **hybridization** between *Helicoverpa armigera* (Hubner) and *H. assulta* Guenee followed by backcrossing of the **hybrids** (F-1) with *H. armigera* produced backcross (BC) lines consisting of fertile females and males. The F1 of *H. armigera* female and *H. assulta* male had only male, no female sex. In this case Haldane's rule applies, and therefore it is proved that the sex chromosomes of *Helicoverpa* species are of ZW type, and the female is the heterozygous sex. This **hybrid** also showed significant heterosis with the heaviest pupal weight, and when it was backcrossed with *H. armigera* female, the sex ratio of the BC offspring was distorted as 1 : 4. The potential utilization of this **hybrid** in genetic controlling *H. armigera* was finally discussed.

Natural hybridization between two swallowtail species *Parnassius nomion* and *Parnassius bremeri* (Lepidoptera, Papilionidae) shown by RAPD-PCR

Zakharov, EV. 2001

Genetic evidence for interspecific **hybridization** between *Parnassius nomion* and *Parnassius bremeri* in nature is presented. To demonstrate **hybridization** between these species, RAPD analysis was used. By testing 25 decamer primers, three and two diagnostic markers were revealed for *P. nomion* and *P. bremeri*, respectively. Out of 28 animals examined, 4 were shown to be interspecific **hybrids**. According to the distribution of diagnostic markers, the interspecific **hybrids** were intermediate with regard to the parental species. Ecological and biological characteristics of two butterfly species that promote their **hybridization** in nature are discussed.

Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*

Naisbit, RE; Jiggins, CD; Mallet, J. 2001

Understanding the fate of **hybrids** in wild populations is fundamental to understanding speciation. Here we provide evidence for disruptive sexual selection against **hybrids** between *Heliconius cydno* and *Heliconius melpomene*. The two species are sympatric across most of Central and Andean South America, and coexist despite a low level of **hybridization**. No-choice mating experiments show strong assortative mating between the species. Hybrids mate readily with one another, but both sexes show a reduction in mating success of over 50% with the parental species. Mating preference is associated with a shift in the adult colour pattern, which is involved in predator defence through Mullerian mimicry, but also strongly affects male courtship probability. The **hybrids**, which lie outside the curve of protection afforded by mimetic resemblance to the parental species, are also largely outside the curves of parental mating preference. Disruptive sexual selection against F-1 **hybrids** therefore forms an additional post-mating barrier to gene flow, blurring the distinction between pre-mating and post-mating isolation, and helping to maintain the distinctness of these **hybridizing** species.

Sex-linked hybrid sterility in a butterfly

Jiggins, CD; Linares, M; Naisbit, RE; Salazar, C; Yang, ZH; Mallet, J. 2001

Recent studies, primarily in *Drosophila*, have greatly advanced our understanding of Haldane's rule, the tendency for **hybrid** sterility or inviability to affect primarily the heterogametic sex (Haldane 1922). Although dominance theory (Turelli and Orr 1995) has been proposed as a general explanation of Haldane's rule, this remains to be tested in female-heterogametic taxa, such as the Lepidoptera. Here we describe a novel example of Haldane's rule in *Heliconius melpomene* (Lepidoptera; Nymphalidae). Female F-1 offspring are sterile when a male from French Guiana is crossed to a female from Panama, but fertile in the reciprocal cross. Male F(1)s are fertile in both directions. Similar female F-1 sterility occurs in crosses between French Guiana and eastern Colombian populations. Backcrosses and linkage analysis show that sterility results from an interaction between gene(s) on the Z chromosome of the Guiana race with autosomal factors in the Panama genome. Large X (or Z) effects are commonly observed in *Drosophila*, but to our knowledge have not been previously demonstrated for **hybrid** sterility in Lepidoptera. Differences in the abundance of male versus female or Z-linked versus autosomal sterility factors cannot be ruled out in our crosses as causes of Haldane's rule. Nonetheless, the demonstration that recessive Z-linked loci cause **hybrid** sterility in a female heterogametic species supports the contention that dominance theory provides a general explanation of Haldane's rule (Turelli and Orr 2000).

Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera : Noctuidae)

Monti, L; Baylac, M; Lalanne-Cassou, B. 2001

S. latifascia and *S. descoinsi* are two closely related species of Noctuid moths. In laboratory conditions they **hybridize**, and F₁, F₂ and backcross **hybrids** may be obtained easily. Some interspecific matings do not succeed due to anatomical incompatibilities resulting from morphological differences in the genitalia of the species. One of these differences involves the clasper, a highly sclerotized process of the male genitalia. The overall contour of the clasper in *S. descoinsi*, *S. latifascia*, F₁, F₂, and backcross **hybrids** was analysed using elliptic Fourier analysis. All outlines were rotated along a common direction defined by two homologous landmarks, centered, and normalized for size. Fourier descriptors were used in a canonical variate analysis. Shape variations along factorial axes, or more generally along any variate of interest, such as size, were modeled by a multivariate regressive approach and visualized by inverse Fourier reconstructions of shapes. This approach allows a direct visualization of the shape transformations, negating the so-called impossibility or difficulty to interpret results of Fourier analyses. The results emphasize the specificity of the male clasper in the parental species. Most **hybrids** and backcrosses segregate from their parents. Many of them are displaced towards one of the parental forms. These results suggest that morphological differences may be controlled by a polygenic system with maternal effect in F₁ and parental dominance in backcross **hybrids**. (C) 2001 The Linnean Society of London.

Parasitism rates in European corn borer (Lepidoptera : Crambidae) larvae collected from six maize hybrids

Clark, TL; Witkowski, JF; Foster, JE. 2001

The parasitoid complex and level of parasitism of European corn borer, *Ostrinia nubilalis* (Hubner), larvae in six maize, *Zea mays* L., **hybrids** was determined in Nebraska during 1995 and 1996. Three parasitoids, *Eriborus terebrans* (Gravenhorst), *Macrocentrus grandii* Goidanich, and *Lixophaga* sp., were reared from field-collected European corn borer larvae. Larvae collected from Hoegemeyer 2626 exhibited the highest percentage parasitism for the 1995 first generation in Lancaster (37.2%) and Dixon (28.6%) counties. No significant differences were identified for the 1995 second generation at both sites because of reduced sample size and high larval mortality caused by naturally-occurring entomopathogens. During 1996, there were several significant differences in percentage parasitism of larvae collected from the **hybrids**. Larvae collected from Northrup King N7070 exhibited the highest first-generation parasitism (23.8%), while larvae collected from Hoegemeyer 1125W exhibited the highest second-generation parasitism (46.0%) in Dixon Co. In Lancaster Co., parasitism of first-generation larvae collected from Hoegemeyer 1125W (10.3%) was only significantly greater than parasitism of larvae collected from Hoegemeyer 2626 (1.1%). Results indicate that European corn borer larval parasitism is significantly affected by the maize **hybrid** planted in the field; however, differences may vary among years and generations as environmental factors affect the maize phenology.

First report of *Gypsonoma aceriana* (Duponchel) (Lepidoptera : Tortricidae), an old world poplar pest, in North America

Miller, WE; LaGasa, EH. 2001

Three adult males of *Gypsonoma aceriana* (Duponchel) were collected in two counties in western Washington State during 1998-99. Although superficially similar to the cottonwood twig borer, *G. haimbachiana* (Kearfott), of eastern North America, *G. aceriana* is easily recognized by its distinctive male genitalia. *G. aceriana* is a notable pest of *Populus* (Salicaceae) species and **hybrids** in plantations and nurseries in Europe, where it is known as the poplar shoot borer. Young larvae mine leaves, and older larvae bore into terminal shoots, often causing gall-like swellings. The species may be established in western Washington State, and could become a significant pest in North America.

Female flight propensity and capability in *Lymantria dispar* (Lepidoptera : Lymantriidae) from Russia, North America, and their reciprocal F-1 hybrids

Keena, MA; Wallner, WE; Grinberg, PS; Carde, RT. 2001

In the laboratory, the timing of both preflight and night behaviors of the Asian strain of female gypsy moths, *Lymantria dispar* L., was regulated primarily by light intensity. The shortest times to initiation of wing fanning and night occurred at 0.1 lux, the lowest light intensity evaluated. A gradual decrease in light intensity, compared with an instantaneous decrease, prolonged time to flight. The highest percentage of female night was observed at 0.1 lux. A higher percentage of females initiated flight when exposed to lower light intensities after the onset of normal scotophase rather than before scotophase. Virgin females were less likely to fly than mated females. Females fanned their wings longer at lower temperatures and when they were capable of only a gliding flight. Females that were night-tested the same day they emerged tended to take longer to initiate flight than those 1-2 d old. At 0.1 lux, the majority of the Asian females, less than 2% of the F-1 **hybrid** females, and none of the North American females exhibited strong, directed flight. Over half of the F-1 **hybrids** glided for a few meters while flapping their wings, whereas none of the North American females exhibited even this level of flight. Thus, female night capability will be reduced when flighted and nonflighted forms initially **hybridize**.

Transcriptional induction of diverse midgut trypsins in larval *Agrotis ipsilon* and *Helicoverpa zea* feeding on the soybean trypsin inhibitor

Mazumdar-Leighton, S; Broadway, RM. 2001

Midgut trypsins insensitive to inhibition by the soybean trypsin inhibitor (STI) were found to be transcriptionally regulated in *A. ipsilon* and *H. zea* larvae feeding on STI, as demonstrated by injections with actinomycin, a transcriptional inhibitor, which abolished the production of these STI-insensitive trypsins. The induced, STI-insensitive trypsins differed from the constitutive, STI sensitive trypsins in their susceptibility to inhibitors based on sizes, suggesting that the induced enzymes

limited access to their active site by blocking bulky inhibitors. Twenty midgut cDNA fragments' were amplified using trypsin-specific PCR primers and at least twelve were shown to encode structurally diverse trypsins. High sequence diversity was observed for both the enzymes encoded by STI-induced mRNAs and those from larvae that had not been exposed to STI. Northern blots showed that midgut mRNAs **hybridizing** to various trypsin cDNA probes were either transcribed de novo or up-regulated following ingestion of STI. Southern **hybridizations** indicated the presence of multiple trypsin gene families in the insect genomes. The complete sequence of a trypsin gene(1) from A. ipsilon (AiT9) revealed the presence of three introns. Comparison of 5' upstream sequences' from AiT9 and AiT6 genes from A. ipsilon revealed putative TATA box and disparate regulatory motifs, within 500 bp of each translational start site. (C) 2001 Elsevier Science Ltd. All rights reserved.

Identification of six chymotrypsin cDNAs from larval midguts of *Helicoverpa zea* and *Agrotis ipsilon* feeding on the soybean (Kunitz) trypsin inhibitor

Mazumdar-Leighton, S; Broadway, RM. 2001

Lepidopteran insects like *Helicoverpa ten* and *Agrotis ipsilon* produce STI-insensitive trypsins in the midgut following ingestion of dietary plant proteinase inhibitors like STI [Broadway, R. M., J. Insect Physiol. 43(9) (1997) 855-874]. In this paper, the effects of dietary STI on a related family of midgut serine proteinases, the chymotrypsins, were investigated. STI-insensitive midgut chymotrypsins were detected in larvae of *H. tea* and *A. ipsilon* feeding on diets containing 1% STI while STI-sensitive chymotrypsins were present in larvae feeding on diets containing 0% STI. These chymotrypsins were unaffected by TPCK, a diagnostic inhibitor of mammalian chymotrypsins but were fully inhibited by chymostatin. Four midgut cDNA libraries were constructed from larvae of each species fed either 0% STI or 1% STI diets. Six full-length cDNAs(1) encoding diverse preprochymotrypsins were isolated (three from *H. zea* and three from *A. ipsilon*) with certain sequence motifs that set them apart from their mammalian counterparts. Northern blots showed that some chymotrypsin mRNA were detected at higher levels while others were down-regulated when comparing insects reared on 0% STI and 1% STI diets. Southern **hybridizations** suggested that (like mammals) both species contained several chymotrypsin genes. A full-length chymotrypsin gene(1) from *H. tea* was sequenced for the first time and the presence of four introns was deduced. A first time comparison of 5' upstream regions(1) from three chymotrypsin genes and two trypsin genes of *A. ipsilon* indicated the presence of putative TATA boxes and regulatory elements. However a lack of consensus motifs in these upstream regions suggested the likelihood of multiple trans factors for regulation of genes encoding digestive proteinases and a complex response mechanism linked to ingestion of proteinase inhibitors. (C) 2001 Elsevier Science Ltd. All rights reserved.

Meiotic pairing of sex chromosome, fragments and its relation to atypical transmission of a sex-linked marker in *Ephesia kuehniella* (Insecta : Lepidoptera)

Marec, F; Tothova, A; Sahara, K; Traut, W. 2001

The physical basis of non-Mendelian segregation of a sex-linked marker was studied in sex-chromosome mutant females of eight ASF ('abnormal segregating females') lines in the flour moth, *Ephesia kuehniella*. Electron microscopical analysis of microspread synaptonemal complexes revealed that in one line, the Z chromosome segment that contained the dz(+) allele was translocated onto an autosome. The resulting quadrivalent visible in early female meiosis was 'corrected' into two bivalents in later stages. This explains autosomal inheritance of the sex chromosome marker in this strain. In the other seven ASF lines, the type of meiotic pairing of an additional fragment (Z(dz+)) of the Z chromosome was responsible for abnormal segregation of the marker gene. In several of these lines, Z(dz+) contained a piece of the W chromosome in addition to the Z segment, as was confirmed by comparative genomic **hybridization** (CGH). Z(dz+) formed three alternative pairing configurations with the original sex chromosomes: (i) a WZZ(dz+) trivalent, (ii) a WZ bivalent and a Z(dz+) univalent or (iii) a ZZ(dz+) bivalent and a W univalent. In the most frequent WZZ(dz+) configuration, Z(dz+) synapsed with Z and, consequently, segregated with W, simulating W linkage. This explains the predominant occurrence of the parental phenotypes in the progeny. Z(dz+) univalents or W univalents, on the other hand, segregated randomly, resulting in both parental and nonparental phenotypes. In two of these lines, the Z(dz+) was transmitted only to females. The results suggest that the W chromosome segment in Z(dz+) of these lines contains a male-killing factor which makes it incompatible with male development. Our data provide direct evidence for the regular transmission of radiation-induced fragments from lepidopteran chromosomes through more than 50 generations. This is facilitated by the holokinetic nature of lepidopteran chromosomes. We conclude that Z(dz+) fragments may persist as long as they possess active kinetochore elements.

Effects of corn plants and corn pollen on monarch butterfly (Lepidoptera : Danaidae) oviposition behavior

Tschenn, J; Losey, JE; Jesse, LH; Obrycki, JJ; Hufbauer, R. 2001

The oviposition behavior of adult insects can have a major impact on the level of exposure of their offspring to natural and artificial toxins. Pollen from Pt-corn **hybrids** represents a novel toxin and very little is known regarding its detection and possible avoidance by ovipositing females. The effect of corn plant proximity and corn pollen presence on the oviposition behavior of the monarch butterfly, *Danaus plexippus* (L.), was assessed in cage and flight chamber studies. The proportions of monarch eggs oviposited on milkweed plants dusted pollen from with a Bt-corn **hybrid**, an untransformed **hybrid**, gravel dust, and undusted control plants were recorded from a cage study. None of the treatments differed significantly in the relative proportion of eggs found. The effect of Bt and untransformed corn plant proximity and corn pollen presence was also assessed in a flight chamber. A significantly higher proportion of eggs (96%) were recovered from patches of milkweed plants not surrounded by corn plants, and a significantly higher proportion of eggs (nearly 70%) were recovered from patches of milkweed plants not dusted with corn pollen. There were no significant differences in the effects of Bt corn plants or corn pollen compared with untransformed plants or pollen. These results may have important implications for the level of exposure of monarch larvae to Pt-corn pollen.

Polyandry in Lepidoptera: a heritable trait in *Spodoptera exigua* Hubner

Torres-Vila, LM; Rodriguez-Molina, MC; Gragera, J; Bielza-Lino, P. 2001

The genetic basis as well as the mode of inheritance of polyandry in *Spodoptera exigua* Hubner was studied in the laboratory by using a simple divergent selection experiment followed by F-1 reciprocal crosses, F-2 and backcrosses. There was an effective response to artificial selection for high (H line) and low (L line) female mating frequency with significant separation of the lines by the second generation of selection. The mean female mating frequency in the parental generation (1.57 matings per female) reached plateaus of 2.50 and 1.25 matings per female in the H and L lines, respectively, after six generations of selection. Selection response becomes saturated at about 90% and 25% levels of polyandry (percentage females re-mating) in the H and L lines, respectively, and consequently mono and polyandric pure strains were not obtained. Polyandry levels in offspring from the H and L lines and their **hybrids** in F-1, F-2 and backcrosses consistently indicate that female mating frequency was more or less proportional to the relative amounts of genes derived from the H and L lines. Such a clear pattern of **hybrid** responses, together with the gradual selective changes under artificial selection, suggests the involvement of a polygenic system. Female mating frequencies from progeny of the two reciprocal F-1 crosses were not significantly different, which suggest that the trait was autosomally inherited. Moreover, female mating frequency of F-1 (pooled) progeny was not significantly different from the mid-parental value, which suggest no dominance. The computation of the Cavalli's joint scaling test consistently confirmed these results yielding values of $d=0.51 > 0.10$ and $h = 0.12 \pm 0.21$. The broad sense heritability estimate was $H^2 = 0.73$. It is concluded that polyandry in *S. exigua* is a polygenic, autosomal heritable trait and that additive genetic variance is available for selection for female mating frequency. The implications of the genetic basis of polyandry are briefly discussed in the context of current theories about this crucial insect mating system.

Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions

Zangerl, AR; McKenna, D; Wraight, CL; Carroll, M; Ficarello, P; Warner, R; Berenbaum, MR. 2001

The widespread planting of corn genetically modified to produce *Bacillus thuringiensis* endotoxin has led to speculation that pollen from these fields might adversely affect nearby nontarget lepidopterans. A previous study of Bt corn engineered with Monsanto event 810 failed to detect an effect of pollen exposure on the black swallowtail, *Papilio polyxenes*, in either the field or the laboratory. Here, we report results of a field study investigating the impact of exposure to pollen from a Bt corn **hybrid** containing Novartis event 176 on two species of Lepidoptera, black swallowtails and monarch butterflies, *Danaus plexippus*. Nearly half of the 600 monarch larvae died within the first 24 h; this and subsequent mortality was not associated with proximity to St corn and may have been due in part to predation. Survivorship of black swallowtails was much higher than that of the monarchs and was also independent of proximity to the transgenic corn. However, despite five rainfall events that removed much of the pollen from the leaves of their host plants during the experiment, we observed a significant reduction in growth rates of black swallowtail larvae that was likely caused by pollen exposure. These results suggest that Bt corn incorporating event 176 can have adverse sublethal effects on black swallowtails in the field and underscore the importance of event selection in reducing environmental impacts of transgenic plants.

Vertical distribution of European corn borer (*Lepidoptera* : Crambidae) egg masses on sweet corn

Spangler, SM; Calvin, DD. 2001

Within-plant height (leaf position) of European corn borer, *Ostrinia nubilalis* (Hubner) egg masses were analyzed in four sweet corn **hybrids** planted across four planting dates from 1994 to 1996. From the five-leaf to 15-leaf vegetative stages, the mean leaf position of an egg mass was typically at the midpoint of available (nonsenescent) leaves. The mean leaf position of egg masses gradually increased from early to late leaf stages as new leaves were added and continued to increase during the first reproductive stage (green tassel). The increase in vertical position during vegetative stages was modeled as $y = -0.766 + 0.653(x)$, where y = nodal leaf position, and x = total leaves expanded ($r(2) = 0.94$). However, when the mean egg mass position was expressed as the mean position within available (expanded and nonsenescent) leaves: no relationship was found. These data indicate that during vegetative stages the mean egg mass position increases relative to ground level, but remains near the midpoint of available available (nonsenescent) leaves. Mean egg position during the next four reproductive stages (anthesis, green silk, brown silk, and postharvest) stabilized at ear height, or within the intermediate two leaf positions above the ear. The distributions of egg masses among leaf positions during the vegetative and reproductive stages generally exhibited normal distributions: where sample sizes were sufficient, 80% (17/21) of the comparisons (having sufficient sample sizes) did not deviate from normality. This study represents the most detailed examination to date on vertical distribution of European corn borer egg masses in corn. Implications of the study in ovipositional ecology and pest management are discussed.

Patterns of postzygotic isolation in Lepidoptera

Presgraves, DC. 2002

I present patterns characterizing the evolution of intrinsic postzygotic isolation in Lepidoptera by analyzing data from the literature on genetic distance, strength of **hybrid** sterility and inviability, biogeography, and natural **hybridization**. Using genetic distance as a proxy for time, I investigate the time-course of the evolution of postzygotic isolation and the waiting times to particular **hybrid** fitness problems. The results show that postzygotic isolation increases gradually as species diverge, but that **hybrid** sterility evolves faster than **hybrid** inviability. The overwhelming preponderance of female-specific **hybrid** problems in Lepidoptera shows that Haldane's rule (the preferential sterility or inviability of the heterogametic sex) is well obeyed. Together the rates and patterns characterizing the accumulation of postzygotic isolation allow several tests of the composite theory of Haldane's rule. Interestingly, comparing these data with those from *Drosophila* reveals that Haldane's rule for sterility evolves faster (if not faster) in Lepidoptera. Finally, I show that a substantial fraction of sympatric species **hybridizes** in nature and that the majority of these suffer some level of **hybrid** sterility or inviability.

Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits

Scriber, JM. 2002

Co-adaptations, co-evolution, and co-speciation between herbivores and their host plants have been topics of interest for several decades. Difficulties in deciphering these relationships as well as physiological, biochemical, and ecological adaptations of herbivorous insects themselves are discussed here in relation to biotic and abiotic environmental factors that create temporal as well as spatial mosaics of genetic variation. Hybridization was shown in swallowtail butterflies (*Papilio*) (Lepidoptera: Papilionidae) to produce some trait concordance, but mostly independent geographic trait clines (physiological, biochemical, and morphological). Strong and extensive genetic introgression of *Liriodendron tulipifera* detoxification abilities was documented northward across the **hybrid** zone, presumably as a result of regional climate warming only during the last 3-4 years. These and other genetic novelties produced by **hybridization** may be important in speciation processes, and they also emphasize the difficulties identifying appropriate taxonomic classifications for discussing any species concept. Host plant detoxification abilities (as 'key innovations') are shown capable of rapid movement between different polyphagous herbivore species independently of the host plant availability and well beyond the insect species geographical range distribution. Part of the difficulty associated with ecologically categorizing herbivore species and identifying affiliated adaptations for host plant use may be related to independent movement of various 'species-diagnostic' traits. Climate-driven local selection regimes could help generate the dynamic variation observed for co-adapted, co-evolved, or non-adapted genotypes, and may produce the confusing and changing patterns of geographic mosaics seen within and among closely related herbivores. Experimental analyses of several factors that could explain the asymmetrical shapes of trait clines across the **hybrid** zone for tiger swallowtail butterflies are discussed.

Host-associated genetic differentiation in the goldenrod elliptical-gall moth, *Gnorimoschema gallaesolidaginis* (Lepidoptera : Gelechiidae)

Nason, JD; Heard, SB; Williams, FR. 2002

Careful study of apparently generalist phytophagous insects often reveals that they instead represent complexes of genetically differentiated host races or cryptic species. The goldenrod elliptical-gall moth, *Gnorimoschema gallaesolidaginis*, attacks two goldenrods in the *Solidago canadensis* complex: *S. altissima* and *S. gigantea* (Asteraceae). We tested for host-associated genetic differentiation in *G. gallaesolidaginis* via analysis of variation at 12 allozyme loci among larvae collected at six sites in Iowa, Minnesota, and Nebraska. *Gnorimoschema gallaesolidaginis* from each host are highly polymorphic (3.6-4.7 alleles/locus and expected heterozygosity 0.28-0.38 within site-host combinations). Although there were no fixed differences between larvae from *S. altissima* and *S. gigantea* at any site, these represent well differentiated host forms, with 11 of 12 loci showing significantly different allele frequencies between host-associated collections at one or more sites. Host plant has a larger effect on genetic structure among populations than does location (Wright's $F_{ST} = 0.16$ between host forms vs. $F_{ST} = 0.061$ and 0.026 among *altissima* and *gigantea* populations, respectively). The estimated F_{ST} between host forms suggests that the historical effective rate of gene flow has been low ($N(e)m$ approximate to 1.3). Consistent with this historical estimate is the absence of detectable recombinant (**hybrid** and introgressant between host form) individuals in contemporary populations (none of 431 genotyped individuals). Upper 95% confidence limits for the frequency of recombinant individuals range from 5% to 9%. Host association is tight, but imperfect, with only one likely example of a host mismatch (a larva galling the wrong host species). Our inferences about **hybridization** and host association are based on new maximum-likelihood methods for estimating frequencies of genealogical classes (in this case, two parental classes, F-1 and F-2 **hybrids**, and backcrosses) in a population and for assigning individuals to genealogical classes. We describe these new methods in the context of their application to genetic structure in *G. gallaesolidaginis*. Population phenograms are consistent with the origin of the host forms (at least in the midwestern United States) via a single host shift: *altissima* and *gigantea* moth populations form distinct lineages with 100% bootstrap support. Genetic structure in *Gnorimoschema* is of particular interest because another gallmaking insect attacking the same pair of hosts, the tephritid fly *Eurosta solidaginis*, includes a pair of host races with partial reproductive isolation. *Gnorimoschema gallaesolidaginis* and *E. solidaginis* therefore represent the first reported case of parallel host-associated differentiation, that is, differentiation by evolutionarily independent insect lineages across the same pair of host plants.

Inferences from a rapidly moving hybrid zone

Dasmahapatra, KK; Blum, MJ; Aiello, A; Hackwell, S; Davies, N; Bermingham, EP; Mallett, T. 2002

Anartia fatima and *Anartia amathea* (Lepidoptera: Nymphalidae) are sister taxa whose ranges abut in a narrow **hybrid** zone in eastern Panama. At the center of the zone, **hybrids** are abundant, although deviations from Hardy-Weinberg and linkage disequilibria are strong, due in part to assortative mating. We measured differences across the zone in four wing color-pattern characters, three allozyme loci, and mitochondrial haplotype. Wing pattern, allozyme, and mitochondrial clines were coincident (i.e., had the same positions) and concordant (i.e., all markers had similar cline shapes, about 28 km wide). Repeated samples demonstrated that the **hybrid** zone has been moving eastwards at an average rate of 2.5 km/year over the past 20 years, accompanied by an equivalent movement of the mtDNA cline. No introgression of mtDNA haplotypes were found in the "wake" of the moving cline, as might be expected for a neutral marker. The concordance of morphological and mtDNA clines between 1994 and 2000, in spite of **hybrid** zone movement, suggests strong epistasis between the mitochondrial genome and nuclear loci. Cline movement is achieved mainly by pure *fatima* immigrating into *amathea* populations; **hybrids** had little effect, and were presumably outcompeted by fitter pure *fatima* genotypes. This movement can be explained if random dispersal of 7-19 km.gen(-1/2) is coupled with a competitive advantage to *A. fatima* genomes of 2-5%. Hybrid zone motion is equivalent to Phase III of Wright's shifting balance. Hybrid zone movement has rarely been considered likely in the past, but our results show that it may be more important in biogeography and evolution than generally realized.

Field bioassays show heterospecific mating preference asymmetry between hybridizing North American *Papilio* butterfly species (Lepidoptera : Papilionidae)

Deering, MD; Scriber, JM. 2002

Few studies of interspecific mating preferences of naturally **hybridizing** species have been done in the field. Yet this is the only way potentially critical habitat-specific factors can be included in mating behavior evaluations. We conducted mate selection (male preference) studies using tethered pairs of heterospecific, size-matched virgin yellow females of *Papilio glaucus* and *P. canadensis* with free-flying male *P. glaucus* populations in south-central Florida. and with free-flying male *P. canadensis* populations in northern Michigan. Florida males clearly preferred the conspecific *P. glaucus* females of the pairs, as indicated by the touches/attempted matings and by the actual copulations observed. In 1997, 66.7% of the total attempts (n = 168) and 94.2% of all copulations (n = 69) were with the conspecific. In 1998 the same pattern of conspecific male preference was observed, with 67.3% of the touches/attempted (n = 49) and 100% of the copulations (n = 30) being with the *P. glaucus* females. In contrast, the natural populations of *P. canadensis* males did not show the expected conspecific preference. In fact, with wild *P. canadensis* males in 1997 a very strong preference was observed for the heterospecific *P. glaucus* females of the pairs, accounting for 75.8% of all touches/attempted matings (n = 483) and 81.7% of all copulations (n = 476). The strong asymmetry in interspecific mating preferences among these **hybridizing** *Papilio* species may reflect an ancestral trait in *P. glaucus* females that elicits strong preference in *P. canadensis* males, due perhaps to previously possessed mate sensory bias or "good genes" indicators in the females. However, reinforcement of reproductive isolation in or near the **hybrid** zone of species overlap (sympatry) cannot be ruled out.

Relationship between the population dynamics of the gypsy moth (*Lymantria dispar* L., Lymantriidae : Lepidoptera) and the genetic polymorphism of the nuclear polyhedrosis virus

Bakhvalov, SA; Bakhvalova, VN; Morozova, OV. 2002

The population dynamics of the gypsy moth and the genomic DNA of the nuclear polyhedrosis virus (NPV) pathogenic for this insect were simultaneously studied using nucleic acid molecular **hybridization** (NAMH). It was demonstrated that population outbreaks of the gypsy moth were accompanied by an increase in its sensitivity to NPV and in the genetic polymorphism of NPV strains circulating in the insect populations. The **hybridization** activity of the virus strains isolated at the phase of gypsy moth population growth was significantly higher ($p < 0.001$) than the activity of the strains isolated at the phase of population decrease.

Feminizing *Wolbachia* in an insect, *Ostrinia furnacalis* (Lepidoptera : Crambidae)

Kageyama, D; Nishimura, G; Hoshizaki, S; Ishikawa, Y. 2002

Wolbachia, which forms a group of maternally inherited bacteria in arthropods, often cause reproduction alterations in their hosts, such as cytoplasmic incompatibility, parthenogenesis, male-killing, **hybrid** breakdown and feminization. To date, *Wolbachia*-induced feminization has been reported only in isopods. Here we report that a *Wolbachia* strain feminizes an insect host, *Ostrinia furnacalis*. Among 79 wild females of *O. furnacalis* examined, *Wolbachia* infection was detected in 13 females. Twelve of the 13 infected females produced all-female progenies, and this trait was maternally inherited. Tetracycline treatment of thelygenic matrilines resulted in the production of all-male progenies. The present findings indicate that the *Wolbachia* infection induces feminization of genetic males in *O. furnacalis*. Differences in the *Wolbachia*-induced feminization in *O. furnacalis* and that in isopods are discussed along with the differences in sex determination mechanisms between insects and isopods. Phylogenetic analysis of the *wsp* gene sequence of *Wolbachia* suggests independent evolutionary origins for the *Wolbachia*-induced feminizations in *O. furnacalis* and in isopods. Our findings over 5 years suggest that the infection has been maintained at a low prevalence in the *O. furnacalis* population.

Hybrid sterility, Haldane's rule and speciation in *Heliconius cydno* and *H. melpomene*

Naisbit, RE; Jiggins, CD; Linares, M; Salazar, C; Mallet, J. 2002

Most genetic studies of Haldane's rule, in which **hybrid** sterility or inviability affects the heterogametic sex preferentially, have focused on *Drosophila*. It therefore remains unclear to what extent the conclusions of that work apply more generally, particularly in female-heterogametic taxa such as birds and Lepidoptera. Here we present a genetic analysis of Haldane's rule in *Heliconius* butterflies. Female F₁ **hybrids** between *Heliconius melpomene* and *H. cydno* are completely sterile, while males have normal to mildly reduced fertility. In backcrosses of male F-1 **hybrids**, female offspring range from completely sterile to fully fertile. Linkage analysis using the Z-linked triose-phosphate isomerase locus demonstrates a "large X" (Z) effect on sterility. Expression of female sterility varies among crosses in this and a previous study of *Heliconius*. Sterility may result from the production of normal but infertile eggs, production of small infertile eggs, or from a complete failure to develop ovarioles, which suggests multiple routes to the evolution of **hybrid** sterility in these *Heliconius* species. These results conform to the expectations of the "dominance" rather than "faster male" theories of Haldane's rule and suggest that relatively few loci are responsible. The two species are broadly sympatric and **hybridize** in the wild, so that female **hybrid** sterility forms one of several strong but incomplete barriers to gene flow in nature. The effect of female sterility is comparable to that of selection against non-mimetic **hybrids**, while mate choice forms a much stronger barrier to gene transfer.

Latitudinal and local geographic mosaics in host plant preferences as shaped by thermal units and voltinism in *Papilio* spp. (Lepidoptera)

Scriber, JM. 2002

Laboratory and field tests support the "voltinism-suitability hypothesis" of host selection at various latitudes as well as in local "cold pockets": The best hosts for rapid development will be selected by herbivorous insects under severe thermal constraints for completion of the generation before winter. *Papilio canadensis* and *P. glaucus* females do select the best hosts for rapid larval growth in Alaska and in southern Michigan, but not in northern Michigan and southern Ohio. In addition to latitudinal patterns, local host preferences of *P. canadensis* are described in relation to "phenological twisting" of leaf suitability for larval growth in cold pockets with "thermally constrained" growing season lengths. White ash leaves (*Fraxinus americana*) have the highest nutritional quality (relative to cherry, aspen, birch, and other local trees) throughout June and July for *P. canadensis* populations inside the cold pocket, but not outside. In all areas outside the cold pockets, even with bud-break occurring much later than other tree species, ash leaves rapidly decline in quality after mid-June and become one of the worse tree host species for larvae. This temperature-driven phenology difference creates a geographic mosaic in host plant suitability for herbivores. However, we also report here that the cold pocket of northern Michigan has basically disappeared since 1998. Implications of these changes are discussed in relation to voltinism and host plant selection. Various physiological and ecological adaptations to thermal unit constraints are evident with both species of naturally **hybridizing** butterflies (*P. canadensis* to the North and *P. glaucus* to the South). The rapid regional climate warming since 1998 in the Great Lakes region has allowed us to document some critical aspects of gene flow via analysis of independent "species-diagnostic" trait clines (including morphological, biochemical, and physiological characters). Narrower black bands on hind-wings, larger fore-wings, larval detoxification abilities for tulip tree leaves, behavioral preference of tulip tree leaves, and species-diagnostic allozymes such as PDG (x-linked) and HK (autosomal) provide evidence of these genodynamics. Laboratory **hybridization** studies (providing known reference groups) and field observations along the **hybrid** zone show that gene flow between *P. glaucus* and *P. canadensis* has recently been extensive across the historical **hybrid** zone.

Comparison of *Bacillus thuringiensis* corn hybrids and insecticide-treated isolines exposed to bivoltine European corn borer (Lepidoptera : Crambidae) in South Dakota

Catangui, MA; Berg, RK. 2002

The performances of *Bacillus thuringiensis*, (Bt) corn **hybrids** and permethrin-treated isolines were evaluated in South Dakota from 1997 to 1999. The corn **hybrids** were exposed to natural seasonal fluctuations of a bivoltine ecotype European corn borer population. Larval injury parameters, grain yields, and gross incomes were quantified during the 3-yr study. The use of either Bt **hybrids** or whorl-applied permethrin granules resulted in significant yield advantages in only 1 out of 3 yr of performance testing. Under high corn borer pressure in 1997, the performances of the Bt corn **hybrids** and permethrin-treated isolines were similar, and significantly better than the untreated isolines. However, injury-free corn did not necessarily translate into higher yields in some of the **hybrid** groups. Grain moisture at harvest, which can result in moisture penalty, or dockage, was significantly higher in some Bt corn **hybrids**. Neither the Bt **hybrids** nor the permethrin-treated isolines produced significant advantages in yields in 1998 and 1999 when infestations of first-generation corn borer were almost nonexistent and infestations of second-generation European corn borers were moderate.

Use of transgenic *Bacillus thuringiensis* Berliner corn hybrids to determine the direct economic impact of the, European corn borer (Lepidoptera : Crambidae) on field corn in eastern Canada

Baute, TS; Sears, MK; Schaafsma, AW. 2002

Transgenic corn expressing *Bacillus thuringiensis* Berliner (Bt corn) (Maximizer and Yieldgard **hybrids**, Novartis Seeds), non-Bt isolines and high-performance (check) **hybrids** were evaluated for European corn borer, *Ostrinia nubilalis*. (Hubner), damage and grain yield in commercial strip plots across Ontario in 1996 and 1997. Bt corn **hybrids** reduced stalk tunneling damage by 88-100%. In 1996, minimal damage was found in locations where only one generation of European corn borer occurred per year. Bt corn proved its greatest potential for reducing the number and length of, cavities below the primary ear in locations where two generations of European corn borer were present. A yield response to using Bt **hybrids** only occurred when levels of tunneling damage exceeded 6 cm in length. European corn borer infestations resulted in a 6 and 2.4% reduction in yield for 1996 and 1997, respectively, when Bt **hybrids** were compared with their non-Bt isolines. A linear relationship was found between tunnel length per plant in centimeters (x) and yield protection (%) obtained from using Bt corn (y) ($y = 1.02 + 0.005x$, $r(2) = 0.7217$). At a premium of \$34.58 Canadian (CDN) per hectare for Bt corn seed, an infestation of at least 6 cm of corn borer tunneling per plant was required to break even at a market price for corn of \$2.50 per bushel CDN. During the period of study, low infestations (0-2 cm) of European corn borer occurred at 25% of the locations assessed, moderate infestations (4-6 cm) occurred at 42% of the locations, and high infestations (>6 cm) occurred at 33% of the locations. At a corn price of \$3.00 per bushel CDN and seed premiums of \$34.58 per hectare CDN, 5 cm of tunneling was required for a return on investment in Bt seed, comprising only 55% of the growers in the study. With infestations of more than 6 cm of tunneling occurring only 33% of the time, a return on seed investment would be realized in only one of three growing seasons. At a seed premium of \$24.70 per hectare CDN per year, at least \$74 per hectare CDN in the year of infestation would be required to make up for the two years of no return. In this study, a \$74 per hectare CDN return at a corn price of \$9.26 per hectare CDN with >16 cm of tunneling damage would have occurred only 7.3% of the time.

Phylogenetic relationships in brown argus butterflies (Lepidoptera : Lycaenidae : Aricia) from north-western Europe

Aagaard, K; Hindar, K; Pullin, AS; James, CH; Hammarstedt, O; Balstad, T; Hanssen, O. 2002

Lycaenid butterflies of the *Aricia agestis-artaxerxes* complex pose an unresolved taxonomic and conservation problem in north-western Europe. Two key issues require resolution: (i) how many species of *Aricia* occur in north-western Europe and what are their distributions?; (ii) how is the morphological variation observed in north-western Europe best explained? We investigated phylogenetic relationships and phylogeographic patterns in this species group using mitochondrial and nuclear markers in comparison with morphological variation. A 325 bp fragment of the mitochondrial cytochrome-b gene was sequenced from 179 individuals representing 18 populations from the UK and Scandinavia. Seventeen enzyme-coding loci were analysed from 538 individuals from the same populations. Highly congruent phylogenies between mitochondrial and allozyme markers demonstrate that the sample is composed of two closely related species, *A. agestis* and *A. artaxerxes*. Both marker types also suggest that Scottish and northern Scandinavian *A. artaxerxes* populations are conspecific, and consequently do not support the endemic status of *A. artaxerxes* in the UK. The subspecies division of British populations of *A. artaxerxes* is also not supported by phylogenetic analyses. Allozyme and mitochondrial analyses cluster two populations from the Peak District, UK, differently. The former suggests that they are *A. artaxerxes* whilst the latter suggests they are *A. agestis*. Further research is required to find the reason for this disagreement, which could be associated with the different dynamics of nuclear and mitochondrial genes across a **hybrid** zone between the two species. (C) 2002 The Linnean Society of London.

Mutations and their use in insect control

Robinson, AS. 2002

Traditional chemically based methods for insect control have been shown to have serious limitations, and many alternative approaches have been developed and evaluated, including those based on the use of different types of mutation. The mutagenic action of ionizing radiation was well known in the field of genetics long before it was realized by entomologists that it might be used to induce dominant lethal mutations in insects, which, when released, could sterilize wild female insects. The use of radiation to induce dominant lethal mutations in the sterile insect technique (SIT) is now a major component of many large and successful programs for pest suppression and eradication. Adult insects, and their different developmental stages, differ in their sensitivity to the induction of dominant lethal mutations, and care has to be taken to identify the appropriate dose of radiation that produces the required level of sterility without impairing the overall fitness of the released insect. Sterility can also be introduced into populations through genetic mechanisms, including translocations, **hybrid** incompatibility, and inherited sterility in Lepidoptera. The latter phenomenon is due to the fact that this group of insects has holokinetic chromosomes. Specific types of mutations can also be used to make

improvements to the SIT, especially for the development of strains for the production of only male insects for sterilization and release. These strains utilize male translocations and a variety of selectable mutations, either conditional or visible, so that at some stage of development, the males can be separated from the females. In one major insect pest, *Ceratitis capitata*, these strains are used routinely in large operational programs. This review summarizes these developments, including the possible future use of transgenic technology in pest control, (C) 2002 Published by Elsevier Science B.V.

Differential patterns of hybridization and introgression between the swallowtails *Papilio machaon* and *P. hospiton* from Sardinia and Corsica islands (Lepidoptera, Papilionidae)

Cianchi, R; Ungaro, A; Marini, M; Bullini, L. 2003

Proportions of **hybridization** and introgression between the swallowtails *Papilio hospiton*, endemic to Sardinia and Corsica, and the holarctic *Papilio machaon*, were characterized using nine fully diagnostic and two differentiated allozyme loci and a mitochondrial DNA marker. Very low frequencies of F-1 **hybrids** were detected in both Sardinia (0-4%, average 1.4%) and Corsica (0-3%, average 0.5%), as well as of first generation backcrosses (B-1). No F-2 were observed, in agreement with the **hybrid** breakdown detected in laboratory crosses. In spite of this minimal current gene exchange, specimens carrying introgressed alleles were found in high proportions in *P. machaon* but in lower proportions in *P. hospiton*. Introgression apparently occurred through past **hybridization** and repeated backcrossing, as evidenced by **hybrid** index scores and Bayesian assignment tests. Levels of introgression were low (0-1%) at two sex-linked loci and mitochondrial DNA, limited (0.4-2%) at three autosomal loci coding for dimeric enzymes, and high (up to 43%) at four autosomal loci coding for monomeric enzymes. Accordingly, selective filters are acting against foreign alleles, with differential effectiveness depending on the loci involved. The low levels of introgression at sex-linked loci and mitochondrial DNA are in agreement with Haldane's rule and suggest that introgression in *P. machaon* proceeds mainly through males, owing to a lower fitness of **hybrid** females. *Papilio machaon* populations showed higher levels of introgression in Sardinia than in Corsica. The role of reinforcement in the present reproductive isolation between *P. machaon* and *P. hospiton* is examined, as well as the evolutionary effects of introgressive **hybridization** between the two species.

Two additional state records of F-1 male interspecific hybrid *Limenitis* (Basilarchia) spp. form "rubidus" Strecker (Lepidoptera : Nymphalidae)

Platt, AP; McClanahan, ET. 2003

State records of single male specimens of the interspecific **hybrid** form "rubidus" (Lepidoptera, Nymphalidae: *Limenitis* (Basilarchia)) are reported from New Mexico and Kansas U.S.A. This form arises from rare mixed matings between *L. archippus* (Cr.) x *L. arthemis astyanax* (Fabr.), two closely related species which are involved in two quite differently colored mimicry complexes. The **hybrids** often occur when one or both of the parental species is or are rare. The New Mexican specimen involves the two southwestern desert subspecies of the parental butterflies. These two state records bring to 51 the known occurrences of these natural **hybrids**, all of which are males. This **hybrid** form has been reported from 20 states and the District of Columbia. Most of the **hybrid** records occur late in the flight season, except in Florida. Phenotypically and behaviorally this form represents a complete breakdown of the different mimetic resemblances present in its two parental species, thus placing this insect at a considerable selective disadvantage in natural populations.

Host choice promotes reproductive isolation between host races of the larch budmoth *Zeiraphera diniana*

Emelianov, I; Simpson, F; Narang, P; Mallet, J. 2003

The chances for sympatric speciation are improved if ecological divergence leads to assortative mating as a by-product. This effect is known in parasites that find mates using host cues, but studies of larch- and pine-feeding races of the larch budmoth (*Zeiraphera diniana*, Lepidoptera: Tortricidae) suggest it may also occur when mate attraction is via sex pheromones that are independent of habitat. We have previously shown that females releasing pheromones on or near their own host attract more males of their own race than if placed on the alternative host. This host effect would enhance assortative mating provided adults preferentially alight on their native hosts. Here we investigate alighting preferences in natural mixed forest using a novel likelihood analysis of genotypic clusters based on three semidiagnostic allozyme loci. Both larch and pine females show a realized alighting preference for their own host of 86%. The equivalent preferences of males were 79% for the larch race and 85% for the pine race. These preferences are also detectable in small-scale laboratory experiments, where alighting preferences of larch and pine races towards their own hosts were, respectively, 67 and 66% in females and 69 and 63% in males. Pure larch race moths reared in the laboratory had alighting choice similar to moths from natural populations, while **hybrids** were intermediate, showing that alighting preferences were heritable and approximately additive. The field estimates of alighting preference, coupled with earlier work on mate choice, yield an estimated rate of natural **hybridization** between sympatric host races of 2.2-3.8% per generation. Divergent alighting choice enhances pheromone-mediated assortative mating today, and is likely to have been an important cause of assortative mating during initial divergence in host use. Because resources are normally 'coarse-grained' in space and time, assortative mating due to ecological divergence may be a more important catalyst of sympatric speciation than generally realized.

Hybridization and postzygotic isolation patterns in pigeons and doves

Lijtmaer, DA; Mahler, B; Tubaro, PL. 2003

The study of the patterns of reproductive isolation in relation to species divergence is critical for the understanding of the process of speciation. Comparative analyses of this kind were previously conducted in *Drosophila*, Lepidoptera, frogs, ducks, and birds in general. In the present study, we used information from the literature to analyze **hybrid** inviability in relation to species divergence in pigeons and doves. Four main patterns arose from this analysis: (1) as in the other groups studied, F-1 **hybrid** inviability gradually increases as species diverge, the time needed to reach total inviability being higher in birds than in the other groups; (2) as expected, the presence of geographic overlap does not influence the evolution of postzygotic isolation; (3) the percentage of unhatched eggs does not differ between **hybrids** of the first generation and the backcrosses, but it increases in the second **hybrid** generation; and (4) pigeons and doves follow Haldane's rule, as found in the other groups studied so far. The similarity between the results of this and previous studies contributes to the growing evidence suggesting that the patterns of the evolution of postzygotic isolation, and the process of speciation in general, are shared among animal groups.

The systematics of the *Trichogramma minutum* species complex (Hymenoptera : Trichogrammatidae), a group of important North American biological control agents: the evidence from reproductive compatibility and allozymes

Pinto, JD; Platner, GR; Stouthamer, R. 2003

Two North American *Trichogramma*, *Trichogramma minutum* Riley, and *Trichogramma platneri* Nagarkatti (the *T. minutum* complex), cannot be distinguished morphologically and their species status has been questioned. Both are commercially available for biological control and are important parasitoids of the eggs of Lepidoptera in agricultural ecosystems. Results are reported of an extensive survey of reproductive compatibility and allozymes at the phosphoglucotransferase (Pgm) locus in collections representing 85 localities throughout much of the geographical range of this species complex. In total, 103 reproductive crosses were performed and 161 collections were analyzed electrophoretically. Results support the recognition of *T. minutum* and *T. platneri* as distinct species. The two are distinguishable electrophoretically at the Pgm locus. High levels of reproductive incompatibility are correlated with the allozymic differences. Although *T. platneri* is western in distribution (W of 115 degrees longitude) and *T. minutum* is primarily eastern, the two are sympatric in the Pacific Northwest. A low level of interspecific **hybridization**

in laboratory studies notwithstanding, there is no evidence that introgression occurs in the zone of sympatry. This study continues to stress the importance of species recognition and the inadvisability of using *T. minutum* and *T. platneri* interchangeably in biological control. (C) 2003 Elsevier Science (USA). All rights reserved.

Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. III. Heterogeneous accumulation of hybrid incompatibilities, degree of dominance, and implications for Haldane's rule

Tao, Y; Hartl, DL. 2003

The genetic basis of Haldane's rule was investigated through estimating the accumulation of **hybrid** incompatibilities between *Drosophila simulans* and *D. mauritiana* by means of introgression. The accumulation of **hybrid** male sterility (HMS) is at least 10 times greater than that of **hybrid** female sterility (HFS) or **hybrid** lethality (HL). The degree of dominance for HMS and HL in a pure *D. simulans* background is estimated as 0.23-0.29 and 0.33-0.39, respectively; that for HL in an F-1 background is unlikely to be very small. Evidence obtained here was used to test the Turelli-Orr model of Haldane's rule. Composite causes, especially, faster-male evolution and recessive **hybrid** incompatibilities, underlie Haldane's rule in heterogametic male taxa such as *Drosophila* (XY male and XX female). However, if faster-male evolution is driven by sexual selection, it contradicts Haldane's rule for sterility in heterogametic-female taxa such as *Lepidoptera* (ZW female and ZZ male). The hypothesis of a faster-heterogametic-sex evolution seems to fit the current data best. This hypothesis states that gametogenesis in the heterogametic sex, instead of in males per se, evolves much faster than in the homogametic sex, in part because of sex-ratio selection. This hypothesis not only explains Haldane's rule in a simple way, but also suggests that genomic conflicts play a major role in evolution and speciation.

Gene flow between great lakes region populations of the Canadian tiger swallowtail butterfly, *Papilio canadensis*, near the hybrid zone with *P. glaucus* (*Lepidoptera* : *Papilionidae*)

Stump, AD; Sperling, FAH; Crim, A; Scriber, JM. 2003

Papilio canadensis were sampled from three locations on either side of Lake Michigan to study gene flow near and through a butterfly **hybrid** zone. Allele frequencies at four polymorphic enzyme loci, as indicated by allozyme electrophoresis, were similar in all samples. Values for F-ST were close to zero, indicating that gene flow is high among these populations, even when separated by Lake Michigan. We developed a mitochondrial DNA marker with diagnostic differences between *P. canadensis* and its parapatric sister species *Papilio glaucus*, based on PCR-RFLP. *P. glaucus* haplotypes of this mtDNA marker and *P. glaucus* alleles of a diagnostic allozyme locus (PGD) were found in *P. canadensis* populations sampled in Michigan's Lower Peninsula but not in the Upper Peninsula or Northern Minnesota. The presence of *P. glaucus* alleles in *P. canadensis* populations could be due to introgression through **hybridization**, or could be remnants of a *P. glaucus* population that was inundated by an influx of *P. canadensis* alleles.

Fumonisin contamination of maize hybrids visibly damaged by *Sesamia*

Avantaggiato, G; Quaranta, F; Desiderio, E; Visconti, A. 2003

Sesamia nonagrioides Lefebvre (*Lepidoptera*: Noctuidae) is the main insect pest of maize cultivated in Mediterranean areas, causing an increase in broken plants, a reduction in yield and a decline in grain quality. An investigation of *Sesamia* attacks and fumonisin accumulation on 25 maize **hybrids** sown as a second crop after wheat has been performed under field conditions in Central Italy in 2000. The **hybrids** tested in this study showed different degrees of insect damage, ranging from 12 to 57% of damaged ears per **hybrid**. Over 50% of the tested **hybrids** showed strong insect damage, with more than 30% of harvested ears visibly damaged by *Sesamia*. Fungal contamination by *Fusarium verticillioides* and *F. proliferatum*, two well-known producers of fumonisins, was detected in both symptomless and insect-damaged samples. Fumonisin analysis of healthy-looking and insect-damaged ear samples of each **hybrid** showed 100% incidence of positive samples, with fumonisin contents ranging from 0.01 to 20 mg kg⁻¹ for healthy-looking ears and from 27 +/- 32 to 287 +/- 221 mg kg⁻¹ for insect-damaged ears. Extremely high levels of fumonisins were found in ear samples visibly damaged by *Sesamia*, with individual values of up to 694 mg kg⁻¹ and average values exceeding 100 mg kg⁻¹ in more than 50% of the **hybrids**. A good correlation ($r = 0.749$) was found between fumonisin contamination and the degree of insect damage by *Sesamia* of the tested **hybrids**, calculated on the basis of percentage of ears visibly damaged by insects and with more than 5% kernel loss. This finding leads to the conclusion that insect damage by *Sesamia* on maize could be used as an early indicator of fumonisin contamination. (C) 2002 Society of Chemical Industry.

Structural characterization and expression analysis of prothoracicotrophic hormone in the corn earworm, *Helicoverpa zea*

Xu, WH; Rinehart, JP; Denlinger, DL. 2003

The cDNA encoding prothoracicotrophic hormone (PTTH), the brain neuropeptide that stimulates the prothoracic glands to synthesize ecdysone, was cloned from the corn earworm *Helicoverpa zea* (Hez). The amino acid sequence deduced from the cDNA indicates a molecular structure that is distinct from the PTTH's reported in other *Lepidoptera*, but all contain an identical proteolytic cleavage site and the seven cysteine residues that are essential for activity. Northern **hybridization** shows a single mRNA present in the brain-subesophageal ganglion complex. Using RT-PCR, we observed constant amounts of PTTH mRNA during larval development but large fluctuations at pupation and prior to adult eclosion. (C) 2003 Elsevier Inc. All rights reserved.

Transgenic *Bacillus thuringiensis* corn hybrid performance against univoltine ecotype European corn borer (*Lepidoptera* : *Crambidae*) in South Dakota

Catangui, MA. 2003

The performances of Bt-corn **hybrids** against univoltine ecotype European corn borer larvae were evaluated in South Dakota from 1997 to 1999. The corn **hybrids** were exposed to natural seasonal fluctuations of a univoltine ecotype European corn borer population. Larval injury parameters, grain yields, and gross incomes were quantified during the 3-yr study. In general, the Bt-corn **hybrids** had significantly higher yields than the untreated non-Bt isolines in 1997 and 1998 when corn borer pressures were high. However, most of the Bt-corn **hybrids** did not produce significant yield advantages in 1999 when the European corn borer pressure was low. Some of the Bt-corn **hybrids** even produced significantly lower yields than their untreated non-Bt counterparts in 1999. The performances of non-Bt isolines that were treated with permethrin granules at whorl stage were similar to Bt-corn **hybrids** in 1998 when the corn borer pressure was high and similar to the untreated non-Bt isolines in 1999 when the corn borer pressure was low. Injury-free corn produced by Bt-corn **hybrids** did not necessarily translate into higher yields in some **hybrid** groups. Grain moisture at harvest, which can result in moisture penalty or dockage, was significantly higher in most Bt-corn **hybrids**. Gross incomes of the Bt-corn **hybrids** were generally higher than the untreated non-Bt isolines when the corn borer infestation was high, but were either similar to or lower than the untreated non-Bt isolines when the corn borer infestation was low.

Enhanced expression of genes in the brains of larvae of *Mamestra brassicae* (*Lepidoptera* : *Noctuidae*) exposed to short daylength or fed Dopa

Uryu, M; Ninomiya, Y; Yokoi, T; Tsuzuki, S; Hayakawa, Y. 2003

The cabbage armyworm, *Mamestra brassicae*, enters diapause in the early pupal stage. Pupal diapause is induced by rearing the larvae under short day lengths. We previously demonstrated that feeding Dopa during last larval instar induces pupal diapause even under long day lengths. In order to elucidate the mechanism by which pupal diapause is induced after experiencing short day lengths or fed Dopa under long day lengths, we analyzed gene expression in the brain of *M. brassicae* larvae under both of these conditions using a subtractive **hybridization** technique. After the secondary screen, 49 clones and 28 clones were identified as short day length or Dopa-feeding specific clones, respectively. All of these genes were sequenced and, using the base sequences of these clones, primers were synthesized. To confirm the genes enhanced specifically by these conditions, quantitative real-time PCR was carried out. This quantitative PCR analysis identified 15 and 1 clone whose expression was enhanced by the short day length conditions or Dopa-feeding, respectively. Among these clones, the gene with a high level of identity to receptor for activated protein kinase C (RACK) from *Heliothis virescens* is the most dramatically up-regulated under both conditions.

Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs

Hui, DQ; Iqbal, J; Lehmann, K; Gase, K; Saluz, HP; Baldwin, IT. 2003

We extend our analysis of the transcriptional reorganization that occurs when the native tobacco, *Nicotiana attenuata*, is attacked by *Manduca sexta* larvae by cloning 115 transcripts by mRNA differential display reverse transcription-polymerase chain reaction and subtractive **hybridization** using magnetic beads (SHMB) from the *M. sexta*-responsive transcriptome. These transcripts were spotted as cDNA with eight others, previously confirmed to be differentially regulated by northern analysis on glass slide microarrays, and **hybridized** with Cy3- and Cy5-labeled probes derived from plants after 2, 6, 12, and 24 h of continuous attack. Microarray analysis proved to be a powerful means of verifying differential expression; 73 of the cloned genes (63%) were differentially regulated (in equal proportions from differential display reverse transcription-polymerase chain reaction and SHMB procedures), and of these, 24 (32%) had similarity to known genes or putative proteins (more from SHMB). The analysis provided insights into the signaling and transcriptional basis of direct and indirect defenses used against herbivores, suggesting simultaneous activation of salicylic acid-, ethylene-, cytokinin-, WRKY-, MYB-, and oxylipin-signaling pathways and implicating terpenoid-, pathogen-, and cell wall-related transcripts in defense responses. These defense responses require resources that could be made available by decreases in four photosynthetic-related transcripts, increases in transcripts associated with protein and nucleotide turnover, and increases in transcripts associated with carbohydrate metabolism. This putative up-regulation of defense-associated and down-regulation of growth-associated transcripts occur against a backdrop of altered transcripts for RNA-binding proteins, putative ATP/ADP translocators, chaperonins, histones, and water channel proteins, responses consistent with a major metabolic reconfiguration that underscores the complexity of response to herbivore attack.

Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operophtera brumata*

Tikkanen, OP; Julkunen-Tiitto, R. 2003

Phenological synchrony between budburst and emergence of larvae is critical for the fitness of many spring-feeding insect herbivores. Therefore, large intraspecific variation in timing of budburst of the host may have a negative effect on the herbivore. We studied how asynchrony between emergence of larvae and budburst affects the fitness of *Operophtera brumata* (Lepidoptera: Geometridae), a major defoliator of *Quercus robur*, which can adapt to the phenology of a single tree. It is known that, in maturing leaves of *Q. robur*, accumulation of condensed tannins has a negative effect on growth of *O. brumata*. However, there is no information about the effect of hydrolysable tannins and other phenolics that are potential antifeedants. In this study, we also analysed changes in secondary chemistry of the foliage of *Q. robur* and how different compounds are correlated with growth and survival of *O. brumata*. The effect of asynchrony on *O. brumata* was studied in rearing experiments. The neonate larvae were incubated without food for different periods of time. The decline in nutritional quality of foliage was estimated by rearing cohorts of larvae with manipulated hatching times on the leaves of ten individual *Q. robur* trees. For the chemical analysis, the foliage of these trees was sampled at regular intervals. In the absence of foliage, mortality of neonate larvae started to increase exponentially soon after the larvae emerged. If the larvae missed budburst, the decline in nutritional quality of the foliage led to increased mortality and lower body mass (= fecundity). Hydrolysable tannins were not significantly correlated with performance of the larvae. Only condensed tannins were found to correlate negatively with the growth and survival of *O. brumata*. Certain individual trees were unsuitable hosts for *O. brumata* because the decline in quality of the foliage was very rapid. Based on regression equations for increasing rate of mortality and decreasing fecundity, we calculated that a relatively small mismatch of +/-30 degree days between budburst and hatching of larvae leads to a 50% decrease in the fitness of *O. brumata*. Thus, large phenological variation within a *Q. robur* stand can limit the colonisation of neighbouring trees by dispersing larvae. Furthermore, the **hybridisation** of moths adapted to phenologically different trees may lead to maladapted phenology of their offspring.

Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates

Halitschke, R; Gase, K; Hui, DQ; Schmidt, DD; Baldwin, IT. 2003

Evidence is accumulating that insect-specific plant responses are mediated by constituents in the oral secretions and regurgitants (R) of herbivores, however the relative importance of the different potentially active constituents remains unclear. Fatty acid-amino acid conjugates (FACs) are found in the R of many insect herbivores and have been shown to be necessary and sufficient to elicit a set of herbivore-specific responses when the native tobacco plant *Nicotiana attenuata* is attacked by the tobacco hornworm, *Manduca sexta*. Attack by this specialist herbivore results in a large transcriptional reorganization in *N. attenuata*, and 161 genes have been cloned from previous cDNA differential display-polymerase chain reaction and subtractive **hybridization** with magnetic beads analysis. cDNAs of these genes, in addition to those of 73 new R-responsive genes identified by cDNA-amplified fragment-length polymorphism display of R-elicited plants, were spotted on polyepoxide coated glass slides to create microarrays highly enriched in *Manduca* spp.- and R-induced genes. With these microarrays, we compare transcriptional responses in *N. attenuata* treated with R from the two most damaging lepidopteran herbivores of this plant in nature, *M. sexta* and *Manduca quinquemaculata*, which have very similar FAC compositions in their R, and with the two most abundant FACs in *Manduca* spp. R. More than 68% of the genes up- and down-regulated by *M. sexta* R were similarly regulated by *M. quinquemaculata* R. A majority of genes up-regulated (64%) and down-regulated (49%) by *M. sexta* R were similarly regulated by treatment with the two FACs. In contrast, few genes showed similar transcriptional changes after H(2)O(2)- and R-treatment. These results demonstrate that the two most abundant FACs in *Manduca* spp. R can account for the majority of *Manduca* spp.-induced alterations of the wound response of *N. attenuata*.

Mimicry: developmental genes that contribute to speciation

Naisbit, RE; Jiggins, CD; Mallet, J. 2003

Despite renewed interest in the role of natural selection as a catalyst for the origin of species, the developmental and genetic basis of speciation remains poorly understood. Here we describe the genetics of Mullerian mimicry in *Heliconius cydno* and *H. melpomene* (Lepidoptera: Nymphalidae), sister species that recently diverged to mimic other *Heliconius*. This mimetic shift was a key step in their speciation, leading to pre- and postmating isolation. We identify 10 autosomal loci, half of which have major effects. At least eight appear to be homologous with genes known to control pattern differences within each species. Dominance has evolved under the influence of identifiable "modifier" loci rather than being a fixed characteristic of each locus. Epistasis is found at many levels: phenotypic interaction between specific pairs of genes, developmental canalization due to polygenic modifiers so that patterns are less sharply defined in **hybrids**, and overall fitness through ecological selection against nonmimetic **hybrid** genotypes. Most of the loci are clustered into two genomic regions or "supergenes," suggesting color pattern evolution is constrained by preexisting linked elements that may have arisen via tandem duplication rather than having been assembled by natural selection. Linkage, modifiers, and epistasis affect the strength of mimicry as a barrier to gene flow between these naturally **hybridizing** species and may permit introgression in genomic

regions unlinked to those under disruptive selection. Mullerian mimics in *Heliconius* use different genetic architectures to achieve the same mimetic patterns, implying few developmental constraints. Therefore, although developmental and genomic constraints undoubtedly influence the evolutionary process, their effects are probably not strong in comparison with natural selection.

Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera : Noctuidae)

Prowell, DP; McMichael, M; Silvain, JF. 2004

Two genetically differentiated forms of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), use different graminaceous host plants, coexist sympatrically throughout their ranges, yet seem to **hybridize**. To address the taxonomic status of the two forms, determine extent and directionality of **hybridization**, and examine host specificity, we compiled multilocus genotypes consisting of mitochondrial DNA (mtDNA) haplotypes, an esterase locus, and eight amplified fragment-length polymorphism (AFLP) loci in moths collected across a broad geographic range. Multilocus analyses indicated 16% of individuals sampled were potentially **hybrids** with a minority being F₁ in origin. Analysis of host use indicated asymmetries in host specificity with one strain specific to corn, *Zea mays* L., and the other strain predominating on pasture grasses and rice, but occasionally using corn. Location of **hybrids** in nature was biased toward cornfields, the habitat used by both strains. To assess genetic divergence of each gene, we calculated their relative strain discriminating ability. Eight AFLP loci collectively had the greatest discriminating power (98%), followed by a single AFLP locus (93%) and mtDNA (91%). Esterase exhibited 89% discrimination. Esterase is X-linked along with an assortative mating trait, suggesting esterase differentiation may be maintained by association with strain-specific fitness genes. Despite strong discrimination of these genes, most of the genome surveyed was not distinct. Cytonuclear comparisons provided evidence for unidirectional matings consistent with mate preference studies. Collectively, these data support introgressive **hybridization** between recently evolved species that are not completely reproductively isolated. Genetic divergence in the presence of gene flow may be a common phase in the speciation process, especially in taxa whose ranges have been altered dramatically by humans.

Genetic (RAPD) diversity between *Oleria onega agarista* and *Oleria onega* ssp (Ithomiinae, Nymphalidae, Lepidoptera) in north-eastern Peru

Gallusser, S; Guadagnuolo, R; Rahier, M. 2004

Oleria onega agarista Felder and Felder and *Oleria onega* ssp. nov. are two Ithomiinae subspecies from northeastern Peru, that differ for some morphological and behavioural traits. Two contact zones are known near the town of Tarapoto: Ahuashiyacu, where both subspecies cohabit but do not seem to **hybridise**, and Estero (near the village of Shapaja), where they apparently **hybridise**. Genetic differences between the two subspecies and between populations were investigated with random amplified polymorphic DNA (RAPD) markers. Both Cluster and Principal Coordinates Analyses (CCoA and PCoA) performed using these data, provided a clear but weak discrimination between the two subspecies. Genetic diversity is much higher within the populations than between them. Moreover, the geographically more distant populations are grouped together by the genetic data. Morphological traits on the wing patterns of the **hybrids** are intermediary between the two butterflies subspecies, while RAPDs data place them closer to *O. onega agarista* than to *O. onega* ssp. The individuals of the Ahuashiyacu population are clearly separated into two groups, those of *O. onega* ssp. and *O. onega agarista*, by both morphology and RAPDs data. Moreover, none of those individuals show RAPD similarity with the **hybrids**, suggesting that **hybridisation** has not occurred in this population.

A genomic BAC library and a new BAC-GFP vector to study the holocentric pest *Spodoptera frugiperda*

d'Alencon, E; Piffanelli, P; Volkoff, AN; Sabau, X; Gimenez, S; Rocher, J; Cerutti, P; Fournier, P. 2004

Two genomic tools for the study of Lepidoptera and the holocentric structure of their chromosomes are presented in this paper. A bacterial artificial chromosome (BAC) library was constructed using nuclear DNA partially digested with HindIII from eggs of *Spodoptera frugiperda*. The library contains a total of 36 864 clones with an average insert size of 125 kb, which corresponds to approximately 11.5 genome equivalents. Hybridization screening of the library was performed with eight single-copy genes, giving an average hit of 10 clones per marker gene. Colinearity between the genome and BACs was demonstrated at the triose phosphate isomerase (tpi) locus. Probing of the library with a PCR fragment internal to the 18S ribosomal gene allowed an estimation of the rDNA locus size close to 115 repeats per haploid genome. A new vector (pBAC3.6eGFP) for transient transfection into *S. frugiperda* cell lines has been constructed. It is based on the BAC vector, pBAC3.6e, in which a gene encoding GFP was inserted under the control of the densovirus P9 promoter. This vector has the advantage to accommodate large genomic inserts and to be transfected in a large lepidopteran host range. It was used to construct a second BAC library from Sf9 cell nuclear DNA in order to allow a comparison between somatic and cell line genome organization. (C) 2003 Elsevier Ltd. All rights reserved.

Genomic evidence for divergence with gene flow in host races of the larch budmoth

Emelianov, I; Marec, F; Mallet, J. 2004

Ecological divergence in the face of gene flow has recently become implicated as a potentially important cause of speciation and adaptive radiation. Here, we develop a genomic approach to test for divergent selection in sympatric host races of the larch budmoth *Zeiraphera diniana* (Lepidoptera: Tortricidae). We analysed hundreds of amplified fragment length polymorphism markers in 92 individuals in sympatric and allopatric populations, and in two backcross broods used to map the markers to individual chromosomes. The results directly confirm the existence of natural **hybridization** and demonstrate strong heterogeneity between chromosomes in terms of molecular divergence between host races (the average level of divergence was $F_{ST} = 0.216$). However, genomic heterogeneity was not found when we analysed divergence between geographically separated populations of the same host race. We conclude that the variance of the level of sympatric divergence among chromosomes is the footprint of divergent selection acting on a few linkage groups, combined with appreciable gene flow that homogenizes between-race variation at the remaining linkage groups. These results, coupled with other recent multilocus analyses of sister species pairs, demonstrate that selection-driven sympatric phase of genetic divergence in the presence of gene flow is a likely feature of speciation.

Discovery of a factor limiting yields in a coconut plantation on peat: The insect pest *Sufetula* spp.

Bonneau, X; Husni, M; Philippe, R; Somchit, N; Jourdan, C; Lubis, N. 2004

On a large coconut plantation planted on a deep peat soil in Sumatra, Indonesia, **hybrid** coconut yields are stabilizing at values well below their potential, even in plots where known production factors have been mastered since the outset: land preparation, water control, and mineral fertilization. To explain this situation, five hypotheses have been proposed. They were tested one by one in field trials. The authors have demonstrated that the main reason for stabilized yields was the Lepidoptera root pest *Sufetula* spp. and conclude by considering lines of research for controlling this pest in the context of rehabilitation and replanting.

Mimicry and the evolution of premating isolation in *Heliconius melpomene* Linnaeus

Jiggins, CD; Estrada, C; Rodrigues, A. 2004

Ecological divergence can cause speciation if adaptive traits have pleiotropic effects on mate choice. In *Heliconius* butterflies, mimetic patterns play a role in mate detection between sister species, as well as signalling to predators. Here we show that male butterflies from four recently diverged parapatric populations of *Heliconius melpomene* are more likely to approach and court their own colour patterns as compared with those of other races. A few exceptions, where males were more attracted to patterns other than their own, suggest that some mimetic patterns are sub-optimal in mate choice. Genotype frequencies in **hybrid** zones between races of *H. melpomene* suggest that mating is random, so reinforcement is unlikely to have played a role in intra-specific divergence. In summary, co-evolved divergence of colour pattern and mate preference occurs rapidly and is likely the first step in *Heliconius* speciation.

Polymorphism in syntopic populations of *Colias crocea* and *C. erate* (Lepidoptera, Pieridae) in the Crimea

Milovanov, AE; Simchuk, AP; Ivashov, AV. 2004

Parallel rows of Completely homologous mutable forms were revealed in syntopic populations of *Colias crocea* and *C. erate*. The studies were based on the examination of genitalia in three serial samplings (total sampling size is 354 specimens: 294 - *C. crocea* Fourc. males and 60 - *C. erate* Esp. males Vic Simferopol, 1997-2000) from the same habitat in the forest-steppe Crimea. The outline of the valve margin is considered as a diagnostic criterion for the identification of species. The electrophoretic testing of 12 allozyme loci in two serial samplings (2000 and 2002) showed statistically significant differences between *C. crocea* and *C. erate* only in the NadH-dehydrogenase locus. These species differed to a lesser degree in the aldehyde-dehydrogenase locus. Paratypical form (**hybrids**) of both species differed from *C. erate* neither by the level of heterozygosity nor by frequencies of rare alleles. A genetic model and adaptive importance of polymorphism in *C. crocea* and *C. erate* is under discussion.

The shape of endemics: Notes on male and female genitalia in the genus *Maniola* (Schrank, 1801), (Lepidoptera, Nymphalidae, Satyrinae)

Grill, A; de Vos, R; van Arkel, J. 2004

Butterflies of the genus *Maniola* are known for their large morphological variation, at the inter- as well as intraspecific level. Given the overlap in wing-patterns, habitat selection, and geographic distribution of various *Maniola* species, genitalia morphology is sometimes the only possibility to tell specimen apart. In this paper we describe diagnostic characters to distinguish different *Maniola* species by means of their genitalia. Included is also the first detailed description and illustration of the genitalia apparatus of the Sardinian endemic *Maniola nurag*. Further, we describe two Sardinian individuals with intermediate characteristics between *Maniola nurag* and *Maniola jurtina*, and propose that they are **hybrids**. Further, we shortly discuss the justification of the species status for the island endemics *Maniola chia* and *Maniola cypricola*.

Hybridization between *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera : Noctuidae): development and morphological characterization of F-1 hybrids

Zhao, XC; Dong, JF; Tang, QB; Yan, YH; Gelbic, I; Van Loon, JJA; Wang, CZ. 2005

Reciprocal **hybridizations** between *Helicoverpa armigera* (Hubner) and *Helicoverpa assulta* (Guenée) were studied. The cross between females of *H. armigera* and males of *H. assulta* yielded only fertile males and sterile individuals lacking an aedeagus, valva or ostium bursae. A total of 492 larvae of the *H.* generation were obtained and 374 of these completed larval development and pupated. Only 203 pupae were morphologically normal males, the remaining 171 pupae were malformed. Larvae and pupae that gave rise to morphologically abnormal adults exhibited longer development times. Sterility was not only associated with malformed external sex organs, but also a range of abnormalities of the internal reproductive system: (i) loss of internal reproductive organs, (ii) with one to three copies of an undeveloped bursa copulatrix; or (iii) with one or two undeveloped testes. Normal male **hybrid** adults showed higher flight activity in comparison with males of both species. In contrast, the cross between females of *H. assulta* and males of *H. armigera* yielded morphologically normal offspring (80 males and 83 females). The interaction of the Z-chromosome from *H. assulta* with autosomes from *H. armigera* might result in morphological abnormalities found in **hybrids** and backcrosses, and maternal-zygotic incompatibilities might contribute to sex bias attributed to **hybrid** inviability.

Hybrid incompatibility is consistent with a hybrid origin of *Heliconius heurippa* Hewitson from its close relatives, *Heliconius cydno* Doubleday and *Heliconius melpomene* Linnaeus

Salazar, CA; Jiggins, CD; Arias, CF; Tobler, A; Bermingham, E; Linares, M. 2005

Shared ancestral variation and introgression complicates the reconstruction of phylogenetic relationships among closely related taxa. Here we use overall genomic compatibility as an alternative estimate of species relationships in a group where divergence is rapid and genetic exchange is common. *Heliconius heurippa*, a butterfly species endemic to Colombia, has a colour pattern genetically intermediate between *H. cydno* and *H. melpomene*: its hindwing is nearly indistinguishable from that of *H. melpomene* and its forewing band is an intermediate phenotype between both species. This observation has led to the suggestion that the pattern of *H. heurippa* arose through **hybridization**. We present a genetic analysis of **hybrid** compatibility in crosses between the three taxa. *Heliconius heurippa* x *H. cydno* and female *H. melpomene* x male *H. heurippa* yield fertile and viable F-1 **hybrids**, but male *H. melpomene* x female *H. heurippa* crosses yield sterile F-1 females. In contrast, Haldane's rule has previously been detected between *H. melpomene* and *H. cydno* in both directions. Therefore, *H. heurippa* is most closely related to *H. cydno*, with some evidence for introgression of genes from *H. melpomene*. The results are compatible with the hypothesis of a **hybrid** origin for *H. heurippa*. In addition, backcrosses using F-1 **hybrid** males provide evidence for a large Z(X)-chromosome effect on sterility and for recessive autosomal sterility factors as predicted by Dominance Theory.

Phylogenetic evaluation of the taxonomic status of *Timandra griseata* and *T. comae* (Lepidoptera : Geometridae : Sterrhinae)

Ounap, E; Viidalepp, J; Saarma, U. 2005

The sterrhine loopers *Timandra griseata* and *T. comae* have been treated as distinct species since 1994. However, morphological differences between the taxa are minor and therefore their status has often been disputed. Here, we present a molecular phylogenetic study, which separates *T. griseata* and *T. comae* into different clades. Altogether, 43 *Timandra* specimens from eight European countries were studied. The phylogeny is based on a comparative sequence analysis of mitochondrial genes coding for the cytochrome C oxidase subunit 1 (COI) and NADH dehydrogenase subunit 1 (ND1). Nevertheless, a single individual of both species was assigned to the "wrong" clade. The symplesiomorphy of *T. griseata* and *T. comae* is considered to be a result of introgressive **hybridization**. Conditions that could lead to the **hybridization** of *T. griseata* and *T. comae* are discussed, as well as the likely distribution history of these taxa in Northern Europe. Results of the current analysis are in favour of retaining the species status of *T. griseata* and *T. comae*.

Ecological speciation without host plant specialization; possible origins of a recently described cryptic *Papilio* species

Scriber, JM; Ording, GJ. 2005

North American *Papilio canadensis* and *P. glaucus* (Lepidoptera: Papilionidae, these *Papilio* = *Pterourus*) have previously been described as having allopatric distributions separated by a narrow **hybrid** zone running from Minnesota to southern New England, and southward in the Appalachian Mountains (possibly to northern Georgia). Recent patterns of **hybridization** and introgression suggest a more complex interaction between the two, possibly even resulting in the formation of a new species (*Pterourus appalachiensis* Pavulaan & Wright, 2002). Recently, extensive northward interspecific introgression of *P. glaucus*-diagnostic traits has been observed in the **hybrid** zone. These include wing bands and other color patterns, the ability to feed on tulip tree leaves, and Hk-100 allozymes; all are autosomally encoded. However, there has been little northward introgression of certain other *P. glaucus* traits (such as facultative diapause and bivoltinism, and Ldh-100 allozymes, both X-linked; and the Y-linked melanic mimicry gene in females). Interspecific recombination of the X-chromosome has evidently occurred, as shown by discordant patterns of X-linked markers. The *P. glaucus* X-linked Pgd-100 and Pgd-50 alleles have introgressed 200-400 km north of the historical **hybrid** zone, yet the *P. glaucus* X-linked Ldh-100 allele has not. The allele frequency shift for both genes is more closely related to the 'thermal landscape' (i.e., accumulated degree-days above a developmental base threshold of 50 degrees F (=10 degrees C)) than to latitude. Delayed post-diapause eclosion of cohorts within the **hybrid** zone, e.g., the New York/Vermont border area, has produced a natural 'false-second generation' flight (a **hybrid** swarm of synchronous males and females, where 2300-2700 degrees F degree-days have accumulated each year since 1998) that is reproductively isolated from flights of both parental species. Moreover, the newly described *P. appalachiensis* exhibits a unique combination of traits. These include obligate diapause, a univoltine habit, and the Ldh-80 or Ldh-40 alleles (as for *P. canadensis*), the Pgd-100 or Pgd-50 alleles (as for *P. glaucus*), and a delayed 'false-second generation' reproductive flight period (as observed in the **hybrid** zone). Since 2001, a rare allele or 'hybrizyme' (Ldh-20) has appeared in this false second generation at high frequencies (40-50%). We hypothesize that strong selection against the facultative diapause (od-)trait (and the linked Ldh-100 allele) in regions with 2800 degrees F degree-days or less, and divergent selection in favor of Pgd-100 (or a closely linked trait) combined with allochronic reproductive isolation, has resulted in recombinational, parapatric, **hybrid** speciation. There is no evidence at present that host-plant shifts or changes in sex pheromones have driven this process, in contrast to many other speciation events in the Lepidoptera.

Independent inheritance of preference and performance in hybrids between host races of *Mitoura* butterflies (Lepidoptera : Lycaenidae)

Forister, ML. 2005

Divergent natural selection contributes to reproductive isolation among populations adapting to different habitats or resources if **hybrids** between populations are intermediate in phenotype and suffer an associated, environmentally dependent reduction in fitness. This prediction was tested using two host races of *Mitoura* butterflies. Thirtyfive F-1 **hybrid** and parental lines were created, larvae were raised on the two host plants, and oviposition preferences were assayed in choice arenas. Larvae from both reciprocal **hybrid** crosses suffered a host-specific reduction in performance: when reared on incense cedar, **hybrid** survival was approximately 30% less than the survival of pure lines of the cedar-associated host race. The performance of **hybrid** larvae reared on the other host, MacNab cypress, was not reduced relative to parental genotypes. Females from both reciprocal **hybrid** crosses preferred to oviposit on incense cedar, the same host that resulted in the reduced survival of **hybrid** larvae. Thus, dominance is implicated in the inheritance of traits involved in both preference and performance, which do not appear to be genetically linked in *Mitoura* butterflies. Gene flow between host races may be reduced because the correlation between preference and performance that was previously described in parental populations is essentially broken by **hybridization**.

Inheritance and plasticity of adult host acceptance in *Yponomeuta* species: implications for host shifts in specialist herbivores

Hora, KH; Roessingh, P; Menken, SBJ. 2005

Changes in host acceptance is an important factor in the host specialization of phytophagous insects, and knowledge of the genetic organization of this behaviour is necessary in order to understand how host shifts occur. Here we describe the inheritance of adult host acceptance (oviposition) in three closely related species of *Yponomeuta* Latreille (Lepidoptera: Yponomeutidae), and their interspecific **hybrids**. *Yponomeuta cagnagellus* (Hubner), a specialist on *Euonymus europaeus* L. (Celastraceae), *Y. malinellus* Zeller, a specialist on *Malus* spp. (Rosaceae), and *Y. padellus* (L.), oligophagous on a number of Rosaceae, were tested for their acceptance of parental hosts in choice tests. Acceptance of *E. europaeus* is semi-dominant in **hybrids** of *Y. cagnagellus* x *Y. padellus*, and in **hybrids** of *Y. cagnagellus* x *Y. malinellus*. The dominance of this acceptance was confirmed in oviposition tests with backcross **hybrids**: backcross **hybrids** F1 x *Y. cagnagellus* oviposited mainly on *E. europaeus* and F1 x *Y. padellus* still deposited more than half of their egg masses on *E. europaeus*. Reciprocal **hybrids** did not differ in their host acceptance, indicating that the trait is autosomal. We further studied the effect of larval food on adult host acceptance ('Hopkins host selection principle') in split full-sib F1 families. Larval diet influenced oviposition only in one of two **hybrid** crosses. The F1 **hybrid** of *Y. padellus* x *Y. cagnagellus*, reared on *Prunus spinosa* L., deposited a significantly lower percentage of egg masses on *E. europaeus* compared to their full-sib sisters fed with *E. europaeus*. We did not find this in the reciprocal cross. However, still more than half of the egg masses are deposited on *E. europaeus* by **hybrids** that have no experience on this host. We conclude that the semi-dominant character of acceptance of *E. europaeus* and a tendency of Rosaceae-feeding *Yponomeuta* to deposit egg masses on this host may have created the opportunity for the host shift of the predecessor of *Y. cagnagellus* from Rosaceae to the Celastraceae. This shift may have been further facilitated by a weak tendency of adults to oviposit on their larval food source.

Response of *Danaus plexippus* to pollen of two new Bt corn events via laboratory bioassay

Mattila, HR; Sears, MK; Duan, JJ. 2005

Laboratory bioassays were conducted to evaluate the response of first instar larvae of the monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Danaidae), a non-target species, to pollen from corn, *Zea mays* L. (Commelinales: Poaceae), from two new corn **hybrids** genetically modified to express different types of insecticidal proteins derived from the bacterium *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Bt). One **hybrid** expresses both Cry1Ab and Cry2Ab2 proteins (MON 810 x MON 84006), active against lepidopteran pests, and the other expresses Cry3Bb1 protein (MON 863), targeted against coleopteran pests. First instar larvae were placed on milkweed leaves (*Asclepias syriaca* L.) (Gentianales: Asclepiadaceae) dusted with doses of either Bt pollen or its nonexpressing (isoline) pollen counterpart ranging from 50 to 3200 grains cm⁻² of milkweed leaves, or no pollen at all. Larvae were exposed to pollen for 4 days, then moved to pollen-free leaves and observed for another 6 days. Survival was observed after 2, 4, and 10 days. Weight gain was estimated after 4 and 10 days, leaf consumption after 2 and 4 days, and larval development after 10 days. Exposure to pollen of the Cry1Ab/Cry2Ab2-Bt expressing **hybrid** reduced larval survival approximately 7.5-23.5% at the dose ranges tested relative to a no pollen control. Larval weight gain and consumption were reduced for larvae exposed to pollen of this **hybrid** and a small minority of larvae (3.1%) never developed past the third instar after 10 days of observation. Exposure to pollen of the Cry3Bb1-Bt expressing **hybrid** had no negative effects on larval mortality, weight gain, consumption, or development relative to the consumption of Bt-free corn pollen. The relevance of these findings to the risk that these Bt corn **hybrids** pose to monarch populations is discussed.

A molecular phylogeny of the hawkmoth genus *Hyles* (Lepidoptera : Sphingidae, Macroglossinae)

Hundsdoerfer, AK; Kitching, IJ; Wink, M. 2005

The hawkmoth genus *Hyles* is one of 15 genera in the subtribe Choerocampina of the subfamily Macroglossinae. Due to a remarkable uniformity, morphological characters usually used to identify and classify Lepidoptera at the species level cannot be used in this genus. Instead, we used DNA sequences comprising about 2300 bp derived from the mitochondrial genes COX 1, COX 11, and tRNA-leucine to elucidate the phylogeny of *Hyles*. The results corroborate the monophyly of *Hyles* but conflict with previous internal classifications of the genus based on morphology. *Hyles* seems to have evolved in the Neotropics during the Oligocene/Eocene epochs and the molecular data (which evolved clock-like) confirm the hypothesis that it is a very young genus that radiated on a global scale rather quickly. We hypothesize its sister group to be one of the genera *Deilephila*, *Theretra* or *Xylophanes*. The Nearctic may have been colonized rapidly by *Hyles* once the land bridge

formed during the Pliocene, since within this same Epoch, the invasion of the Palaearctic appears to have proceeded from the East, via the Bering route. The colonization of Australia appears to have occurred rather early in Hyles radiation, although the route is not clear. We propose that the radiation of the Hyles euphorbiae-complex s. str. (HEC) occurred as recently as the Pliocene/Pleistocene boundary and that its roots can still be reconstructed in Asia. Hyles dahlia is closely related to the HEC, but a sister group relationship to the HEC s. str. cannot be corroborated unequivocally. HEC population ranges appear to have tracked climate oscillations during the Pleistocene Ice Ages, resulting in **hybridization** around the Mediterranean Sea as they repeatedly intermingled. Comparison of the phylogeny with food plant affiliations leads us to hypothesize that Euphorbia monophagy evolved at least two times independently within Hyles. (c) 2005 Elsevier Inc. All rights reserved.

Survey results for the sugarcane pest, *Blastobasis graminea* (Lepidoptera : Coleophoridae), in Texas and Louisiana in 2002

White, WH; Adamski, D; Brown, J; Reagan, TE; Villanueva-Jimenez, JA; Mendez-Lopez, M; Way, MO. 2005

Recent discoveries of *Blastobasis graminea* Adamski (Lepidoptera: Coleophoridae) in Mexico have prompted interests that this insect pest, originally discovered in South America, may be moving northward. A survey in Texas and Louisiana was conducted in 2002 to determine if *B. graminea* has extended its range into the U.S. Surveys included five nights of blacklight trapping in Texas and three nights of blacklight trapping plus diurnal surveys of 23 fields in Louisiana. Field surveys in Louisiana included examination not only of sugarcane (interspecific **hybrids** of *Saccharum* spp.) but also of maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.) Moench, as well as non-cultivated gramineous species. We did not collect *B. graminea* during blacklight trappings nor did we detect it in association with cultivated host species (i.e., sugarcane, maize, and sorghum). We did, however, discover *B. graminea* in smooth cordgrass, *Spartina alterniflora* Loisel., in Louisiana in a non-agricultural environment. Finding *B. graminea* only in cordgrass suggests the possibility that rather than expanding its geographic range, *B. graminea* may be a widespread tropical species that is expanding its host range from native grasses to sugarcane. Finding *B. graminea* in Louisiana represents a new U.S. record for the species. Also, from this survey, new U.S. hosts records for *Pyroderces badia* (Hodges) (Lepidoptera: Cosmopterigidae) and *Dicymolomia julianalis* (Walker) (Lepidoptera: Crambidae), moths reared from grain sorghum seedheads, are documented.

The lesser wanderer butterfly, *Danaus petilia* (Stoll 1790) stat. rev. (Lepidoptera : Danainae), reinstated as a species

Lushai, G; Zalucki, MP; Smith, DAS; Goulson, D; Daniels, G. 2005

The lesser wanderer butterfly, *Danaus* (Anosia) *chrysippus petilia* (Stoll) (Lepidoptera: Danainae), has been treated as a subspecies for the last 100 years. New mitochondrial DNA sequence data for *D. petilia*, in conjunction with allozyme, structural, morphometric and pattern characters, constitute a compelling case for its specific rank. The holotype of *D. petilia* has never been located and, as the type location is uncertain, a neotype is designated. Fresh material was collected and examined for this project. *Danaus petilia* and *D. chrysippus* have been separated at Lydekker's Line for an estimated 1.1 million years, and they remain interfertile. However, as an allopatric taxon, diagnosable from *D. chrysippus*, *D. petilia* merits specific status under the phylogenetic species concept. The following pairs of *D. chrysippus* subspecies are considered to be synonyms, the first member of each pair having priority: *chrysippus* + *aegyptius* (subspecies), *klugii* + *infumata* (**hybrid** phenotypes) and *orientis* + *liboria* (subspecies).

Diapause and voltinism in western and 2-year-cycle spruce budworms (Lepidoptera : Tortricidae) and their hybrid progeny

Nealis, VG. 2005

Breeding experiments and rearing under variable controlled conditions have revealed that western and 2-year-cycle spruce budworms (*Choristoneura occidentalis* Freeman and *C. biennis* Freeman, respectively) from British Columbia, Canada, and their **hybrid** progeny have the inherent capacity for a variable number of diapause events and hence voltinism. While all crosses have at least one diapause, variability in the relative frequency of a second diapause is determined by genetic traits modified by the photoperiod and, to a lesser extent, temperature experienced during the larval stages. Second diapause appears fixed in *C. biennis* but is facultative and most frequent at short photophases (121:12D) in *C. occidentalis*. Hybrids and backcrosses had responses intermediate to the parental responses under all environmental conditions. The occurrence of a facultative third diapause in all crosses underlines the inherent capacity for flexibility in voltinism in these species. These results are discussed in the context of past, present, and future distributions of alternative life cycles in closely related species.

Impact of pollen grains from Bt transgenic corn on the growth and development of Chinese tussah silkworm, *Antheraea pernyi* (Lepidoptera : Saturniidae)

Li, WD; Wu, KM; Wang, XQ; Wang, GR; Guo, YY. 2005

The tussah silkworm, *Antheraea pernyi* (Lepidoptera: Saturniidae), is an important natural resource for the silk industry and has been cultured using wild host plants for > 2,000 yr in China. To clarify whether there is any risk from pollen of Cry1Ab-containing corn varieties on this insect, the frequency of pollen dispersal and deposition of corn pollen near cornfields and impact on the development of tussah silkworm larvae were studied separately in the field and laboratory. The field survey showed that the pollen density was the highest inside the cornfield with a value of approximate to 1,000 grains/cm². The pollen deposition rapidly declined with distance from the edge of the cornfield as expected in most cases. No significant differences were observed in the amounts of pollen deposited on glass slides positioned at different heights from the ground at each distance. In the laboratory bioassays, there were no significant differences in the larval mortality and weight of Chinese tussah silkworm between treatment with pollen grains from a transgenic corn line and a nontransgenic corn control at a density of 1,000 pollen grains/cm². Also no significant negative impact was found for efficiency of conversion of digested food (ECD), efficiency of conversion of ingested food (ECI), and efficiency of approximate digestion of food (AD) at the level of 1,000 pollen grains/cm². The results of this study suggest that the impact on the Chinese tussah silkworm of Bt corn pollen from the **hybrid** to be commercialized in China is negligible in the natural environment.

Individual sequences in large sets of gene sequences may be distinguished efficiently by combinations of shared sub-sequences

Gibbs, MJ; Armstrong, JS; Gibbs, AJ. 2005

Background: Most current DNA diagnostic tests for identifying organisms use specific oligonucleotide probes that are complementary in sequence to, and hence only **hybridise** with the DNA of one target species. By contrast, in traditional taxonomy, specimens are usually identified by 'dichotomous keys' that use combinations of characters shared by different members of the target set. Using one specific character for each target is the least efficient strategy for identification. Using combinations of shared bisectially-distributed characters is much more efficient, and this strategy is most efficient when they separate the targets in a progressively binary way. Results: We have developed a practical method for finding minimal sets of sub-sequences that identify individual sequences, and could be targeted by combinations of probes, so that the efficient strategy of traditional taxonomic identification could be used in DNA diagnosis. The sizes of minimal sub-sequence sets depended mostly on sequence diversity and sub-sequence length and interactions between these parameters. We found that 201 distinct cytochrome oxidase subunit-1 (CO1) genes from moths (Lepidoptera) were distinguished using only 15 sub-sequences 20 nucleotides long, whereas only 8-10 sub-sequences 6-10 nucleotides long were required to distinguish the CO1 genes of 92 species from the 9 largest orders of insects. Conclusion: The presence/absence of sub-sequences in a set of gene sequences can be used like the questions in a traditional dichotomous taxonomic key; **hybridisation** probes complementary to such sub-sequences should provide a very efficient means for identifying individual species, subtypes or genotypes. Sequence diversity and sub-sequence length are the major

factors that determine the numbers of distinguishing sub-sequences in any set of sequences.

Verification of a European corn borer (*Lepidoptera* : *Crambidae*) loss equation in the major corn production region of the northeastern United States

Dillehay, BL; Calvin, DD; Roth, GW; Hyde, JA; Kulda, GA; Kratochvil, RJ; Russo, JM; Voight, DG. 2005

Field studies in Pennsylvania and Maryland were conducted during 2000, 2001, and 2002 to test the applicability of published yield loss relationships developed in central Pennsylvania for European corn borer, *Ostrinia nubilalis* (Hubner), management in warmer, longer season corn, *Zea mays* L., grain production regions of the northeastern United States. Both isoline **hybrids** and non-Bt lead **hybrids** were compared against *Bacillus thuringiensis* (Bt) **hybrids** to measure effects of the pest on yield. The European corn borer economic analysis model was used to make site-specific predictions of loss per European corn borer larva for comparison with measured yield loss per larva. Although the model did not predict loss per larva at a field level, it did predict loss at a regional level. The model predicted an overall percentage of yield loss per larva of 2.69 +/- 0.12% over the region, which was similar to the measured yield loss per larva of 2.66 +/- 0.59% for isoline **hybrids** and 3.08 +/- 0.51% for lead **hybrids**. The model, on average, provided a good prediction of percentage of yield loss per larva within the climatic zones of 1100-1700 degree-days (DD) (base threshold 12.5 degreesC). Our results suggest that the yield loss relationship developed in Central Pennsylvania, when matched to the timing of third instar second generation European corn borer stalk tunneling is adequate for major corn grain production zones of the northeast United States.

Clinal variation in populations of the common blue butterfly *Polyommatus icarus* Rott. (*Lepidoptera*, *Lycaenidae*)

Artemyeva, EA. 2005

Geographical trends in variation of wing pattern characters of the common blue butterfly *Polyommatus icarus* Rott. have been established. Peripheral populations were shown to be more rich and diverse phenotypically than those of the central parts of the species range. Phenotypic diversity of the populations increased from the center to the periphery of the range. The range boundaries were characterized by strong fluctuations of frequencies of wing pattern phenotypes. Hybrid areas were found on the part of the range examined.

Strikingly variable divergence times inferred across an Amazonian butterfly 'suture zone'

Whinnett, A; Zimmermann, M; Willmott, KR; Herrera, N; Mallarino, R; Simpson, F; Joron, M; Lamas, G; Mallet, J. 2005

'Suture zones' are areas where **hybrid** and contact zones of multiple taxa are clustered. Such zones have been regarded as strong evidence for allopatric divergence by proponents of the Pleistocene forest refugia theory, a vicariance hypothesis frequently used to explain diversification in the Amazon basin. A central prediction of the refugia and other vicariance theories is that the taxa should have a common history so that divergence times should be coincident among taxa. A suture zone for Ithomiinae butterflies near Tarapoto, NE Peru, was therefore studied to examine divergence times of taxa in contact across the zone. We sequenced 161.9 bp of the mitochondrial COI/COII region in 172 individuals of 31 species from across the suture zone. Inferred divergence times differed remarkably, with divergence between some pairs of widespread species (each of which may have two or more subspecies interacting in the zone, as in the genus *Melinaea*) being considerably less than that between **hybridizing** subspecies in other genera (for instance in *Oleria*). Our data therefore strongly refute a simple hypothesis of simultaneous vicariance and suggest that ongoing parapatric or other modes of differentiation in continuous forest may be important in driving diversification in Amazonia.

Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies

Lukhtanov, VA; Kandul, NP; Plotkin, JB; Dantchenko, AV; Haig, D; Pierce, NE. 2005

The reinforcement model of evolution argues that natural selection enhances pre-zygotic isolation between divergent populations or species by selecting against unfit **hybrids**(1,2) or costly interspecific matings(3). Reinforcement is distinguished from other models that consider the formation of reproductive isolation to be a by-product of divergent evolution(4,5). Although theory has shown that reinforcement is a possible mechanism that can lead to speciation(6-8), empirical evidence has been sufficiently scarce to raise doubts about the importance of reinforcement in nature(6,9,10). *Agrodiaetus* butterflies (*Lepidoptera*: *Lycaenidae*) exhibit unusual variability in chromosome number. Whereas their genitalia and other morphological characteristics are largely uniform, different species vary considerably in male wing colour, and provide a model system to study the role of reinforcement in speciation. Using comparative phylogenetic methods, we show that the sympatric distribution of 15 relatively young sister taxa of *Agrodiaetus* strongly correlates with differences in male wing colour, and that this pattern is most likely the result of reinforcement. We find little evidence supporting sympatric speciation: rather, in *Agrodiaetus*, karyotypic changes accumulate gradually in allopatry, prompting reinforcement when karyotypically divergent races come into contact.

Is the last glaciation the only relevant event for the present genetic population structure of the meadow brown butterfly *Maniola jurtina* (*Lepidoptera* : *Nymphalidae*)?

Schmitt, T; Rober, S; Seitz, A. 2005

Phylogeographical studies are available for a considerable number of European species, but few analyses exist for temperate species with very large and fairly continuous populations that are also absent from Northern Europe. Therefore, we studied the butterfly *Maniola jurtina* as a model for this group. The species has two major genetic lineages (mean genetic distance between lineages: 0.033; F-CT: 0.052), most probably evolving in glacial differentiation centres in the western and eastern Mediterranean. The onset of this differentiation might have been the beginning of the last glacial stage maximum some 40 kyr BP. A **hybrid** zone between these two lineages exists in western Central Europe. No genetic substructures have been found within the two lineages (F-SC: 0.017) and average genetic distances are very small. Therefore, it is highly probable that postglacial expansion was of the phalanx type. There is, at most, very limited differentiation at regional and local scales. However, the genetic diversity within populations is high (means: A: 2.68; H-E: 17.2%; P: 78%), as would be predicted for such a common species. Comparison of these results with a published allozyme analysis revealed a similar phylogeographical pattern, but lower genetic diversity in the latter. Morphological patterns of wings and genitalia show similar geographical patterns as allozyme data. (c) 2005 The Linnean Society of London.

A case of natural hybridization between *Hyalophora cecropia* and *Hyalophora columbia* (*Lepidoptera* : *Saturniidae*) in Nova Scotia

Bridgehouse, DW. 2006

Progeny of a wild female of *Hyalophora cecropia* (Linnaeus) (*Lepidoptera*: *Saturniidae*) were reared and appear to be natural **hybrids** resulting from a mating with *Hyalophora columbia* (Smith).

Multilocus analyses of admixture and introgression among hybridizing *Heliconius* butterflies

Kronforst, MR; Young, LG; Blume, LM; Gilbert, LE. 2006

Introgressive **hybridization** is an important evolutionary process and new analytical methods provide substantial power to detect and quantify it. In this study we use variation in the frequency of 657 AFLP fragments and DNA sequence variation from 15 genes to measure the extent of admixture and the direction of interspecific gene flow among three *Heliconius* butterfly species that diverged recently as a result of natural selection for Mullerian mimicry, and which continue to **hybridize**. Bayesian clustering based on AFLP genotypes correctly delineated the three species and identified four *H. cydno*, three *H. pacheus*, and three *H. melpomene* individuals that were of mixed ancestry. Gene genealogies revealed substantial shared DNA sequence variation among all three species and coalescent simulations based on the Isolation with Migration (IM) model pointed to interspecific gene flow as its cause. The IM simulations further indicated that interspecific gene flow was significantly asymmetrical, with greater gene flow from *H. pacheus* into *H. cydno* ($2Nm = 4.326$) than the reverse ($2Nm = 0.502$), and unidirectional gene flow from *H. cydno* and *H. pacheus* into *H. melpomene* ($2Nm = 0.294$ and 0.252 , respectively). These asymmetries are in the directions expected based on the genetics of wing patterning and the probability that **hybrids** of various phenotypes will survive and reproduce in different mimetic environments. This empirical demonstration of extensive interspecific gene flow is in contrast to a previous study which found little evidence of gene flow between another pair of **hybridizing** *Heliconius* species, *H. himera* and *H. erato*, and it highlights the critical role of natural selection in maintaining species diversity. Furthermore, these results lend support to the hypotheses that phenotypic diversification in the genus *Heliconius* has been fueled by introgressive **hybridization** and that reinforcement has driven the evolution of assortative mate preferences.

Effects of fall armyworm (*Lepidoptera* : *Noctuidae*) interstrain mating in wild populations

Nagoshi, RN; Meagher, RL; Nuessly, G; Hall, DG. 2006

Fall armyworm is a significant agricultural pest in the United States, affecting most notably sweet corn and turfgrass. Two morphologically identical strains, rice strain (R-strain) and corn strain (C-strain), exist that differ physiologically and behaviorally and can be identified by mitochondrial haplotyping. Recent studies of overwintering populations in Florida indicate that the mitochondrial lineage associated with the R-strain itself consists of two genetically distinct subgroups, with one having molecular markers consistent with interstrain **hybridization** between R-strain females and C-strain males. To test this possibility and examine the ramifications of interstrain mating on population behavior and strain fidelity, larvae and adult males were tested for genetic marker combinations representative of the host strains and potential **hybrids**. These Studies showed a sexually dimorphic distribution pattern for a sex-linked marker that is a predicted result of interstrain mating. Despite evidence of substantial interbreeding in the overwintering sites, both FR and the strain-diagnostic mitochondrial markers still showed the plant host and habitat biases associated with the host strains, indicating that strain integrity was largely maintained. However, there is evidence that the two R-strain subpopulations differ in habitat distribution in a manner suggestive of the "**hybrid**" genotype being less specific in its plant host preference. The existence of a genetically distinct **hybrid** subpopulation must be taken into account when evaluating fall armyworm population dynamics and infestation patterns in overwintering areas.

An endemic population of western poplar clearwing moths (*Lepidoptera* : *Sesiidae*) invades a monoculture of hybrid poplar

Brown, JJ; Kittelson, NT; Hannon, ER; Walsh, DB. 2006

Western poplar clearwing, *Paranthrene robiniae* (Hy. Edwards) (*Lepidoptera*: *Sesiidae*), is endemic in Pacific Northwest riparian habitats at low population densities. These moths have colonized commercial **hybrid** poplar (*Populus* spp.) plantings. Moth populations increased rapidly and became a major pest. Trap catches of male moths in mid-season surveys increased 190-fold from 95 in 2001 to > 18,500 in 2002 across 6,597 ha of poplars monitored. The outbreak of western poplar clearwings was widespread in 2002. Pheromone-baited traps placed one trap per 81.75 ha over 13,274 ha of commercial poplars captured > 108,000 male moths in 2002. Damage to commercial poplars included girdling of saplings and burrows in limbs and trunks of trees. Repeated applications of chlorpyrifos failed to reduce the abundance of moths in 2002. Two management strategies over two separate plantations of approximately 6,500 ha each were contrasted. Future control strategies recommend a halt to the use of contact insecticides that target adult moths. Short-term (3-5 yr) control should involve a pheromone-based mating disruption strategy followed eventually by selection of a clone that is less susceptible to *P. robiniae* attack.

Genetic analysis of larval host-plant preference in two sibling species of *Helicoverpa*

Tang, QB; Jiang, JW; Yan, YH; van Loon, JJA; Wang, CZ. 2006

The genetic basis of larval host-plant preference was investigated in reciprocal F-1, F-2, and backcrossed generations derived from **hybrid** crosses between the generalist species *Helicoverpa armigera* (Hubner) and the closely related specialist species *Helicoverpa assulta* (Guenee) (*Lepidoptera*: *Noctuidae*). Host-plant preference for cotton [*Gossypium arboreum* L. (*Malvaceae*)] and pepper [*Capiscum frutescens* L. (*Solanaceae*)] of fifth-instar caterpillars was tested by using a two-choice leaf-disk assay. *Helicoverpa armigera* and *H. assulta* were significantly different in their feeding preferences, but the difference was not significant in the reciprocal **hybrids**, which showed there were no maternal/cytoplasmic effects. Comparisons of feeding preference between different groups of females or males demonstrated that the trait was not controlled by sex-linked loci. The distributions of feeding preference index values for crosses that carried similar complements of autosomal genes were not significantly different, whereas crosses with different complements of autosomal genes were associated with significantly different feeding preferences, indicating that feeding preference of the two species for cotton and pepper, respectively, is controlled by autosomal genes. It was found that one major autosomal locus affected this feeding preference, with the *H. armigera*-derived alleles being partially dominant to those carried by *H. assulta*. The genetic analysis of **hybrids** contributes to understand the evolution of feeding preference in these closely related species.

Inheritance of olfactory preferences I. Pheromone-mediated behavioral responses of *Heliothis subflexa* x *Heliothis virescens* hybrid male moths

Vickers, NJ. 2006

Shifts in male preference for qualitatively different pheromone blends appear to have played a fundamental role in the divergence of olfactory communication and evolution of moth species. As an initial step in documenting the genetic complexity underlying such shifts, we characterized the behavioral responses of **hybrid** male moths created by mating two heliothine moth species, *Heliothis subflexa* and *Heliothis virescens*. Between 67 and 96% of **hybrid** males flew upwind and contacted the pheromone source when presented with a blend consisting of (Z)-11-hexadecenal (Z11-16:Ald), (Z)-9-hexadecenal (Z9-16:Ald), and (Z)-11-hexadecenol (Z11-16:OH) in a 1:0.5: 0.1 ratio that has previously been shown to be attractive to *H. subflexa* males. In addition, an *H. virescens* blend of Z11-16:Ald and (Z)-9-tetradecenal (Z9-14:Ald) enhanced by the addition of Z11-16:OH (in a 1:0.05:0.1 mixture) was attractive to **hybrid** males (26-64% source contact), but significantly fewer males reached the odor source compared to the blend containing Z9-16:Ald. A blend in which the dosage of Z9-14:Ald was doubled, however, was equally attractive (75-77% source contact) as the Z9-16:Ald-containing blend. Consecutive presentation of two blends revealed that individual **hybrid** males responded equally well to blends containing either Z9-14:Ald or Z9-16:Ald. Together these results suggest that in addition to Z11-16:Ald, **hybrid** males: (1) required either Z9-16:Ald (like *H. subflexa* males) or Z9-14:Ald (like *H. virescens* males); (2) required the presence of Z11-16:OH (*H. subflexa* dominant); (3) were not adversely affected by the presence of Z11-16:Ac (*H. subflexa* dominant). The behavioral response phenotype of **hybrid** males was therefore influenced by genetic factors inherited from both parental species. Copyright (c) 2006 S. Karger AG, Basel.

DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera : Tachinidae)

Smith, MA; Woodley, NE; Janzen, DH; Hallwachs, W; Hebert, PDN. 2006

Insect parasitoids are a major component of global biodiversity and affect the population dynamics of their hosts. However, identification of insect parasitoids is often difficult, and they are suspected to contain many cryptic species. Here, we ask whether the cytochrome c oxidase I DNA barcode could function as a tool for species identification and discovery for the 20 morphospecies of *Belvosia* parasitoid flies (Diptera: Tachinidae) that have been reared from caterpillars (Lepidoptera) in Area de Conservacion Guanacaste (ACG), northwestern Costa Rica. Barcoding not only discriminates among all 17 highly host-specific morphospecies of ACG *Belvosia*, but it also raises the species count to 32 by revealing that each of the three generalist species are actually arrays of highly host-specific cryptic species. We also identified likely **hybridization** among *Belvosia* by using a variable internal transcribed spacer region 1 nuclear rDNA sequence as a genetic covariate in addition to the strategy of overlaying barcode clusters with ecological information. If general, these results will increase estimates of global species richness and imply that tropical conservation and host-parasite interactions may be more complex than expected.

Oviposition preference and larval survival of *Chilo partellus* (Lepidoptera : Pyralidae) on Napier grass (*Pennisetum purpureum*) trap crops

Van den Berg, J. 2006

Bioassays were conducted to identify varieties of Napier grass (*Pennisetum purpureum*) that may be used as trap crops for the gramineous stem- borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Oviposition preference was determined in two-choice tests with maize and Napier grass in the laboratory while larval survival was determined under greenhouse conditions. Twenty- one different Napier varieties and *P. purpureum* x *P. glaucum* **hybrids** were evaluated and compared to maize in these bioassays. The varieties were collected from nurseries, from the wild and from farmers using them as a source of animal fodder. Results from two-choice tests indicated that Napier grasses were highly preferred for oviposition by *C. partellus* moths. However, Napier grasses were poor larval hosts, and a rapid decline in larval numbers was observed during the first week after egg hatch. Larval survival on maize was significantly higher than on Napier grass where virtually no larvae survived. The evaluated Napier grass varieties therefore have the characteristics required of trap crops. Preference for oviposition on hairy leaf surfaces of Napier grass instead of maize was most likely influenced by leaf volatiles since oviposition preference was negatively correlated to leaf hairiness.

Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera : Noctuidae), as a potential pest of transgenic Cry1Ab *Bacillus thuringiensis* corn hybrids in South Dakota

Catangui, MA; Berg, RK. 2006

Injuries caused by the western bean cutworm, *Striacosta albicosta* (Smith), on transgenic Cry1Ab *Bacillus thuringiensis* (Bt) corn **hybrids** were documented and quantified. The western bean cutworm is an emerging or potential pest of transgenic Bt corn in South Dakota. The proportion of ears infested with western bean cutworm larvae in the Cry1Ab Bt corn **hybrids** were 18-20, 38-70, and 0-34% in 2000, 2003, and 2004, respectively. The Cry1Ab Bt corn **hybrids** were almost completely free of European corn borer infestations. Untreated conventional corn **hybrids** were less infested with western bean cutworm larvae but more infested with European corn borer larvae. The proportion of ears infested with European corn borer larvae alone were 33,58-80, and 8-25% in 2000,2003, and 2004, respectively. Infestations with western bean cutworm alone were 28, 8-28, and 13-19%, respectively. Proportion of ears simultaneously infested with both western bean cutworm and European corn borer larvae were much lower than single infestations by either species alone, indicating niche overlap and competition. Simultaneous infestations by the two species on untreated conventional corn **hybrids** were only 8,0-18, and 0-1% in 2000,2003, and 2004. The corn grains harvested from injured ears were also analyzed for fumonisin and aflatoxin through quantitative enzyme-linked immunosorbent assays. More mycotoxins were found in 2003 when the levels of insect infestation in the corn ears were higher than in 2004. Results from this study underscore the need to investigate other emerging or potential arthropod pests of transgenic Bt corn **hybrids** in addition to the western bean cutworm.

Behavioral and electrophysiological responses of *Helicoverpa assulta*, *H. armigera* (Lepidoptera : Noctuidae), their F-1 hybrids and backcross progenies to sex pheromone component blends

Zhao, XC; Yan, YH; Wang, CZ. 2006

Two sibling species, *Helicoverpa assulta* and *Helicoverpa armigera* both use (Z)-9-hexadecenal and (Z)-11-hexadecenal as their sex pheromone components but in almost reversed ratios, 93:7 and 3:97, respectively. *H. assulta* and *H. armigera* males performed upwind flight in response to the *H. assulta* sex pheromone blend (93:7). *H. armigera* responded strongly to the *H. armigera* blend (3:97), whereas *H. assulta* males remained inactive upon exposure to this blend. Both species gave clear dose-dependent electrophysiological responses to (Z)-11-hexadecenal. However, (Z)-9-hexadecenal evoked strong dose-dependent electrophysiological responses in *H. assulta* males but not in *H. armigera*. The two male F-1 **hybrids** exhibited similar behavioral responses to two sex pheromone blends and electrophysiological responses to two pheromone components as *H. armigera* males. This indicated that *H. armigera* genes appear dominant in determining the behavioral response and electrophysiological responses. Behavioral and electrophysiological responses of backcrosses of male F-1 **hybrids** (*H. armigera* female x *H. assulta* male) with female *H. assulta* and *H. armigera* were close to that of *H. assulta* and *H. armigera*, respectively. However, backcrosses of female F-1 **hybrids** (*H. assulta* female x *H. armigera* male) with male *H. assulta* and *H. armigera* showed reduced behavioral responses but normal electrophysiological responses compared to males of the respective parental line.

The green-veined white (*Pieris napi* L.), its Pierine relatives, and the systematics dilemmas of divergent character sets (Lepidoptera, Pieridae)

Chew, FS; Watt, WB. 2006

The butterfly *Pieris napi* (L.) and relatives exemplify recently evolving taxa, exhibiting variation that makes their evolutionary dynamics interesting, but their systematics difficult. Wing-pattern characters commonly used to distinguish these Holarctic insects display both genetic polymorphism and environmentally-cued polyphenism. Often, these causes of variation are confounded, impairing the characters' phylogenetic usefulness. DNA sequences of four mitochondrial genes offer an independent view of pierine phylogeny. Sampling diverse relatives within family Pieridae assists resolution of the *P. napi* complex, suggests previous underestimation of clade diversity in subfamily Pierinae, and shows that other genera near *Pieris* also display confusions of wing-pattern-based phylogenetic inference. The European *P. napi* is sister to all North American taxa, and is well diverged from them all in sequences. The North American taxa comprise a northern subclade including *Pieris oleracea*, and questionably distinct *Pieris angelika*, and a southern subclade including distinct *Pieris virginiensis*, *Pieris marginalis*, and *Pieris macdunnoughii*, and other regional entities yet to be clarified. Weak bootstrap support for some nodes in this group arises from a closeness of sequence identity rather than character conflict; more sequence data and denser geographical sampling may resolve these nodes more clearly. Evidence of reproductive isolation, from other experimental **hybridization** studies, agrees with the DNA results where these conflict with other divergent character sets. The system offers much promise for a deeper understanding of character evolution in relation to phyletic differentiation. (c) 2006 The Linnean Society of London.

Identification, purification, cloning and expression of a novel receptor for *Bacillus thuringiensis* Cry1A delta-endotoxins in the brush border membranes of the *Helicoverpa armigera* (Lepidoptera : Noctuidae)

Insecticidal crystal proteins of *Bacillus thuringiensis* are effective in controlling agriculturally and biomedically harmful insects. However, little is known about the mechanism of insecticidal activity of these proteins. We report here a 65 kDa Protein present in the extract of the larval midgut membrane of *Helicoverpa armigera* as putative receptor for Bt CryIA delta-endotoxin, on the basis of binding affinity to Cry1Aa, Cry1Ab and Cry1Ac but not to Cry2A. The protein has been highly purified by a combination of chromatography, electrophoresis and isoelectrofocussing techniques. The isolated protein exists as an oligodimer in its native form. The purified protein exhibits amino-peptidase activity. N-terminal sequence of the purified protein shows no homology to protein sequences in the Gen bank (NCBI) protein database. Degenerate primers were designed, based on N-terminal sequence of the purified protein and **hybridization** of Probe with mRNAs of *Helicoverpa armigera* indicated sequence complementarity. The structural gene of this purified protein was cloned in pGEX-4T-3 expression vector. The cloned Protein exhibited binding properties, aminopeptidase activity and other characteristics of native protein of *Helicoverpa armigera*. Larval mortality of *Helicoverpa armigera* to CryIA toxin was considerably reduced when the larvae were pre-fed a diet containing antibodies to the 65 kDa protein, presumably due to blocking of the receptor sites in BBMVs.

Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless

Kronforst, MR; Young, LG; Kapan, DD; McNeely, C; O'Neill, RJ; Gilbert, LE. 2006

Sexual isolation is a critical form of reproductive isolation in the early stages of animal speciation, yet little is known about the genetic basis of divergent mate preferences and preference cues in young species. *Heliconius* butterflies, well known for their diversity of wing color patterns, mate assortatively as a result of divergence in male preference for wing patterns. Here we show that the specific cue used by *Heliconius cydno* and *Heliconius pachinus* males to recognize conspecific females is the color of patches on the wings. In addition, male mate preference segregates with forewing color in **hybrids**, indicating a genetic association between the loci responsible for preference and preference cue. Quantitative trait locus mapping places a preference locus coincident with the locus that determines forewing color, which itself is perfectly linked to the wing patterning candidate gene, *wingless*. Furthermore, yellow-colored males of the polymorphic race *H. cydno* alithea prefer to court yellow females, indicating that wing color and color preference are controlled by loci that are located in an inversion or are pleiotropic effects of a single locus. Tight genetic associations between preference and preference cue, although rare, make divergence and speciation particularly likely because the effects of natural and sexual selection on one trait are transferred to the other, leading to the coordinated evolution of mate recognition. This effect of linkage on divergence is especially important in *Heliconius* because differentiation of wing color patterns in the genus has been driven and maintained by natural selection for Mullerian mimicry.

Critical yield components and key loss factors of tropical cucumber crops

Bacci, L; Picanco, MC; Gonring, AHR; Guedes, RNC; Crespo, ALB. 2006

The critical components and the key factors of yield losses in autumn-winter and spring-summer cucumber crops were determined and quantified in Vicosa county, State of Minas Gerais, Brazil, in areas cultivated with gynoceium **hybrids** of the groups Aodai (Sprint 440 II) and "Caipira" (Vlasstar). The data were used to build crop life tables. The overall losses in the autumn winter and spring-summer cultivations were of 53.5 and 66.6 ton ha⁻¹, representing 76.3% and 59.2% of the overall crop yield potential. A critical component of yield loss was flower abortion in both cultivation periods. Fruit loss in the autumn-winter cultivation period was due to deficient pollination and production of non-marketable fruit size, and fruit damage caused by *Diaphania* spp. (Lepidoptera: Pyralidae), factors that also affected losses in the spring-summer cultivation period, followed by the physiological disturbance caused by nitrogen deficiency. (c) 2006 Elsevier Ltd. All rights reserved.

Comparative susceptibility of European corn borer, southwestern corn borer, and sugarcane borer (Lepidoptera : Crambidae) to Cry1Ab protein in a commercial *Bacillus thuringiensis* corn hybrid

Huang, FN; Leonard, BR; Gable, RH. 2006

One field strain each of the European corn borer, *Ostrinia nubilalis* (Hubner); southwestern corn borer, *Diatraea grandiosella* Dyar; and sugarcane borer, *Diatraea saccharalis* (F.); were collected from cornfields in northeastern Louisiana. Susceptibilities of the field strain and a corresponding laboratory strain of the three borer species to Cry1Ab protein in DK69-70 *Bacillus thuringiensis* (Bt) corn **hybrid** were determined by exposing neonates to intact leaf tissues from whorl stage plants or by feeding neonates or third instars on a meridic diet treated with different concentrations of Cry1Ab protein extracted from Bt corn leaves. Mortality and growth of larvae were evaluated after 2 and 4 d posttreatment in the bioassays by using intact leaf tissues or after 7 d in the bioassays by using diet incorporating Cry1Ab protein. *D. saccharalis* was the least susceptible species to Cry1Ab protein among the three species, followed by *D. grandiosella*, whereas *O. nubilalis* was most susceptible. The 2-d mortality of *D. saccharalis* neonates on intact Bt leaf tissues was lower than that of *O. nubilalis* and *D. grandiosella*. All neonates of *O. nubilalis* were killed on the diet treated with Cry1Ab protein at 0.5 and 1 mg/kg. The mortality of *D. grandiosella* was > 75% at 1 mg/kg, but it was < 6% for *D. saccharalis* at 1 mg/kg. The LC50 values of *D. saccharalis* were 3- and 11-fold higher than those of *D. grandiosella* and *O. nubilalis*, respectively. The LC90 values of *D. saccharalis* were 8- and 32-fold higher than those of *D. grandiosella* and *O. nubilalis*, respectively. Larval growth of the three species on Cry1Ab-treated diet was inhibited, but the inhibition was greater for *O. nubilalis* and *D. grandiosella* than for *D. saccharalis*. The lower susceptibility of *D. saccharalis* to Cry1Ab protein suggests that it is necessary to verify if a high-dose Bt corn for *O. nubilalis* and *D. grandiosella* is also a high dose for *D. saccharalis*.

Effect of corn hybrids expressing the coleopteran-specific Cry3Bb1 protein for corn rootworm control on aboveground insect predators

Ahmad, A; Wilde, GE; Whitworth, RJ; Zolnerowich, G. 2006

Field and laboratory studies were conducted to determine the effect of transgenic *Bacillus thuringiensis* (Bt) corn, *Zea mays* L. (YieldGard Rootworm), expressing the Cry3Bb1 protein on aboveground nontarget insect predators (minute pirate bug, ladybird beetles, and carabids). Visual counts of adult and immature *Orius insidiosus* (Say), *Coleomegilla maculata* (DeGeer), *Hippodamia convergens* Gurin-Meneville, and *Scymnus* spp. occurring in Bt corn and its non-Bt isolate were made at Manhattan, KS, in 2002 and at Manhattan and Scandia, KS, in 2003. No significant differences were found between the Bt corn and non-Bt isolate plots in the abundance (number per plant) of *O. insidiosus*, *C. maculata*, *H. convergens*, and *Scymnus* spp. Field predation on *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) egg masses was also observed during the silking stage of corn at Manhattan and Scandia in 2003. No significant differences were observed among treatments in predation rate for predators with chewing versus sucking mouthparts. Two laboratory studies determined the effect of Cry3Bb1 protein expressed in Bt corn pollen on *C. maculata* and carabids. The larvae of *C. maculata* were reared on Bt pollen, non-Bt pollen, or greenbugs, *Schizaphis graminum* (Rondani). The duration of larval and pupal stages, developmental time from egg batch to adult emergence, percentage of survival, and elytra length were compared among treatments. There were no significant differences in developmental time of larvae fed pollen or greenbugs during their first two instars. However, significantly prolonged development of the third (1d) and fourth instars (2d) was observed for larvae fed greenbugs only. Total time for larval development was significantly longer for larvae that fed on greenbugs versus larvae fed on pollen. No significant differences were observed among treatments in the percentage of larvae that pupated or pupal stage duration. Larvae that fed on greenbugs had higher pupal and adult weights compared with pollen-fed larvae. However, pupal and adult weights did not vary between the Bt and non-Bt pollen treatments. No significant differences occurred in longevity and elytra length of beetles among all treatments. Two carabid species, *Harpalus caliginosus* F. and *Harpalus pensylvanicus* DeGeer, were reared on moistened dog food sprinkled with Bt or non-Bt corn pollen. No significant differences in mortality of *H. caliginosus* and *H. pensylvanicus* were detected among any of the treatments. There was no significant effect of Bt pollen on fecundity and egg viability of *H. caliginosus*. Our studies showed that YieldGard Rootworm had no effect on the selected coleopteran predators; therefore, this Bt corn **hybrid** could be used in an integrated pest management system.

Evolutionary history and patterns of differentiation among European *Maniola* butterflies (Lepidoptera : Satyrinae)

Grill, A; Gkiokia, E; Alvarez, N. 2006

Phylogenetic relationships of European *Maniola* butterflies are reconstructed using molecular sequences from two regions of the mitochondrial DNA, cytochrome oxidase subunit I (COI) and cytochrome b (Cytb). A total of 988 base pairs (486 for Cytb, and 502 for COI) were aligned for 15 individuals of *Maniola* and an outgroup, species. The phylogenetic tree obtained through Bayesian inference analysis of the combined data sets shows evidence that the island endemic *M. chia* is indistinguishable from *M. jurtina* on the basis of the mtDNA genes studied. Net nucleotide divergence between *M. jurtina* and *M. chia* is 0.4%, but 2% between the *M. jurtina* and the *M. nurag* clade. A phenetically distinct entity of individuals from Sardinia appears to be a **hybrid** between *M. nurag* and *M. jurtina*. The southern and northern European ecotypes of *M. jurtina*, which differ in the summer aestivation period of the southern type, are not structured genetically at the level of coding mtDNA genes. Divergence time between *M. nurag* and *M. jurtina* was estimated to be 1.1 to 1.2 million years. Speciation most likely took place in the early Pleistocene as a consequence of the isolation of Sardinia, when the sea reflooded the Mediterranean basin after the Messinian crisis (about 5 million years ago).

Wing pattern evolution and the origins of mimicry among North American admiral butterflies (Nymphalidae : Limenitis)

Mullen, SP. 2006

The evolution of wing pattern diversity in butterflies has emerged as a model system for understanding the origins and maintenance of adaptive phenotypic novelty. Admiral butterflies (genus *Limenitis*) are an attractive system for studying wing pattern diversity because mimicry is common among the North American species and **hybrid** zones occur wherever mimetic and non-mimetic wing pattern races meet. However, the utility of this system has been limited because the evolutionary relationships among these butterflies remain unclear. Here I present a robust species-level phylogeny of *Limenitis* based on 1911 bp of two mitochondrial genes (COI and COII) and 904 bp of EF1-alpha for all five of the Nearctic species/wing pattern races, the majority of the Palearctic species, and three outgroup genera; *Athyma*, *Moduza* (*Limenitidini*), and *Neptis* (*Limenitidinae*: *Neptini*). Maximum-likelihood and Bayesian analyses indicate that the North American species are a well-supported, monophyletic lineage that is most closely related to the widespread, Palearctic, Poplar admiral (*L. populi*). Within North America, the Viceroy (*L. archippus*) is the basal lineage while the relationships among the remaining species are not well resolved. A combined maximum-likelihood analysis, however, indicates that the two western North America species (*L. lorquini* and *L. weidemeyerii*) are sister taxa and closely related to the wing pattern subspecies of the polytypic *Limenitis arthemis* species complex. These results are consistent with (1) an ancestral host-shift to Salicaceae by the common ancestor of the Poplar admiral and the Nearctic admiral lineage, (2) a single colonization of the Nearctic, and (3) a subsequent radiation of the North American forms leading to at least three independent origins of mimicry. (c) 2006 Elsevier Inc. All rights reserved.

Natural hybridization in heliconiine butterflies: the species boundary as a continuum

Mallet, J; Beltran, M; Neukirchen, W; Linares, M. 2007

Background: To understand speciation and the maintenance of taxa as separate entities, we need information about natural **hybridization** and gene flow among species. Results: Interspecific **hybrids** occur regularly in *Heliconius* and *Eueides* (Lepidoptera: Nymphalidae) in the wild: 26-29% of the species of *Heliconiina* are involved, depending on species concept employed. Hybridization is, however, rare on a per-individual basis. For one well-studied case of species **hybridizing** in parapatric contact (*Heliconius erato* and *H. himera*), phenotypically detectable **hybrids** form around 10% of the population, but for species in sympatry **hybrids** usually form less than 0.05% of individuals. There is a roughly exponential decline with genetic distance in the numbers of natural **hybrids** in collections, both between and within species, suggesting a simple "exponential failure law" of compatibility as found in some prokaryotes. Conclusion: Hybridization between species of *Heliconius* appears to be a natural phenomenon; there is no evidence that it has been enhanced by recent human habitat disturbance. In some well-studied cases, backcrossing occurs in the field and fertile backcrosses have been verified in insectaries, which indicates that introgression is likely, and recent molecular work shows that alleles at some but not all loci are exchanged between pairs of sympatric, **hybridizing** species. Molecular clock dating suggests that gene exchange may continue for more than 3 million years after speciation. In addition, one species, *H. heurippa*, appears to have formed as a result of **hybrid** speciation. Introgression may often contribute to adaptive evolution as well as sometimes to speciation itself, via **hybrid** speciation. Geographic races and species that coexist in sympatry therefore form part of a continuum in terms of **hybridization** rates or probability of gene flow. This finding concurs with the view that processes leading to speciation are continuous, rather than sudden, and that they are the same as those operating within species, rather than requiring special punctuated effects or complete allopatry. Although not qualitatively distinct from geographic races, nor "real" in terms of phylogenetic species concepts or the biological species concept, **hybridizing** species of *Heliconius* are stably distinct in sympatry, and remain useful groups for predicting morphological, ecological, behavioural and genetic characteristics.

No genomic mosaicism in a putative hybrid butterfly species

Kronforst, MR; Salazar, C; Linares, M; Gilbert, LE. 2007

Recent descriptions of **hybrid** animal species have spurred interest in this phenomenon, but little genomic data exist to support it. Here, we use frequency variation for 657 amplified fragment length polymorphism (AFLP) markers and DNA sequence variation from 16 genes to determine whether the genome of *Heliconius pacheus*, a suspected **hybrid** butterfly species, is a mixture of the putative parental species, *Heliconius cydno* and *Heliconius melpomene*. Despite substantial shared genetic variation among all three species, we show that the genome of *H. pacheus* is not a mosaic; both AFLP and DNA sequence data overwhelmingly associate *H. pacheus* with just one of the potential parents, *H. cydno*. This pattern also applies to the gene wingless, which is tightly linked to the locus that determines forewing colour-one specific *H. pacheus* trait that has been hypothesized to have originated from *H. melpomene*. As a whole, the data support a traditional, bifurcating model of speciation in which *H. pacheus* split from a common ancestor with *H. cydno* without a genetic contribution from *H. melpomene*. However, comparison of our data to DNA sequence data for another putative **hybrid** *Heliconius* species, *Heliconius heurippa*, suggests that the *H. heurippa* genome may be a mosaic.

Genetic isolation and cryptic variation within the *Lycaena xanthoides* species group (Lepidoptera : Lycaenidae)

Oliver, JC; Shapiro, AM. 2007

Species exist as biological entities with patterns of discontinuous phenotypic variation. However, the distinctness of taxa is called into question when morphological intermediates exist in areas of sympatry, reflecting either gene flow among variants of a species or **hybridization** between different species. Studying the partitioning of genetic variation provides a means to discern between the two possibilities. We used genetic and morphometric approaches to investigate the degree of isolation among the three members of the *Lycaena xanthoides* species group. *Lycaena xanthoides*, *L. editha*, and *L. dione* are predominantly allopatric and have been treated both as three separate species and as a single polytypic species. Using 618 bp of the mitochondrial gene COII, we found little phylogenetic resolution, but significant among-taxon genetic variance partitioning. Divergence among these taxa has been relatively recent, as evidenced by relatively low pairwise sequence divergence. Also, the existence of two well-supported clades within *L. xanthoides sensu stricto*, concordant with the Transverse Ranges of southern California, indicates divergence within this taxon, and a possible cryptic species. Significant morphological differentiation between *L. editha* and *L. xanthoides* supports the hypothesis that these taxa represent separate gene pools. Populations occurring in a narrow zone where the two species' ranges approach are characterized by intermediate morphology, suggesting incomplete morphological divergence or recent **hybridization**. These findings highlight the utility of genetic data in inferring species boundaries and the

identification of cryptic lineages.

Limited hybridization along a large contact zone between two genetic lineages of the butterfly *Erebia medusa* (Satyrinae, Lepidoptera) in Central Europe

Schmitt, T; Muller, P. 2007

Genetic lineages evolving during glacial isolation frequently come into contact as the result of postglacial range expansions. Hybridization often occurs along these contact zones. In Europe, the high mountain systems of the Alps and Pyrenees are well known for their **hybrid** belts. This article studies the contact zone of the Woodland Ringlet *Erebia medusa* in a Hercynian mountain area in the Czech-German border region not exceeding 1500 m a.s.l. Hybrid populations between an eastern and a western genetic lineage were detected by AMOVA-based tests, principal component analysis and neighbour joining analysis. Over most of the range of the Czech-German Border Mts, the ridges separate the western and the eastern genetic lineage from each other. However, two important **hybrid** areas were detected: (1) the watershed of the Ohre river in the north-west of that area, a major valley system passing through these mountains and (2) the high plateaux of the Sumava Mts in the south-east, an extended area of high elevation. The location of **hybrid** populations in geographical vicinity to non-**hybrid** populations and the generally low F-IS (2.1%) make reduced fitness of **hybrid** individuals little likely. The **hybrid** populations have intermediate genetic diversity between the genetically poor western and the genetically rich eastern lineage populations.

The effects of Pleistocene glaciations on the phylogeography of *Melitaea cinxia* (Lepidoptera : Nymphalidae)

Wahlberg, N; Saccheri, I. 2007

Partial (600 bp) sequences of mitochondrial cytochrome oxidase I (COI) gene were used to infer the phylogeography of *Melitaea cinxia* (Lepidoptera: Nymphalidae) across the entire distributional range of the species, encompassing north Africa and Eurasia. Cladistic analysis of 49 distinct haplotypes (haplotype and nucleotide diversity were 0.95 and 0.027, respectively) revealed strong phylogeographic structure in *M. cinxia*, characterised by four major clades: Morocco; Western (Iberia, France, Italy); Central (central and northern Western Europe, Balkans, Greece, Anatolia, Levant); and Eastern (eastern Baltic, Urals, Iran, Siberia, China); separated by average pairwise distances of between 2 and 6 percent. This pattern is consistent with the location of southern glacial refugia in the Iberian, Italian and Balkan peninsulas, as well as multiple eastern refugia. The Western clade is further structured into south-central Iberian, northern Iberian (and French) and southern Italian sub-clades; and the Eastern clade into Near Eastern and Far Eastern sub-clades; with weaker phylogeographical concordance within the Central clade, except for a large area in central and northern Western Europe which is monomorphic for COI haplotype. The Baltic and eastern Europe have been primarily colonized by the Far Eastern sub-clade, rather than the Central (Balkan) clade, highlighting the importance of including Near and Far Eastern populations in phylogeographic studies of Palearctic species. Maps showing them extent of clades and sub-clades suggest several regions of secondary contact and possible **hybridization**. Interspecific comparison of representative *M. cinxia* haplotypes supports a monophyletic origin of all *M. cinxia*.

Morphological and molecular evidence of a possible hybrid zone of *Leptidea sinapis* and *L. reali* (Lepidoptera : Pieridae)

Verovnik, R; Glogovcan, P. 2007

Overlapping measurements in the length of the genitalia of *Leptidea sinapis*/*reali* collected in Slovenia triggered an investigation of a possible natural **hybridization** between these two well known sibling species of butterflies. Random polymorphic DNA (RAPD) was used to generate species specific markers and sequences of the cytochrome oxidase subunit one gene for determination of the progeny. RAPD's clustering and mitochondrial DNA (mtDNA) phylogeny were congruent with the taxonomic placement of specimens of both species, but slightly incongruent with the results of the analysis of genital morphology. Two specimens with *L. reali* genitalia measurements, but genetically belonging to *L. sinapis*, had species specific RAPD markers of both species indicating probable **hybrid** origin. All the specimens with genitalia of intermediate length were also genetically assigned to *L. sinapis* indicating a possible one way introgression as predicted from their genitalia morphology. *Leptidea sinapis* was found predominantly in xerothermic habitats in Slovenia, whereas *L. reali* was more of a generalist except in the sub-Mediterranean region where it is limited to humid meadows.

Segregation of F-2 interspecific hybrid growth performance and wing color patterns relative to parental species in the *Papilio machaon* species group (Lepidoptera : Papilionidae)

Scriber, JM. 2007

A strange *Papilio* female specimen with a prominent yellow wing band type very much like *P. brevicauda* (and also seen in *P. machaon*, and *P. zelicaon*) was captured in the forested mountains of southwest Vermont, USA. Despite its *brevicauda*-like appearance, it had mt-DNA of *P. polyxenes*. In eastern North America the *P. brevicauda* (Maritime Provinces of Canada) is currently believed to reflect previous (ancient) **hybridization** between ancestral *P. machaon* and *P. polyxenes*. It is uncertain whether the *brevicauda*-like wing trait features in the odd Vermont female may be a result of some introgression of *P. machaon* or *P. brevicauda* with *P. polyxenes* in eastern North America as seen also in the Ozark Mountains with *P. joanae*. Nonetheless, the significance of wing trait analyses in relation to **hybrid** zones with *P. polyxenes* remains relevant. The segregation of wing color patterns and hostplant use abilities (efficiencies and growth rates) were examined for laboratory hand-paired F-2 **hybrids** of *P. zelicaon* x *Papilio polyxenes* relative to the parental species and primary **hybrids**. The black adult color pattern characterizing *P. polyxenes* is presumed to be inherited as a simple autosomal dominant. These **hybrids** were examined to determine: (i) how F-1 primary **hybrid** offspring perform relative to the parental types in survival, growth rates, and growth efficiencies; and (ii) whether these larval traits and the adult color patterns might be linked with wing morphology in the F-2 **hybrids**. While the F-1 **hybrids** grew as well as the *P. polyxenes* parent (*P. zelicaon* was significantly less efficient and slower in growth than either of these), we could not convincingly determine whether the growth performances of the F-2 segregants were linked with the parental wing patterns.

Pre- and postzygotic isolation and Haldane rule effects in reciprocal crosses of *Danaus erippus* and *Danaus plexippus* (Lepidoptera : Danainae), supported by differentiation of cuticular hydrocarbons, establish their status as separate species

Hay-Roe, MM; Lamas, G; Nation, JL. 2007

Hybridization experiments and cuticular hydrocarbon analyses were performed to determine the extent of the reproductive isolation present to clarify the taxonomic status of *Danaus erippus* (Cramer, 1775), which has often been classified as a subspecies of *Danaus plexippus* (Linnaeus, 1758). The results of crosses between *D. erippus* and *Danaus plexippus nigrippus* (Haensch, 1909) showed pre- and postzygotic isolation. Pupal inviability, and Haldane rule effects (male **hybrids** only, the homogametic sex in butterflies) were observed after the crosses. These results reinforce the hypothesis that *D. plexippus* and *D. erippus* are separate, reproductively isolated species. However, we have also investigated the cuticular hydrocarbons in *D. erippus* and *D. p. nigrippus* with temperature-programmed gas chromatography. The chromatographic profiles of peaks were similar in both taxa, with retention indices in the range 2100-3797. The profiles were characterized by the presence of 15 peaks whose retention indices were in the range 2501-3772. Although no major qualitative differences between the two species were observed, we did find some quantitative differences. A principal component analysis showed a clear separation between members of *D. erippus* and *D. p. nigrippus*, suggesting that semiochemical differences might have become differentiated after isolation, perhaps in response to different physiological pressures. (c) 2007 The Linnean Society of London, Biological Journal of the Linnean Society, 2007, 91, 445-453.

Genetic differentiation and natural hybridization between the Sardinian endemic *Maniola nurag* and the European *Maniola jurtina*

Grill, A; Rajimann, LEL; Van Ginkel, W; Gkioka, E; Menken, SBJ. 2007

The Mediterranean island of Sardinia is known for its multitude of unique genetic lineages. We view one of them in a larger phylogeographic context. The endemic Sardinian Meadow Brown butterfly, *Maniola nurag*, is restricted to the mountainous areas of the island, whereas its widespread close relative, *Maniola jurtina*, also occurs on the coast. At intermediate altitudes the species' distributions overlap. There, a number of individuals exhibit phenotypic characteristics intermediate between the two species. We examined patterns of intra- and interpopulation variation in 10 *M. nurag* populations from Sardinia and 16 *M. jurtina* populations from Sardinia and continental Europe, as well as 17 intermediate individuals, sampled in 1999-2002, by means of allozyme markers, combining it with a morphometric analysis based on 18 wing-characters of 52 males. At the 15 loci studied (aldolase, aat-1, aat-2, g6pdh, gpd, idh-1, idh-2, mdh-1, mdh-2, mpi, me, leu-ala, pgi, pgm, and 6pgdh), 76 different alleles were detected, 63 of which were shared by *M. nurag* and *M. jurtina*. None of the loci was found to be alternatively fixed between the two species. In that respect, this study testifies to the difficulties that may arise when trying to identify **hybrids** from genotypic data. Levels of genetic variation in island populations (*M. jurtina*: H-o = 0.137-0.189; *M. nurag*: H-o = 0.141-0.270) were comparable to those of mainland *M. jurtina* (110 = 0.141-0.236). A Bayesian admixture analysis supported the hypothesis of mixed (**hybrid**) ancestry of individuals occurring at intermediate altitudes. Similarly, neighbour-joining and unweighted pair-group method with arithmetic averaging (UPGMA) analyses, as well as morphometrics hinted at the existence of a *Maniola*-**hybrid** zone in Sardinia at intermediate altitudes. We discuss the results in the light of the phylogeography of other Sardinian taxa with the aim to reach a general understanding of the biogeographic history of this island's endemic species.

Diversification of host use in two polyphagous butterflies: differences in oviposition specificity or host rank hierarchy?

Mercader, RJ; Scriber, JM. 2007

Novel host usage may represent an initial step towards diversification or radiation onto novel hosts within an evolutionary lineage, particularly if a shift in host plant preference ranking takes place. Polyphagous stages of evolutionary lineages may represent transitional states in which novel host associations are more likely to develop, but may be more difficult to detect experimentally. The polyphagous sister species *Papilio glaucus* L. and *Papilio canadensis* (Lepidoptera: Papilionidae; these *Papilio* = *Pterourus*) are known to exhibit differences in host-plant use, despite significant overlap in host-use abilities, providing an opportunity to examine how host shifts in polyphagous species may occur and what the implications for future divergence may be. In particular, we were interested in (i) determining whether differences in oviposition behavior of these species were due to changes in specificity or shifts in host-plant hierarchy, (ii) whether the varying preference for primary hosts also affected the preference for secondary hosts, and (iii) what the oviposition preferences of a new **hybrid** swarm population are. We examined more than 40 000 oviposition bouts from more than 400 *P. glaucus*, *P. canadensis*, and **hybrid** females placed in seven-, three-, or two-choice assays. In each of the choice assays, leaves from plants in different plant families of varying suitability for *P. glaucus* and *P. canadensis* larvae were used. We found the primary difference between *P. glaucus* and *P. canadensis* to be limited to a Z-linked shift in host rank hierarchy due to an acceptance of *Populus tremuloides* Michx. (Salicaceae) and reduced specificity for *Liriodendron tulipifera* L. (Magnoliaceae) in *P. canadensis*. In addition, we found the absence of the Z-linked oviposition acceptance of *P. tremuloides* in a recently formed allochronically separated **hybrid** swarm population found in *P. canadensis* territory at the northern border of the *P. glaucus* and *P. canadensis* **hybrid** zone.

Molecular divergence of the W chromosomes in pyralid moths (Lepidoptera)

Vitkova, M; Fukova, I; Kubickova, S; Marec, F. 2007

Most Lepidoptera have a WZ/ZZ sex chromosome system. We compared structure of W chromosomes in four representatives of the family Pyralidae-Ephestia kuehniella, Cadra cautella, Plodia interpunctella, and Galleria mellonella-tracing pachytene bivalents which provide much higher resolution than metaphase chromosomes. In each species, we prepared a W-chromosome painting probe from laser-microdissected W-chromatin of female polyploid nuclei. The Ephestia W-probe was cross-**hybridized** to chromosomes of the other pyralids to detect common parts of their W chromosomes, while the species-specific W-probes identified the respective W chromosome. This so-called Zoo-FISH revealed a partial homology of W-chromosome regions between E. kuehniella and two other pyralids, C. cautella and P. interpunctella, but almost no homology with G. mellonella. The results were consistent with phylogenetic relationships between the species. We also performed comparative genomic **hybridization**, which indicated that the W chromosome of C. cautella is composed mainly of repetitive DNA common to both sexes but accumulated in the W chromosome, whereas E. kuehniella, P. interpunctella, and G. mellonella W chromosomes also possess a large amount of female specific DNA sequences, but differently organized. Our results support the hypothesis of the accelerated molecular divergence of the lepidopteran W chromosomes in the absence of meiotic recombination.

The effect of Bt maize on Sesamia calamistis in South Africa

Van den Berg, J; Van Wyk, A. 2007

Bt maize, Zea mays L. (Poaceae) expressing Cry 1Ab insecticidal proteins was introduced for control of Busseola fusca (Fuller) (Lepidoptera: Noctuidae) and Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) in South Africa after its development for control of crambid borers in North America. In the light of the reportedly lower toxicity of Bt maize to certain Noctuidae borers, the effect of Bt maize was evaluated on Sesamia calamistis (Hampson) (Lepidoptera: Noctuidae). The characteristic larval behaviour of S. calamistis may result in reduced exposure to Bt toxin and subsequent high levels of survival. Larvae do not feed on plant whorls like other borer species but penetrate stems directly from behind leaf sheaths where eggs are laid. Greenhouse and laboratory bioassays were done with three Bt maize **hybrids** and their iso-**hybrids**. 'Whole plant methods' were used and potted plants artificially infested with eggs or larvae and survival recorded over time. Larval survival was also determined on different plant parts (whorls, stems, tillers, and ears) over time. Bt maize was shown to be highly toxic to S. calamistis. No larvae survived longer than 12-18 days on Bt maize plants in any of the experiments. Adults did not differentiate between Bt and non-Bt plants in oviposition choice experiments. Sesamia calamistis is polyphagous and occurs in mixed populations with other borer species with which it shares many parasitoid species in Africa. The ecological impact of local extinction of S. calamistis caused by this highly effective transgenic event is therefore not expected to be great.

cDNA characterization and expression analysis of two arylphorin-like hexameric protein genes from the diamondback moth, Plutella xylostella (L.)

Ashfaq, M; Sonoda, S; Tsumuki, H. 2007

We cloned and characterized two hexameric storage protein genes, PxAry1 and PxAry2, from Plutella xylostella and investigated the expression pattern in different developmental stages and in response to treatment by a juvenile hormone (JH) analog. The complete coding sequences of PxAry1 and PxAry2 are comprised of 2,097 and 2,094 bp with 699 and 698 amino acid residues, respectively. Signal peptides of 16 amino acids are predicted at the N-termini. According to both the phylogenetic analysis and amino acid composition (> 16% aromatic amino acids), PxAry1 and PxAry2 belong to the arylphorin-like protein genes. Analysis using Northern **hybridization** and RT-PCR showed varying levels of genes expression in the developmental stages with a small difference between sexes. Expression of both genes in fourth instar larvae was suppressed after treatment with a JH-analog. Southern **hybridization** revealed the presence of multiple arylphorin genes in the genome. Arch.

Parasitoids of leafmining lepidoptera. in spontaneous growth plants in an organic citrus orchard in Montenegro, RS, Brazil

dos Santos, JP; Dal Soglio, FK; Redaelli, LR; Costa, VA; Foelkel, E. 2007

This work aimed to check if *Phyllocnistis citrella* attacks spontaneous growth plants present in an organic citrus orchard, to identify in these plants other leafmining Lepidoptera and its parasitoids, and to verify if the parasitoids species are the same ones reported for *R. citrella*. The work was conducted in Montenegro, RS, in an organic orchard of the **hybrid** 'Murcott' (*Citrus sinensis* x *C. reticulata*). Samplings were taken every other week, from May 2003 to May 2004, with a ring of 0.28 m(2) being randomly thrown in the lines and interlines of 30 trees. All the plants with mines inside of the ring were collected. The screening of the material was made in laboratory. Eleven species of leafmining Lepidoptera and 12 species of microhymenopteran parasitoids were found. Some genera identified that this study had been already reported in several regions of the world with parasitizing species *P. citrella*, such as *Chrysocharis*, *Closterocerus*, *Sympiesis* (Hymenoptera: Eulophidae) and *Bracon* (Hymenoptera: Braconidae).

Inheritance of female flight in *Lymantria dispar* (Lepidoptera : Lymantriidae)

Keena, MA; Grinberg, PS; Wallner, WE. 2007

A clinal female flight polymorphism exists in the gypsy moth, *Lymantria dispar* L., where female flight diminishes from east to west across Eurasia. A Russian population where females are capable of sustained ascending flight and a North American population with females incapable of flight were crossed: parentals, reciprocal F, **hybrids**, double reciprocal F, **hybrids**, and all possible back-crosses to both the parental lines were compared. Heritabilities were estimated using a threshold model, female offspring on female parent regressions, and joint-scaling analyses. Heritability of female flight capability measured using a free flight test was at least 0.60, and variation in wing size, muscle strength, and flight behaviors contributed to the flight polymorphism. Relative wing size varied continuously and had a heritability of 0.70. Environmental variation accounted for > 90% of the variation in female preflight weight and relative flight muscle strength, as estimated by an inverted female's ability to right herself. Preflight walking behavior and early deposition of eggs were each inherited through a single gene with two co-dominant alleles. There was no evidence for sex-linkage or maternal effects in female flight capability or associated traits. Continued vigilance to exclude and eradicate introductions of strains capable of female flight in North America is warranted even in areas where no females fly, because some of the alleles needed for full flight capability may not be present in the North American populations, and some flight capability is maintained in the **hybrids** that could increase the rate of spread of *L. dispar*.

A glassy-winged sharpshooter cell line supports replication of *Rhopalosiphum padi* virus (Dicistroviridae)

Boyapalle, S; Pal, N; Miller, WA; Bonning, BC. 2007

Rhopalosiphum padi virus (RhPV) (family Dicistroviridae; genus Cripavirus) is an icosahedral aphid virus with a 10 kb positive-sense RNA genome. To study the molecular biology of RhPV, identification of a cell line that supports replication of the virus is essential. We screened nine cell lines derived from species within the Lepidoptera, Diptera and Hemiptera for susceptibility to RhPV following RNA transfection. We observed cytopathic effects (CPE) only in cell lines derived from hemipterans, specifically GWSS-Z10 cells derived from the glassy winged sharp shooter, *Homalodisca*, coagulata and DMII-AM cells derived from the corn leaf hopper, *Dalbulus maidis*. Translation and appropriate processing of viral gene products, RNA replication and packaging of virus particles in the cytoplasm of GWSS-Z10 cells were examined by Western blot analysis, Northern blot **hybridization** and electron microscopy. Infectivity of the GWSS-Z10 cell derived-virus particles to the bird cherry-oat aphid, *R. padi*, was confirmed by RT-PCR and Western blot. The GWSS-Z10 cell line provides a valuable tool to investigate replication, structure and assembly of RhPV. (c) 2006 Elsevier Inc. All rights reserved.

Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push-pull strategy

Khan, ZR; Midega, CAO; Wadhams, LJ; Pickett, JA; Mumuni, A. 2007

We evaluated eight Napier grass [*Pennisetum purpureum* Schumacher (Poaceae)] varieties, used in various parts of eastern Africa as fodder, for their potential role as trap plants in the management of the African stemborer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) through a push-pull strategy. Oviposition preference, larval orientation, settling, arrest and dispersal, feeding, mortality and survival, and development were determined for each of these varieties under laboratory and screen house conditions. Two-choice tests showed that only two of the varieties tested (cv. Bana and cv. Uganda Hairless) were preferentially chosen by gravid female moths for oviposition over a susceptible maize variety, cv. Western Hybrid 502. Larval preference was, however, highly variable. Larval feeding by first instars on the maize leaves was more intense and significantly more than on leaves of all the Napier grass varieties evaluated. Food consumed and amounts assimilated by the third instars over a 24-h period were not different among larvae fed on stems of maize and those fed on stems of the various Napier grass varieties. Larval survival was significantly lower on all the Napier grass varieties (below 3%) than on maize (about 44%). Similarly, larval development was about 2-3 weeks longer on majority of the Napier grass varieties. It was concluded that cv. Bana had potential for use as a trap plant in the management of *B. fusca* because it was more preferred by the moths for oviposition, equally preferred as maize by the larvae for orientation, settling, and arrest, and allowed minimal survival of the larvae. It can thus be used with such 'push' plants as *Desmodium* spp. (Fabaceae) in a 'push-pull' strategy, but the effectiveness of such a strategy would strictly depend on proper establishment and management of these companion plants.

Discordant divergence times among Z-chromosome regions between two ecologically distinct swallowtail butterfly species

Putnam, AS; Scriber, JM; Andolfatto, P. 2007

We investigate multi locus patterns of differentiation between parental populations of two swallowtail butterfly species that differ at a number of ecologically important sex-linked traits. Using a new coalescent-based approach, we show that there is significant heterogeneity in estimated divergence times among five Z-linked markers, rejecting a purely allopatric speciation model. We infer that the Z chromosome is a mosaic of regions that differ in the extent of historical gene flow, potentially due to isolating barriers that prevent the introgression of species-specific traits that result in **hybrid** incompatibilities. Surprisingly, a candidate region for a strong barrier to introgression, *Ldh*, does not show a significantly deeper divergence time than other markers on the Z chromosome. Our approach can be used to test alternative models of speciation and can potentially assign chronological order to the appearance of factors contributing to reproductive isolation between species.

Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach

Roe, AD; Sperling, FAH. 2007

Accurate delimitation of species boundaries is especially important in cryptic taxa where one or more character sources are uninformative or are in conflict. Rather than relying on a single marker to delimit species, integrative taxonomy uses multiple lines of evidence such as molecular, morphological, behavioural and geographic

characters to test species limits. We examine the effectiveness of this approach by testing the delimitation of two cryptic Nearctic species of *Dioryctria* (Lepidoptera: Pyralidae) using three independent molecular markers [cytochrome c oxidase I (COI), second internal transcribed spacer unit (ITS2), and elongation factor 1 α (EF1 α)], forewing variation and larval host plant association. Although mitochondrial DNA (mtDNA) haplotypes do not form reciprocally monophyletic clades, restricted gene flow between COI haplotype groups, and concordance with ITS2 genotypes, forewing variation and host plant associations support delimitation of two Nearctic species: eastern *Dioryctria reniculelloides* and western *Dioryctria pseudotsugella*. Conversely, EF1 α genotype variation was incongruent with the two previous markers. A case of discordance between COI and ITS2 was detected, suggesting either introgression due to **hybridization** or retained ancestral polymorphism due to incomplete coalescence. This study is consistent with other similar literature where molecular loci in closely related species progress from shared to fixed haplotypes/alleles, and from polyphyletic to reciprocally monophyletic relationships, although loci may vary in these characteristics despite maintenance of genomic integrity between distinct species. In particular, mtDNA in other studies generally showed a lower rate of fixation of differences than did X-linked or autosomal loci, reinforcing the need to use an integrative approach for delimiting species.

Limited hybridization along a large contact zone between two genetic lineages of the butterfly *Erebia medusa* (Satyrinae, Lepidoptera) in Central Europe (vol 45, pg 39, 2007)

Schmitt, T; Muller, P. 2007

Molecular phylogeny and dating of an insular endemic moth radiation inferred from mitochondrial and nuclear genes: The genus *Galagete* (Lepidoptera : Autostichidae) of the Galapagos Islands

Schmitz, P; Cibois, A; Landry, B. 2007

Galagete is a genus of microlepidoptera including 12 nominate species endemic to the Galapagos Islands. In order to better understand the diversification of this endemic insular radiation, to unravel relationships among species and populations, and to get insight into the early stages of speciation, we developed a phylogenetic reconstruction based on the combined mitochondrial cytochrome oxidase I (555 bp) and II (453 bp), and the nuclear elongation factor-1 α (711 bp) and wingless (351 bp) genes. Monophyly of the genus is strongly supported in the Bayesian and maximum likelihood analyses suggesting a single colonization event by a common ancestor. Two cases of paraphyly observed between species are hypothesized to represent imperfect species limits for *G. espanolaensis* nested within the *G. turritella* clade, and introgressive **hybridization** or lineage sorting in the case of the population of *G. protozona* from Santa Fe nested within the *G. gnathodoxa* clade. A geologically calibrated, relaxed molecular clock model was used for the first time to unravel the chronological sequence of an insular radiation. The first split occurring within the *Galagete* lineage on the archipelago is estimated at 3.3 \pm 0.4 million years ago. The genus radiated relatively quickly in about 1.8 million years, and gives an estimated speciation rate of 0.8 species per million years. Although the colonization scenario shows a stochastic dispersal pattern, the arrival of the ancestor and the diversification of the radiation coincide with the chronological emergence of the major islands. (c) 2007 Elsevier Inc. All rights reserved.

Reinforcement of mate preference among hybridizing *Heliconius* butterflies

Kronforst, MR; Young, LG; Gilbert, LE. 2007

Recent models of mate preference evolution suggest that direct selection on alleles at preference loci and correlated evolution of preference with locally adapted mating cues are more likely to drive the evolution of assortative mate preference than reinforcement. Mate preference evolution in mimetic *Heliconius* butterflies has been attributed to all three forms of selection, but here we show that reinforcement has been critical. By examining geographical variation in assortative mating and male mate preference among seven populations of three **hybridizing** *Heliconius* species from Costa Rica, we found pronounced character displacement of preference such that sexual isolation was enhanced in areas of interspecific contact. Of the different explanations for the evolution of assortative mate preference, only reinforcement is dependent on interspecific contact in this system. Thus, the observed pattern of reproductive character displacement of mate preference is best explained as a product of indirect selection generated by natural selection against nonmimetic **hybrids**.

Impact of the stem borer, *Dectes texanus*, on yield of the cultivated sunflower, *Helianthus annuus*.

Michaud, JP; Grant, AK; Jyoti, JL. 2007

Foliar and soil-drench insecticide treatments were used in attempts to manipulate infestation of cultivated sunflower plants, *Helianthus annuus* LeConte (Asterales: Asteraceae) by *Dectes texanus* LeConte, (Coleoptera: Cerambycidae) a serious pest of sunflowers in the High Plains of the USA. Seed yields were assessed on a per-plant basis for both oilseed and confection type sunflower **hybrids** in two years. Both insecticide treatments (foliar lambda-cyhalothrin and soil-drench carbofuran) improved yield of oilseed sunflowers in 2004, but not in 2005. Yield of confection **hybrids** was improved by a systemic fungicide (thiophanate methyl) in 2005, but insecticides did not improve yield in either year. Both insecticide treatments gave good control of various stalk-boring insects such as *Cylindrocropturus adspersus* (Coleoptera: Curculionidae), *Mordellistena* sp. (Coleoptera: Mordellidae), and *Pelochrista womanana* (Lepidoptera: Tortricidae), but neither gave better than 50% control of *D. texanus*. Plants were sorted according to the presence or absence of *D. texanus* larvae and no reduction was found in total seed weight, seed size, or oil content as a result of infestation. However, mature larvae of *D. texanus* girdle stalks at the base in preparation for overwintering, a behavior that reduced stalk breakage force by 34-40%, leading to yield losses through lodging. At harvest in 2005, there were differences between cultivars and among treatments in the proportions of *D. texanus* larvae that had girdled their plants at harvest. It was concluded that further research aimed at reducing crop losses to *D. texanus* should focus on means of delaying stalk desiccation and/or deterioration, factors that appear to trigger girdling behavior.

Structure and composition of the assemblage of parasitoids associated to *Phyllocnistis citrella* pupae stainton (Lepidoptera : Gracillariidae) in citrus orchards in southern Brazil

Jahnke, SM; Redaelli, LR; Diefenbach, LMG; Dal Soglio, FK. 2007

The structure and composition of the assemblage of pupal parasitoids of *Phyllocnistis citrella* Stainton, the citrus leafminer, were studied in two citrus orchards (*Citrus deliciosa* Tenore cv. Montenegrina and *Citrus sinensis* (L.) Osbeck x *Citrus reticulata* Blanco **hybrid** Murcott), in Montenegro County (29 degrees 68'S and 51 degrees 46'W), southern Brazil. At fortnightly samplings, from July 2001 to June 2003, all the new shoots from 24 randomly selected trees were inspected. The species richness reached five native species in the Murcott orchard, and six in Montenegrina. In Murcott, the presence of *Ageniaspis citricola* (Hymenoptera: Encyrtidae), an exotic species, was detected in the first year of sampling, probably migrating from the nearby areas where it had been released for the miner control. In Montenegrina, its presence was only registered in the second year. *A. citricola* in both areas was dominant and changed the community structure of parasitoid complex of *P. citrella* in both orchards.

Influence of mulberry cultivars *Morus* spp. on the production and quality of silkworm *Bombyx mori* L. (Lepidoptera : Bombycidae) cocoons

Meneguim, AM; Lovato, L; da Silva, RZ; Yamaoka, RS; Nagashima, GT; Pasini, A. 2007

The success of sericulture greatly depends on the availability of mulberry cultivars with agronomic characteristics that maximize the production and quality of silkworm, *Bombyx mori* L., cocoons. The effect of the cultivars Miura (standard) and Korin, and the **hybrids** SM 14, SM 63, SK 1, SK 4 and FM 86 was evaluated on silkworm development, and on cocoon production and quality, at the Instituto Agronomico do Parand, INPAR, in Londrina, PR, Brazil (23 +/- 3 degrees C; 61 +/- 8% RH). The experiment was arranged in a randomized block design with four replicates. Larvae feeding either on 'SK 4' or on 'Korin' showed a higher weight compared to that of larvae feeding on 'Miura'. Cocoon weight (both sexes) and pupae weight (male) obtained with larvae fed on SK 4 were higher compared with that of insects that fed on 'Miura'; there was no difference among the remaining treatments. Silk net weight was independent of the cultivar used. Cocoon shell weight was similar amongst insects raised on 'Miura' and on the other mulberry materials. The percentage of cocoon spinning was higher than 90% in all treatments, with the spinning concentrating in the first two days. The proportion of first grade cocoons varied from 80% (SK4) to 90%. The results obtained with the studied cultivars were either similar or superior to that of the standard cultivar Miura with emphasis to 'SK 4', thus showing the potential use of these cultivars in sericulture in the state of Parand.

Widespread mito-nuclear discordance with evidence for introgressive hybridization and selective sweeps in *Lycaeides*

Gompert, Z; Forister, ML; Fordyce, JA; Nice, CC. 2008

We investigated the extent and potential cause(s) of mitochondrial introgression within the polytypic North American *Lycaeides* species complex (Lepidoptera). By comparing population genetic structure based on mitochondrial DNA (COI and COII) and nuclear DNA (251 polymorphic amplified fragment length polymorphism markers), we detected substantial mito-nuclear discordance, primarily involving a single mitochondrial haplotype (h01), which is likely due to mitochondrial introgression between differentiated *Lycaeides* populations and/or species. We detected reduced mitochondrial genetic diversity relative to nuclear genetic diversity in populations where mitochondrial haplotype h01 occurs, suggesting that the spread of this haplotype was facilitated by selection. We found no evidence that haplotype h01 is associated with increased fitness (in terms of survival to eclosion, fresh adult weight, and adult longevity) in a polymorphic *Lycaeides melissa* population. However, we did find a positive association between mitochondrial haplotype h01 and infection by the endoparasitic bacterium *Wolbachia* in one out of three lineages tested. Linkage disequilibrium between mitochondrial haplotype h01 and *Wolbachia* infection status may have resulted in indirect selection favouring the spread of haplotype h01 in at least one lineage of North American *Lycaeides*. These results illustrate the potential for introgressive **hybridization** to produce substantial mito-nuclear discordance and demonstrate that an individual's mitochondrial and nuclear genome may have strikingly different evolutionary histories resulting from non-neutral processes and intrinsic differences in the inheritance and biology of these genomes.

No simple sum: seasonal variation in tannin phenotypes and leaf-miners in hybrid oaks

Yarnes, CT; Boecklen, WJ; Salminen, JP. 2008

Parent-**hybrid** asymmetries in the biosynthesis of defense chemistry are believed to affect the distribution of herbivorous insects within plant **hybrid** zones. While tannins are often produced as complex, dynamic mixtures, the ecological effects of biological and ontogenetic variation in tannin metabolism within **hybrid** zones are poorly understood. Here, we examine correlations between the seasonal variation of absolute and relative concentrations of individual ellagitannins and total proanthocyanidins in the *Quercus gambelii* x *Q. grisea* **hybrid** complex and a community of leaf-mining Lepidoptera (Gracillariidae, Heliozelidae, Nepticulidae, Tischeriidae), and examine the interactive effects between tannins on leaf-miner abundance. We found strong seasonal variation in oak tannin phenotypes, but two general phenotypes are characteristic of oaks of the *Q. gambelii* x *Q. grisea* complex, based upon biosynthetic variation in high molecular weight ellagitannins. Leaf-miner community structure was significantly correlated with the dominant tannin phenotypes in the *Q. gambelii* x *Q. grisea* **hybrid** zone in mid-summer (July, August). Additionally, leaf-miner density was significantly negatively correlated with the ratio of vescalagin-derivatives and total proanthocyanidins in August and September. Overall, **hybridization** affects biological and ontogenetic variation in ellagitannin and proanthocyanidin biosynthesis within the *Q. gambelii* x *Q. grisea* **hybrid** complex and this variation correlates with herbivore distribution and abundance. Future studies of phenolic chemistry in plant-herbivore interactions in **hybrid** zones should include a more rigorous quantification of tannin phenotypes as complex, temporally dynamic mixtures.

Patterns of genitalic morphology around suture zones in North American *Lycaeides* (Lepidoptera : Lycaenidae): Implications for taxonomy and historical biogeography

Lucas, LK; Fordyce, JA; Nice, CC. 2008

Within the North American *Lycaeides* (Hubner) fauna, there are at least three major lineages that exhibit extensive morphological and ecological variation, especially at suture zones where these lineages meet. We examined male genitalic morphology in *Lycaeides* populations spanning much of North America to evaluate the current taxonomy and to address questions about the patterns of morphological variation at suture zones and potential evolutionary processes responsible for the patterns. Our genitalic measurements were based on those of V. Nabokov who revised North American *Lycaeides* taxonomy in the 1940s. Canonical discriminant analysis validated Nabokov's original species designations, but it did not support many of his subspecific designations. Populations at a suture zone in the Great Lakes region are similar to populations on the east side of this zone. Populations at a western suture zone in the Sierra Nevada and adjacent ranges exhibit intermediate morphology between lineages on either side of this suture zone. We tested the hypothesis that contemporary gene flow contributes to the patterns of morphology in suture zones by testing for the increased variance in quantitative traits that is expected in a **hybrid** swarm. Based on a comparison of variances from populations within and outside of these suture zones, there is no evidence of current **hybridization** between lineages, with the sole exception of the population sample from the White Mountains of eastern California. The intermediate morphology and the general absence of increased variance within western suture zone populations imply that **hybridization** may have been important in the evolution of North American *Lycaeides*.

Considering evolutionary processes in the use of single-locus genetic data for conservation, with examples from the Lepidoptera

Forister, ML; Nice, CC; Fordyce, JA; Gompert, Z; Shapiro, AM. 2008

The increasing popularity of molecular taxonomy will undoubtedly have a major impact on the practice of conservation biology. The appeal of such approaches is undeniable since they will clearly be an asset in rapid biological assessments of poorly known taxa or unexplored areas, and for discovery of cryptic biodiversity. However, as an approach for diagnosing units for conservation, some caution is warranted. The essential issue is that mitochondrial DNA variation is unlikely to be causally related to, and thus correlated with, ecologically important components of fitness. This is true for DNA barcoding, molecular taxonomy in general, or any technique that relies on variation at a single, presumed neutral locus. Given that natural selection operates on a time scale that is often much more rapid than the rates of mutation and allele frequency changes due to genetic drift, neutral genetic variation at a single locus can be a poor predictor of adaptive variation within or among species. Furthermore, reticulate processes, such as introgressive **hybridization**, may also constrain the utility of molecular taxonomy to accurately detect significant units for conservation. A survey of published genetic data from the Lepidoptera indicates that these problems may be more prevalent than previously suspected. Molecular approaches must be used with caution for conservation genetics which is best accomplished using large sample sizes over extensive geography in addition to data from multiple loci.

Host plants of lepidoptera leafminers in citrus orchard in Montenegro-RS, Brazil

Dos Santos, JP; Redaelli, LR; Soglio, FK. 2008

This study aimed to verify and to identify Lepidopterous leafminers in plants of spontaneous growth present in citrus orchard and to verify the occurrence of "citrus leafminer", *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), on those plants of spontaneous growth. The work was conducted in Montenegro, RS, in a tangor 'Murcott' **hybrid** orchard. Samplings were taken every fortnightly, from May, 2003 to May, 2004, collecting in each occasion all plants and or branches with mines found in a area delimited by an 0.28 m(2) arch thrown in the lines and between lines of 30 randomly chosen trees. The host plants of the leafminers were collected for identification. Eleven species of Lepidoptera leafminers were found distributed in six families, collected in 15 species of host plants of nine botanical families. The community of plants of spontaneous growth in the studied area hosted a huge diversity of Lepidoptera leafminers, including possible new families; however it did not hosted *P. citrella*.

Reproductive biology and pollination mechanisms of *Epidendrum secundum* (Orchidaceae). Floral variation: a consequence of natural hybridization?

Pansarin, ER; Amaral, MCE. 2008

The phenology, flower morphology, pollination mechanism and reproductive biology of *Epidendrum secundum* were studied in a semi-deciduous forest at the Serra do Japi (SJ), and in the Atlantic rain forest of Picinguaba, both natural reserves in the State of Sao Paulo, southeastern Brazil. *E. secundum* flowers all year round, with a flowering peak between September and January. This species is either a lithophytic or terrestrial herb in the SJ, whereas, in Picinguaba, it grows mainly in disturbed areas along roadsides. *E. secundum* is pollinated by several species of diurnal Lepidoptera at both study sites. In Picinguaba, where *E. secundum* is sympatric with *E. fulgens* and both share the same pollinators, pollen transference between these two species was recorded. *E. secundum* is self-compatible but pollinator-dependent. It is inter-compatible with *E. fulgens*, producing fertile seeds. In contrast to the population of the SJ, in the Picinguaba region, floral morphology is quite variable among plants and some individuals present flowers with characteristics in-between both sympatric species, suggesting that natural **hybridization** occasionally occurs. The anthropogenic perturbation is probably the cause of the occurrence of *E. secundum* in the Picinguaba region, enabling its contact with *E. fulgens*.

Widespread decoupling of mtDNA variation and species integrity in *Grammia* tiger moths (Lepidoptera: Noctuidae)

Schmidt, BC; Sperling, FAH. 2008

We investigate the diversity of the North American tiger moth genus *Grammia* Rambur (Lepidoptera: Noctuidae) by comparing mitochondrial DNA (mtDNA) 'barcode' fragments of cytochrome oxidase I with non-molecular characters such as morphology, ecology, behaviour and distribution. Mitochondrial DNA genealogy is strikingly at odds with morpho-species taxonomy for most of the 28 sampled species, as haplotypic polyphyly not only is taxonomically widespread, but involves multiple shared haplotypes among two to four species. Morpho-ecological traits show that those species sharing haplotypes are often not closely related. Furthermore, high mtDNA divergences occur within species. Haplotypic variation is highly discordant with species taxonomy, but variation at a continental scale reveals significant geographic structuring of haplogroups, transcending morpho-species boundaries. A nested clade analysis and comparison of non-molecular with mtDNA data indicate that most discordance between mtDNA and taxonomy in *Grammia* is explained best by taxonomically and geographically widespread ongoing **hybridization** events resulting in mtDNA introgression. We hypothesize that broad areas of sympatry, interspecifically compatible genitalic structure, and species overlap in pheromone components facilitate **hybridization**, with disparate interspecies abundances promoting mitochondrial introgression. The molecular evolution of *Grammia* challenges the view that interspecific gene exchange occurs rarely and is restricted to recently diverged species. These results show the value of mtDNA in detecting cryptic **hybridization**, while highlighting the inherent dangers of drawing taxonomic conclusions based solely on mtDNA.

Geometric morphometrics reveal male genitalia differences in the *Lasiommata megera*/paramegaera complex (Lepidoptera, Nymphalidae) and the lack of a predicted hybridization area in the Tuscan Archipelago

Dapporto, L. 2008

The recognition of specific demarcation between allopatric populations, as for island endemics, is particularly difficult according to the Biological Concept of Species. However, the recognition of the specific status of island taxa is decisive in evaluating important areas of endemism for conservation purposes. The recent taxonomic inflation of European butterfly species calls into question the validity and objectivity of taxonomic practices, and of databases dependent on them, which are used in biogeography and conservation. In this paper I applied rigorous morphometric analyses instead of visual evaluation to solve the long debated question of whether *Lasiommata paramegaera* butterfly from Sardinia and Corsica should be considered as a separated entity from the widespread *Lasiommata megera*. First, I carried out analyses of male genitalia shape comparing the populations from Corsica and Tuscany. Thereafter, I also searched for possible **hybridization** evidence in the sole area where it is strongly predicted: the Tuscan Archipelago. These islands are located between Tuscany and Corsica and there is large evidence of a continuous immigration of butterflies from these two main sources. I found that Corsican *L. paramegaera* and Tuscan *L. megera* can be clearly distinguished on the basis of genitalia shape. Furthermore, the *Lasiommata* population of each islet clearly maintains the characteristics of one of the two species without evidence of intermediate individuals. In conclusion, I suggest that the two entities should be considered as separate species maintaining their homogeneity by a reduced gene flow across sea, mating avoidance and/or by some depletion of F1 **hybrids** in developmental or mating success.

Genetic independence of female signal form and male receiver design in the almond moth, *Cadra cautella*

Allison, JD; Roff, DA; Carde, RT. 2008

Efficient signalling requires coordination of signal form and receiver design. To maintain signal function, parallel changes in signaller and receiver traits are required. Genetic correlation and co-evolution among signal and response traits have been proposed to preserve signal function (i.e. coordination) during the evolution of mate recognition systems. Empirical studies have provided support for both mechanisms; however, there is debate regarding the interpretation of some of these studies. Tests for a genetic correlation typically **hybridize** divergent signalling systems and look at **hybrid** signal form and receiver design, or impose artificial selection on signal form and look for an indirect response to selection in receiver design. Some of the **hybridization** studies did not achieve reassortment of genes from the parental types, whereas some of the artificial selection studies incorporated random mating in their designs. As a result of these limitations, the **hybridization** studies cannot discriminate between genetic correlation and co-evolution with primarily additive genetic effects underlying signal and response traits. Similarly, the artificial selection experiments cannot discriminate between genetic correlation because of linkage disequilibrium and co-evolution. This study examined the mating preferences of male almond moths, *Cadra cautella*, before and after female moths were artificially selected (using a design incorporating assortative mating) for novel pheromone blend ratios. Our results demonstrate the absence of a genetic correlation between signal and response traits in the almond moth.

Notes on some *Athis inca* ssp collected in Mexico (Lepidoptera: Castniidae)

Gonzalez, JM; Hernandez-Baz, F; Vinciguerra, R. 2008

Information on two Mexican subspecies of *Athis inca* (*A. inca inca* and *A. inca orizabensis*), as well as a possible natural **hybrid** of both, is presented.

Molecular characterization of host strains of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) in Southern Brazil

Machado, V; Wunder, M; Baldissera, VD; Oliveira, JV; Fiuza, LM; Nagoshi, RN. 2008

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is a Neotropical species found from Argentina to the United States of America. In its distribution area, two genetically distinct strains are found that differ in their plant host distribution. The strains are morphologically identical, and they can only be reliably distinguished by molecular markers. In this study, we characterized caterpillars collected in corn, *Zea mays* L., and rice, *Oryza sativa* L., plantations in the state of Rio Grande do Sul, Brazil. The mitochondrial haplotype typical of the rice strain was found in 100% of the caterpillars collected from that crop, whereas 83% of the caterpillars obtained from corn were of the corn strain haplotype. A sex-linked tandem repeat element called FR was previously shown to have a strain-biased distribution in North American Populations. We demonstrated that the same element is found in Brazilian fall armyworm and that it also organized in large tandem repeat clusters that are most frequently observed in caterpillars collected from rice and that display the rice strain mitochondrial haplotype. These results indicate that the Brazilian and North American fall armyworm populations are virtually indistinguishable with respect to the known genetic markers that are diagnostic of strain identity. Furthermore the data are consistent with other studies suggesting substantial gene flow between these widely separated populations. The strain-biased distribution of the FR element makes it a potentially useful marker to estimate interstrain **hybridization** frequencies of this species in Brazil.

The lock-and-key mechanisms of the internal genitalia of the Noctuidae (Lepidoptera): How are they selected for?

Mikkola, K. 2008

In the Noctuidae, the owlet moths, the internal genitalia, i.e. the aedeagus and vesica (penis) in the males, and the bursa copulatrix in the females, together form a lock-and-key mechanism (LKM). The species-specific structures have their counterparts in the opposite sex. The internal LKM constitutes a specific reproductive isolation mechanism (lock-and-key hypothesis), which seem to be the rule in the ditrysian Lepidoptera, and also Occurs in the Carabidae (Coleoptera) and some other insects. In contrast, the external genitalia rarely have species-specific counterparts in the sexes. Several results indicate the presence of LKMs: In the Noctuidae, (1) heterospecific differences in the male vesica may prevent sperm transfer or lead to mechanical failure during copulation, (2) the more complicated the specific genitalia structures, the more aberrations may occur even in conspecific copulations, and (3) in many species pairs and groups, and in one large genus, *Apamea*, the structures in the opposite sexes show a strictly specific correspondence, but, (4) when there is precopulatory isolation due to differences in pheromone production or perception, the internal genitalia may be identical. Conversely, in the *Colias* butterflies (Pieridae), (5) frequent heterospecific **hybridization** is associated with the similarity of the internal genitalia. The LKMs seem to protect genomes against alien genes, supposedly selected for because of the lower fitness of specimens with an imprecise LKM and/or inferiority of **hybrids**. In the literature, the diversity of the noctuid genitalia has been ascribed to sexual selection, because the females were classified as polyandrous. Most species produce the main part of their eggs monandrously, and remate, if at all, in their old age, and are thus successively monandrous and polyandrous. The allopatric divergence in the structure of the internal genitalia of 39 Holarctic pairs of sister species of Noctuidae is suggested to be due to genetic drift. The insecure function of the female pheromones and external genitalia of males are illustrated with the aid of original photographs.

Genetic basis of sex pheromone blend difference between *Helicoverpa armigera* (Hubner) and *Helicoverpa assulta* (Guenee) (Lepidoptera : Noctuidae)

Wang, HL; Ming, QL; Zhao, CH; Wang, CZ. 2008

The two closely related moth species, *Helicoverpa armigera* and *H. assulta*, are sympatric in China. Both species use a mixture of (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-hexadecenal (Z9-16:Ald) as their sex pheromones but in widely different ratios. Hybridization and backcrossing experiments between *H. armigera* and *H. assulta* were conducted and sex pheromone compositions of the parent species, their F₁ **hybrids** and backcrosses were compared to study the genetic basis of the production of their sex pheromone blend composition. Results show that the difference in sex pheromone blend ratios of these *Helicoverpa* species is mainly controlled by an autosomal locus with two alleles, with the allele from *H. armigera* being almost completely dominant over that derived from *H. assulta*. (C) 2008 Elsevier Ltd. All rights reserved.

Frequency and severity of western bean cutworm (Lepidoptera : Noctuidae) ear damage in transgenic corn hybrids expressing different *Bacillus thuringiensis* cry toxins

Eichenseer, H; Strohhahn, R; Burks, J. 2008

The frequency and severity of corn ear damage caused by western bean cutworm, *Striacosta albicosta* (Smith), were measured on transgenic corn, *Zea mays* L., **hybrids** expressing two different insecticidal *Bacillus thuringiensis* (Bt) (Berliner) Cry toxins (Bt) selected to protect against damage caused by larval European corn borer, *Ostrinia nubilalis* (Hubner). A field cage experiment deliberately infested with western bean cutworm egg masses resulted in less damage in the **hybrid** expressing the Cry1F protein and supported fewer western bean cutworm larvae than its non-Bt isolate. Corn **hybrids** expressing Cry1F, grown in small plot field experiments at three locations over two separate years and exposed to natural western bean cutworm infestations suffered less damage than non-Bt or Bt-**hybrids** expressing a Cry1Ab protein. Later maturing **hybrids** suffered more damage than shorter-season **hybrids**. Finally, corn ears observed in strip trials for several years in diverse agronomic conditions in farmer-cooperator fields corroborated the in-plant protection conferred by corn **hybrids** expressing the Cry1F protein in small plot field trials.

Phyllocnistis citrella Stainton population fluctuation in Citrus deliciosa and Murcott hybrid Citrus sinensis x Citrus reticulata

de Jesus, CR; Redaelli, LR; Dal Soglio, FK. 2008

To evaluate the population dynamics of *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae), the citrus leafminer, in tangerine *Citrus deliciosa* Tenore var. *Montenegrina* and tangor 'Murcott' *Citrus sinensis* L Osbeck X *Citrus reticulata* Blanco in organically managed orchards, in Montenegro (29 degrees 68'S e 51 degrees 46'W), RS, fortnightly samples were carried out from July 2001 to June 2003. Sampled shoots were examined in the lab and the number (if leaves, presence or absence of mines and the number of mines, eggs, larvae and pupae) were recorded. In both orchards there were no records of *P. citrella* mines in the first leaf flush, from August to October. In the first year, the highest mine densities were recorded in the middle of November, beginning of January and in April for both orchards. In the following year, the highest mine and larval densities were in January and April for *C. deliciosa* var *Montenegrina* and from December to March for 'Murcott'. Although the number of shoots has always been greater in *C. deliciosa*, the citrus leafminer colonization and establishment followed the same pattern for both orchards. Minimum and medium temperature and relative humidity were the abiotic factors showing the strongest influence in the numbers of *P. citrella* mines and larvae.

Host race formation in the leaf-mining moth *Acrocercops transecta* (Lepidoptera : Gracillariidae)

Ohshima, I. 2008

Genetic differentiation in ecological traits plays an important role in the reproductive isolation of phytophagous insects. The present study aims to elucidate the genetic changes involved during the process of host shifts, by combining analyses for (1) host adaptations, (2) pre- and postmating isolation, and (3) phylogeny among populations, using a leaf-mining moth, *Acrocercops transecta*. This species is associated with *Juglans ailanthifolia* and *Lyonia ovalifolia*. Transplantation of the larvae demonstrated that the *Juglans*-associated population completely failed to survive on *Lyonia*, whereas the *Lyonia*-associated population survived on *Juglans* as well as on *Lyonia*. Females of respective host-associated populations oviposited on their natal host plant only. An mtDNA-based phylogeny clearly separated the *Lyonia*-associated population from the *Juglandaceae*-associated population, and indicated that the *Lyonia*-associated population once evolved from the *Juglandaceae*-associated population. These results indicate that the processes of host shifting from juglandaceous species to *Lyonia* involved genetic changes both in larval ability to use host plants and in host preference of females. The derived *Lyonia*-associated population has retained the potential to assimilate the ancestral host, *Juglandaceae*. Mating between the two host-associated populations was successful for both directions of crossing, and there were no significant differences in egg hatchability between **hybrids** and control crosses. No adults emerged when the F-1 **hybrid** larvae were maintained on *Lyonia*; however, on *Juglans* the F-1 **hybrid** larvae grew to adulthood as well as in the control, suggesting a lack of genomic incompatibilities between the two host-associated populations. In conclusion, the results showed that the two host-associated populations are host races that are partially reproductively isolated, and that the differences in performance and preference function as strong barriers against gene flow between the host races. (c) 2008 The Linnean Society of London.

Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing

Vera, JC; Wheat, CW; Fescemyer, HW; Frilander, MJ; Crawford, DL; Hanski, I; Marden, JH. 2008

We present a de novo assembly of a eukaryote transcriptome using 454 pyrosequencing data. The Glanville fritillary butterfly (*Melitaea cinxia*; Lepidoptera: Nymphalidae) is a prominent species in population biology but had no previous genomic data. Sequencing runs using two normalized complementary DNA collections from a genetically diverse pool of larvae, pupae, and adults yielded 608 053 expressed sequence tags (mean length = 110 nucleotides), which assembled into 48 354 contigs (sets of overlapping DNA segments) and 59 943 singletons. BLAST comparisons confirmed the accuracy of the sequencing and assembly, and indicated the presence of c. 9000 unique genes, along with > 6000 additional microarray-confirmed unannotated contigs. Average depth of coverage was 6.5-fold for the longest 4800 contigs (348-2849 bp in length), sufficient for detecting large numbers of single nucleotide polymorphisms. Oligonucleotide microarray probes designed from the assembled sequences showed highly repeatable **hybridization** intensity and revealed biological differences among individuals. We conclude that 454 sequencing, when performed to provide sufficient coverage depth, allows de novo transcriptome assembly and a fast, cost-effective, and reliable method for development of functional genomic tools for nonmodel species. This development narrows the gap between approaches based on model organisms with rich genetic resources vs. species that are most tractable for ecological and evolutionary studies.

Structure and Distribution of a Strain-Biased Tandem Repeat Element in Fall Armyworm (Lepidoptera: Noctuidae) Populations in Florida, Texas, and Brazil

Nagoshi, RN; Armstrong, JS; Silvie, P; Meagher, RL. 2008

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major economic pest throughout the Western Hemisphere. There exist two morphologically identical but genetically distinct strains (corn-strain and rice-strain) that differ in their host plant preferences. These strains can be distinguished by polymorphisms in the mitochondrial cytochrome oxidase I gene. There is also a tandem-repeat genetic element called FR that is found in large sex-linked clusters primarily in the rice strain, as characterized by sampling of fall armyworm populations in the southeastern United States. It was recently shown that the FR element is also present in Brazil, where it exhibits a similar strain-biased distribution. In this article, the analysis of FR was extended to populations in southern Texas, one of the principle overwintering locations for fall armyworm that infests the continental United States. DNA sequence analysis and an optimized polymerase chain reaction (PCR)-based method demonstrated that FR sequences are present in Texas and show the same distribution pattern as observed in Florida. The distribution of FR in Florida has remained relatively unchanged over a 4-yr period, suggestive of polymorphic equilibrium and the existence of at least partial barriers to the generation of interstrain **hybrids**. The implications of these findings on our understanding of interstrain mating behavior and the utility of the modified detection method to study fall armyworm populations are discussed.

Isolation and characterization of a baculovirus associated with the insect parasitoid wasp, *Cotesia marginiventris*, or its host, *Trichoplusia ni*

Grasela, JJ; McIntosh, AH; Shelby, KS; Long, S. 2008

A multiple nucleopolyhedrovirus (MNPV) was isolated from *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) larvae that had been stung by the parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). The wild type virus was plaque purified by infecting a *Heliothis subflexa* (BCIRL-HsAM1) cell line and isolating several clones. The mean estimated genomic size of this virus based on PstI, BstEII, StyI, HindIII restriction profiles was estimated to be 106 +/- 2.5 kbp (mean +/- SE). A clone designated as TnMNPV/CmBCL9 was used in bioassays against several lepidopteran pests and in comparative studies with the baculoviruses AcMNPV, AgMNPV, AfMNPV, PxMNPV and HzSNPV of *Autographa californica*, *Anticarsia gemmatilis*, *Anagrapha falcifera*, *Plutella xylostella*, and *Helicoverpa zea*, respectively. Infectivity studies showed that TnMNPV/CmBCL9 was highly infectious for *Heliothis subflexa* and *T. ni*, with an LC50 value 0.07 occlusion bodies/mm(2) in both species and also infectious for *H. zea* and *Heliothis virescens* with LC50 values of 0.22 and 0.27 occlusion bodies/mm(2), respectively. Restriction endonuclease analysis of the isolate and selected baculoviruses revealed profiles that were very similar to AfMNPV but different from the restriction endonuclease profiles of the other baculoviruses. Hybridization studies suggest that the TnMNPV/CmBCL9 was closely related to AfMNPV and AcMNPV-HPP. Further support for this comes from a phylogenetic analysis employing a split-graphs network, comparing the polh, egt, and p10 genes from TnMNPV/CmBCL9 with those from other baculoviruses and suggests that this virus is closely related to the AcMNPV variants, AfMNPV and RoMNPV of *Rachiplusia ou*.

A BAC-based integrated linkage map of the silkworm *Bombyx mori*

Yamamoto, K; Nohata, J; Kadono-Okuda, K; Narukawa, J; Sasanuma, M; Sasanuma, S; Minami, H; Shimomura, M; Suetsugu, Y; Banno, Y; Osoegawa, K; de Jong, PJ; Goldsmith, MR; Mita, K. 2008

Background: In 2004, draft sequences of the model lepidopteran *Bombyx mori* were reported using whole-genome shotgun sequencing. Because of relatively shallow genome coverage, the silkworm genome remains fragmented, hampering annotation and comparative genome studies. For a more complete genome analysis, we developed extended scaffolds combining physical maps with improved genetic maps. Results: We mapped 1,755 single nucleotide polymorphism (SNP) markers from bacterial artificial chromosome (BAC) end sequences onto 28 linkage groups using a recombining male backcross population, yielding an average inter-SNP distance of 0.81 cM (about 270 kilobases). We constructed 6,221 contigs by fingerprinting clones from three BAC libraries digested with different restriction enzymes, and assigned a total of 724 single copy genes to them by BLAST (basic local alignment search tool) search of the BAC end sequences and high-density BAC filter **hybridization** using expressed sequence tags as probes. We assigned 964 additional expressed sequence tags to linkage groups by restriction fragment length polymorphism analysis of a nonrecombining female backcross population. Altogether, 361.1 megabases of BAC contigs and singletons were integrated with a map containing 1,688 independent genes. A test of synteny using Oxford grid analysis with more than 500 silkworm genes revealed six versus 20 silkworm linkage groups containing eight or more orthologs of *Apis* versus *Tribolium*, respectively. Conclusion: The integrated map contains approximately 10% of predicted silkworm genes and has an estimated 76% genome coverage by BACs. This provides a new resource for improved assembly of whole-genome shotgun data, gene annotation and positional cloning, and will serve as a platform for comparative genomics and gene discovery in Lepidoptera and other insects.

Asymmetrical thermal constraints on the parapatric species boundaries of two widespread generalist butterflies

Mercader, RJ; Scriber, JM. 2008

1. The sibling species *Papilio glaucus* and *Papilio canadensis* meet in a narrow **hybrid** zone believed to be maintained by temperature thresholds acting independently on both species. The present study tests if this assertion is true for the cold-adapted species, *P. canadensis*, which is presumed to be limited by the effect of high temperatures in late summer and/or autumn on pupal survival. 2. Three experiments were conducted examining the effects of: (i) short periods of high temperature stress in autumn, (ii) prolonged warm temperatures in autumn, and (iii) temperatures simulating warmer winters/longer springs upon the survival of *P. canadensis* and *P. glaucus*. 3. Results indicated that short periods of high temperatures did not induce the high C mortality rates required to be the key factor limiting the range of *P. canadensis*. However, *P. canadensis* did exhibit a considerably lower tolerance to high temperature extremes, prolonged warm temperatures in autumn, and conditions simulating shorter/warmer winters than *P. glaucus*. 4. Differences in temperature tolerance throughout the pupal stage are likely to be a significant factor in maintaining the southern range limit of *P. canadensis*. Further warming as may occur during climate change, particularly in winter and spring, will likely affect the dynamics of southerly populations of *P. canadensis*.

Trade-Off Between Sensitivity and Specificity in the Cabbage Looper Moth Response to Sex Pheromone



Hemmann, DJ; Allison, JD; Haynes, KF. 2008

The evolution of male moth responses to pheromone blends may be constrained by a trade-off between two response traits: sensitivity and breadth of response. Population genetic simulations predict that if sensitivity and breadth of response are negatively correlated (i.e., a trade-off exists), then selection will favor males with narrow response phenotypes and high sensitivity. Although sensitivity-breadth of response trade-offs are generally assumed to exist and are implicit to the shape of male preference function, this study is the first to provide empirical support measuring behavior at the level of the individual. Previous studies with the cabbage looper, *Trichoplusia ni*, have documented the existence of a mutant pheromone strain. While mutant females produce a pheromone blend significantly different from wild-type females, mutant males respond equally to the wild-type and mutant pheromone blends. This study used wind tunnel bioassays to document that relative to wild-type males, mutant males had broader response profiles but lower pheromone sensitivity. While wild-type male responses were highest to the wild-type pheromone blend, mutant males did not discriminate among pheromone blends. These results are consistent with a trade-off between breadth of response and sensitivity. Pure wild-type and mutant lines were crossed and **hybrid** males assayed. Both **hybrid** types (maternal wild-type and maternal mutant **hybrids**) responded similarly. Hybrid males had response profiles similar to wild-type males and the reduced sensitivity observed in mutant males. These results suggest a possible **hybrid** disadvantage and a putative mechanism for reinforcement of male pheromone response traits.

Digestive alpha-amylases from *Tecia solanivora* larvae (Lepidoptera: Gelechiidae): response to pH, temperature and plant amylase inhibitors

Valencia-Jimenez, A; Arboleda, JW; Avila, AL; Grossi-de-Sa, MF. 2008

The biochemical properties of the digestive alpha-amylase from *Tecia solanivora* larvae, an important and invasive insect pest of potato (*Solanum tuberosum*), were studied. This insect has three major digestive alpha-amylases with isoelectric points 5.30, 5.70 and 5.98, respectively, which were separated using native and isoelectric focusing gels. The alpha-amylase activity has an optimum pH between 7.0 and 10.0 with a peak at pH 9.0. The enzymes are stable when heated to 50 degrees C and were inhibited by proteinaceous inhibitors from *Phaseolus coccineus* (70% inhibition) and *P. vulgaris* cv. Radical (87% inhibition) at pH 6.0. The inhibitors present in all amaranth **hybrid** inhibited 80% of the activity at pH 9.0. The results show that the alpha-amylase inhibitor from amaranth seeds may be a better candidate to make genetically-modified potatoes resistant to this insect than inhibitors from common bean seeds.

Reproductive compatibility of several East and West African *Cotesia sesamiae* (Hymenoptera : Braconidae) populations and their crosses and backcrosses using *Sesamia calamistis* (Lepidoptera : Noctuidae) as the host

Gounou, S; Chabi-Olaye, A; Poehling, HM; Schulthess, F. 2008

The relative importance of the braconid *Cotesia sesamiae*, a gregarious larval parasitoid of lepidopteran stemborers, varies greatly with region in Africa; while the most common parasitoid of noctuid stemborers in eastern Africa, it is rare in western Africa. Thus, several strains of *C. sesamiae* from Kenya are envisaged for introduction into western Africa. The present study investigates the reproductive compatibility between four populations of *C. sesamiae* from West Africa and Kenya with the noctuid *Sesamia calamistis* as the host using reciprocal crosses as well as backcrosses of **hybrid** females with males of the parental populations. Searching time of the male for the female and mating period varied significantly with couple and ranged between 0.78-1.9 min and 3.4-12.8 s, respectively. Crosses that involved females from inland Kenya (KI) did not yield any female offspring. However, backcrosses of **hybrid** female bearing a KI male genome with a KI male yielded both female and male offspring. Thus, there was a partial reproductive incompatibility between KI and West African populations which suggested that the latter were infected with *Wolbachia* sp. However, this should not affect the efficacy of a population introduced from East into West Africa, as there is a high degree of sib-mating in this gregarious parasitoid species. It was concluded that the regional differences in the relative importance of *C. sesamiae* was due to differences in the insect and plant host range of the different populations.

Host strain specific sex pheromone variation in *Spodoptera frugiperda*

Groot, AT; Marr, M; Schofl, G; Lorenz, S; Svatos, A; Heckel, DG. 2008

Background: The fall armyworm *Spodoptera frugiperda* (Lepidoptera; Noctuidae) consists of two distinct strains with different host plant preferences for corn and rice. To assess whether pheromonal-mediated behavioral isolation accompanies the habitat isolation on different host plants, we compared the sex pheromone composition among females of the two strains. Pheromone glands were extracted with or without injection of pheromone biosynthesis activating neuropeptide (PBAN). To assess the mode of inheritance of this variation, we also analyzed the pheromone composition of F-1 **hybrid** females. Results: Relative to intra-strain variation, the pheromone composition of the two strains differed significantly. Corn strain females contained significantly more of the second most abundant pheromone compound Z11-16:Ac (m), and significantly less of most other compounds, than rice strain females. When females were injected with PBAN before their glands were extracted, the differences between the strains were less pronounced but still statistically significant. The pheromone composition of **hybrid** females showed a maternal inheritance of the major component Z9-14:Ac (M) as well as of Z11-16:Ac (m). Most other compounds showed an inheritance indicating genetic dominance of the corn strain. The within-strain phenotypic correlations among the various components were consistent with their hypothesized biosynthetic pathway, and between-strain differences in the correlation structure suggested candidate genes that may explain the pheromone differences between the two strains. These include Delta 9- and Delta 11 desaturases, and possibly also a Delta 7-desaturase, although the latter has not been identified in insects so far. Conclusion: The two host strains of *S. frugiperda* produce systematically differing female sex pheromone blends. Previously- documented geographic variation in the sexual communication of this species did not take strain identity into account, and thus may be partly explained by different strain occurrence in different regions. The finding of pheromone differences reinforces the possibility of incipient reproductive isolation among these strains, previously shown to differ in the timing of nocturnal mating activity and host plant use. Finding the genetic basis of the pheromone differences, as well as these other biological traits, will help to elucidate the role of premating isolation in the continuing differentiation of these two strains that may eventually lead to speciation.

Taxonomy, ecology, genetics and conservation status of the pale imperial hairstreak (*Jalmenus eubulus*) (Lepidoptera:Lycaenidae): a threatened butterfly from the Brigalow Belt, Australia

Eastwood, R; Braby, MF; Schmidt, DJ; Hughes, JM. 2008

The taxonomic status of *Jalmenus eubulus* Miskin stat. rev. is revised and considered to be specifically distinct from *J. evagoras* (Donovan) based on fundamental differences in morphology, ecology and genetics. Miskin's holotype is fixed by monotypy and illustrated, with type locality Rockhampton, Queensland. Fixed differences in the mitochondrial genomes of *J. eubulus* and *J. evagoras* in which the mean pairwise divergence is only 0.85% indicate absence of matrilineal gene flow, whereas allozyme data show significant structure within and between populations of both species consistent with recent diversification. Underlying causes for the observed genetic patterns are investigated. The two species are parapatric, with a narrow range of overlap along the Great Escarpment in south-eastern Queensland. *Jalmenus eubulus* is restricted to vegetation communities comprising brigalow-dominated old-growth open-forests and woodlands in the Brigalow Belt (with larvae monophagous on *Acacia harpophylla* F. Muell. ExBenth), whereas *J. evagoras* occurs in a range of disturbed eucalypt woodlands/open-forests predominantly in montane and coastal areas east of this bioregion (with larvae polyphagous on *Acacia* species other than *A. harpophylla*). The conservation status of *J. eubulus* is considered to be vulnerable nationally and critically endangered in New South Wales according to International Union for Conservation of Nature (IUCN) criteria. Nationally, the geographic range has an estimated area of occupancy of less than 2000 km², is severely fragmented, and the extent or quality of its habitat, which is poorly conserved, continues to decline. It is recommended that the taxon be used as an indicator for identification of remnant old-growth forest for conservation planning, as well as a flagship for the conservation of invertebrate biodiversity associated with this threatened ecological community.

Establishment of *cotesia flavipes* (Hymenoptera : braconidae) in sugarcane fields of Ethiopia and origin of founding population

Assefa, Y; Mitchell, A; Conlong, DE; Muirhead, KA. 2008

Cotesia flavipes (Cameron) (Hymenoptera: Braconidae) is used as a classical biological control agent against *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), a serious exotic pest of cereal crops in eastern and southern Africa. This parasitoid has been introduced into several African countries for the control of *C. partellus* in maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.), but it has never been released in Ethiopia. It is hypothesized that it spread into Ethiopia from populations released in Kenya and Somalia to become the predominant parasitoid of *C. partellus* in maize and sorghum fields of the country. In recent surveys conducted in Ethiopia, *C. flavipes* was recovered from *C. partellus* in sugarcane, *Saccharum* L. spp. **hybrids**, at a site > 2,000 km from the nearest known release sites in Kenya and Somalia. These findings question published hypotheses that estimate the dispersal rate of *C. flavipes* to be 60 km per year in Africa, and they suggest that since its release in Africa this parasitoid has developed strains adapted to searching particular host plants infested by particular stem borers. The anomalies between our results and previous reports evoked the hypothesis that *C. flavipes* in Ethiopian sugarcane might be a different strain. To test this hypothesis, we compared partial COI gene sequences of *C. flavipes* collected from sugarcane in Ethiopia and those of specimens from other African countries to determine the origin of the Ethiopian population. In addition, COI sequences were obtained for *C. flavipes* from other continents. The *C. flavipes* population established in Ethiopian sugarcane is most closely related to the populations released against *C. partellus* in maize in other parts of Africa, which were derived from the original population imported from Pakistan. The dispersal rate of the parasitoid was estimated to be > 200 km per year.

Comparative efficacy of Bt maize events MON810 and Bt11 against *Sesamia calamistis* (Lepidoptera: Noctuidae) in South Africa

Van Wyk, A; Van den Berg, J; Van Rensburg, JBJ. 2009

Maize, expressing Cry1 Ab insecticidal proteins produced by the bacterium *Bacillus thuringiensis* (Bt), was introduced for control of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in South Africa in 1998. In the light of the reportedly lower toxicity of Bt maize to certain noctuid borers, the effect of Bt maize was evaluated on *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) in South Africa. The characteristic larval behaviour of *S. calamistis* may result in reduced exposure to Bt toxin and subsequent high levels of survival since larvae do not feed on plant whorls like other borer species, but penetrate stems directly from behind leaf sheaths. Growth and survival of larvae were determined in a greenhouse bioassay with two Bt maize **hybrids** (Monsanto event MON810 and Syngenta event Bt11) and their non-Bt, isoline **hybrids**. Potted plants were artificially infested with 1st instar larvae. Percentage larval survival and mean larval mass were recorded over time. Bt maize of both events was shown to be highly toxic to *S. calamistis*. No larvae survived longer than 9 days on plants of either of the Bt events. *S. calamistis* is stenophagous and occurs in mixed populations with other borer species, with which it shares several parasitoid species in Africa. The ecological impact of local extinction of *S. calamistis* caused by this highly effective transgenic event is therefore not expected to be great. (C) 2008 Elsevier Ltd. All rights reserved.

Rapid assessment of the sex of codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) eggs and larvae

Fukova, I; Neven, LG; Barcenas, NM; Gund, NA; Dalikova, M; Marec, F. 2009

Two different methods were tested to identify the sex of the early developmental stages of the codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) with a WZ/ZZ (female/male) sex chromosome system. First, it was shown that the sex of all larval stages can be easily determined by the presence or absence of sex chromatin, which is formed by the female-specific W chromosome in interphase nuclei. This trait can also be used to identify the sex of newly hatched larvae but it does require care and accuracy. Secondly, a new sexing technique was developed based on a molecular marker of the codling moth W chromosome. Flanking regions of an earlier described W-specific sequence (CpW2) were isolated and sequenced and a 2.74 kb sequence (CpW2-EcoRI), specific for the W chromosome, was obtained. Several PCR tests were conducted, which confirmed that the CpW2-EcoRI sequence is a reliable marker for the sex identification in codling moth samples of different geographical origin. In addition, a fragment of a codling moth gene, period (Cpper) was isolated and sequenced. Results of southern **hybridization** of the Cpper probe with female and male genomic DNA suggested that the Cpper gene is located on the Z chromosome. Then a multiplex PCR assay was developed, which co-amplified the CpW2-EcoRI sequence to identify the W chromosome and the Z-linked Cpper sequence, which served as a positive control of accurate processing of tested samples. The multiplex PCR provides an easy and rapid identification of the sex of embryos and early larval instars of the codling moth.

Female sex pheromones in the host races and hybrids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Lima, ER; McNeil, JN. 2009

The three major pheromone components found in gland extracts of both rice and corn strain *Spodoptera frugiperda* virgin females were Z9-14:Ac, Z11-16:Ac and Z7-12:Ac. However, both the concentrations and relative proportions of the compounds varied significantly between strains as a function of female age and the time during the scotophase that the glands were extracted. The concentration of Z9-14:Ac found in the glands of **hybrid** females, coming from corn x rice and rice x corn crosses differed significantly, but in both cases did not differ significantly from that of maternal line females. The results suggest that synthesis is controlled, at least in part, by the W chromosome. Given the intraspecific variability reported in other species of Lepidoptera, both with respect to female pheromone production and male responsiveness, it would appear rather unlikely that these differences alone would be sufficient to ensure reproductive isolation of the two strains.

Microsatellite markers for the hybridizing tiger swallowtails, *Papilio glaucus* and *P. canadensis*, and their applicability to historic specimens and congeneric species

Nowak, C; Brown, CM; Hellmann, JJ. 2009

We report the development of microsatellite markers for the sister species *Papilio glaucus* and *P. canadensis* (Papilionidae: Lepidoptera). All 16 markers displayed a high degree of variation in both species, ranging from eight to 24 alleles per locus. Substantial heterozygote deficits were observed for several loci, indicating the presence of null alleles. All markers were successfully used to genotype dried samples from a historical collection. Cross-species amplification with six additional *Papilio* species showed that most loci can be used to study genetic variation in other closely related species of tiger swallowtails.

Phylogeography and counter-intuitive inferences in island biogeography: evidence from morphometric markers in the mobile butterfly *Maniola jurtina* (Linnaeus) (Lepidoptera, Nymphalidae)

Dapporto, L; Bruschini, C; Baracchi, D; Cini, A; Gayubo, SF; Gonzalez, JA; Dennis, RLH. 2009

Distribution of mobile organisms on near-continent islands is mainly shaped by factors operating over ecological rather geological time. However, the phylogeography of single species has the potential to expose historical factors at work. In the present study, West Mediterranean populations of the butterfly *Maniola jurtina* are studied using geometric morphometrics. The distribution of the two well established lineages (*Maniola jurtina jurtina* in the Atlanto-Mediterranean area and *Maniola jurtina janira* in the Central-Eastern-Mediterranean area) on 12 islands and the adjoining continents are compared. The south-western lineage unexpectedly occurs on islands close to shores occupied by the eastern lineage. We have modelled the distribution of the lineages using three different hypotheses: (1) a contemporary isolation model, which predicts lineage occupancy of islands is linked to relative distances from neighbouring continental areas; (2) a refugial hypothesis, which predicts one lineage to be the ancestral one for the whole region studied, and then successively replaced over part of it; (3) a changing geography hypothesis, which predicts the two lineages to have evolved in their currently occupied areas, continuously sourcing islands subsequent to the Wurm maximum glaciation. Of the three models, the refugial hypothesis is most highly correlated with the observed pattern, suggesting that Mediterranean islands may function as refugia during cold periods, much as the three mainland peninsulas of Iberia, Italy and Greece are known to have done. Thereafter, **hybridization** on the nearest and smallest islands has occurred, with the entire process supporting the notion of the joint influence of factors in ecological and geological time. (C) 2009 The Linnean Society of London, Biological Journal of the Linnean Society, 2009, 98, 677-692.

Interspecific and intraspecific comparisons of ejaculates in the cotton bollworm *Helicoverpa armigera* and the tobacco budworm *H. assulta*

Teng, ZQ; He, Q; Li, HT; Zhang, QW. 2009

The evolution of reproductive isolation is a prerequisite in the formation of new species. Although there are numerous studies on ejaculates in lepidopteran insects, ejaculate comparisons among sibling species have not been adequately addressed to understand possible reproductive barriers to **hybridization**. Here, we examined the interspecific and intraspecific variations of ejaculates in the sibling noctuid moths *Helicoverpa armigera* and *Helicoverpa assulta*. We found that there were considerable variations in the number of apyrene and eupyrene sperm and the length of eupyrene sperm. Male pupal mass explained not only a significant proportion of the variation in apyrene sperm number in both *H. armigera* and *H. assulta*, but also a significant proportion of the variation in eupyrene sperm number in *H. assulta*. There was a significant positive relationship between the number of eupyrene sperm and the number of apyrene sperm in both species. No difference in the length of eupyrene sperm was found between them; however, ejaculates of *H. armigera* had many more eupyrene sperm than *H. assulta* had. In *H. armigera*, large males generally mated with large females. The evolutionary consequences of these differences are discussed in this paper.

Introgression as a likely cause of mtDNA paralogy in two allopatric skippers (Lepidoptera: Hesperidae)

Zakharov, EV; Lobo, NF; Nowak, C; Hellmann, JJ. 2009

Gene transfer between species during interspecific **hybridization** is a widely accepted reality in plants but is considered a relatively rare phenomenon among animals. Here we describe a unique case of mitochondrial DNA (mtDNA) paralogy in the skipper genus, *Erynnis*, that involves well-diverged allopatric species. Using molecular evidence from both mitochondrial and nuclear genomes, we found high levels of intraspecific divergence in the mitochondrial genome within *E. propertius* (over 4% pair-wise sequence divergence) but no such differentiation in the nuclear genome. Sequence comparisons with related *Erynnis* suggest that past, but recent and infrequent introgression between *E. propertius* and *E. horatius* is the most reasonable explanation for the observed pattern of mtDNA paralogy. This example of putative introgression highlights the complexity of mtDNA evolution and suggests that similar processes could be operating in other taxa that have not been extensively sampled. Our observations reinforce the importance of involving multiple genes with different modes of inheritance in the analysis of population history of congeneric taxa. Heredity (2009) 102, 590-599; doi:10.1038/hdy.2009.26; published online 18 March 2009

Assessment of Napier millet (*Pennisetum purpureum* x *P. glaucum*) and sorghum (*Sorghum bicolor*) trap crops for the management of *Chilo partellus* on maize

Hari, NS; Jindal, J. 2009

Two Napier millet (*Pennisetum purpureum* x *P. glaucum*) **hybrids**, namely PBN 83 and PBN 233 and one sorghum (*Sorghum bicolor*) variety, SL 44, were assessed for their potential role as a trap crop in the management of the stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) on maize. Oviposition preference and larval survival and development were determined for different test plants under laboratory and screen house conditions. Further, field dispersal of *C. partellus* larvae was assessed between Napier millet and maize crops. Results from no-choice and dual-choice tests indicated that Napier millet **hybrids** were preferred for oviposition over maize by *C. partellus* moths. Sorghum was, however, not preferred over maize in this respect. Napier millet **hybrids** were poor larval hosts, and a rapid decline in larval numbers was noticed within the first five days after hatching and virtually no larvae survived to pupation. Leaf area eaten by the borer larvae was significantly less on these **hybrids** than on maize or sorghum. Plant damage was more severe in maize and sorghum than Napier millet **hybrids**. No appreciable larval shift was noticed from Napier millet **hybrids** to the adjoining maize crop. The evaluated Napier millet **hybrids**, therefore, had potential for use as trap crop in *C. partellus* management. Sorghum, however, did not hold promise in this respect,

Evaluation of Economically Important Traits from Sixteen Parental Strains of the Silkworm *Bombyx mori* L (Lepidoptera: Bombycidae)

Zanatta, DB; Bravo, JP; Barbosa, JF; Munhoz, REF; Fernandez, MA. 2009

The classification and characterization of silkworm strains are important for sericulture, which is supported by the constant development of new **hybrids**. In this study, 16 parental strains of *Bombyx mori* L from the germplasm banks of the Universidade Estadual de Maringá - UEM, and Associação dos Criadores de Bicho-da-Seda de Nova Esperança e Regiões Serícolas do Paraná - ACESP, were evaluated regarding biological and productive traits economically important. The Chinese C122-B and C121-A, and the Japanese HA-A and HA-B strains yielded the highest cocoon weight, which is related to the raw silk percentage. Our data will be useful in breeding programs for the production of superior silkworm strains and **hybrids**.

Toward the physiological basis for increased *Agrotis ipsilon* multiple nucleopolyhedrovirus infection following feeding of *Agrotis ipsilon* larvae on transgenic corn expressing Cry1Fa2

Schmidt, NR; Haywood, JM; Bonning, BC. 2009

Larvae of the black cutworm, *Agrotis ipsilon* Hufnagel, were more susceptible to infection by *A. ipsilon* multiple nucleopolyhedrovirus (AgipMNPV: Baculoviridae) after feeding on Herculex (R) I, a transgenic corn **hybrid** expressing the *Bacillus thuringiensis* (Bt)-derived toxin Cry1Fa2 compared to larvae fed on isoline corn. We investigated the physiological basis for increased susceptibility to virus infection following exposure to Herculex (R) I by analyzing the midgut pH, gut protease activity and peritrophic matrix structure which are important factors for both Bt toxin action and baculovirus infection. No significant treatment differences were found in the pH of anterior midgut, central midgut or posterior midgut in larvae fed Herculex (R) I or isoline diets. Analysis of soluble and membrane-associated gut proteinase activities from larvae fed Herculex (R) I or isoline diets indicated that membrane-associated aminopeptidase activity and soluble chymotrypsin-like proteinase activity were significantly lower in Herculex (R) I-fed larvae compared to isoline-fed larvae. The number and relative molecular masses of soluble chymotrypsin-like proteinases did not differ. Baculoviruses were not susceptible to in vitro degradation by bovine chymotrypsin, suggesting that chymotrypsin degradation of baculovirus occlusion-derived virus did not result in reduced infection of larvae fed on isoline diet. Scanning electron micrographs of the peritrophic matrices of Herculex (R) I-fed larvae and isoline-fed larvae indicated that Herculex (R) I did not result in damage to the peritrophic matrix that could facilitate subsequent baculovirus infection. Additional research is required to further delineate the physiological basis for enhanced baculovirus infection following exposure to sublethal doses of Bt toxins. (C) 2009 Elsevier Inc. All rights reserved.

Survey for Winter Moth (Lepidoptera: Geometridae) in Northeastern North America With Pheromone-Baited Traps and Hybridization With the Native Bruce Spanworm (Lepidoptera: Geometridae)

Elkinton, JS; Boettner, GH; Sermac, M; Gwiazdowski, R; Hunkins, RR; Callahan, J; Scheufele, SB; Donahue, CP; Porter, AH; Khirmian, A; Whited, BM; Campbell, NK. 2010

We used pheromone-baited traps to survey the distribution of winter moth, *Operophtera brumata* (L.) (Lepidoptera: Geometridae), a new invasive defoliator from Europe in eastern New England. The traps also attracted Bruce spanworm, *Operophtera bruceata* (Hulst) (Lepidoptera: Geometridae), native to North America. We distinguished between the two species by examining male genitalia and sequencing the mitochondrial cytochrome oxidase subunit I (COI) gene, the DNA barcoding region. In 2005, we recovered winter moths at sites stretching from eastern Long Island, southeastern Connecticut, all of Rhode Island, eastern Massachusetts, coastal New Hampshire, and southern coastal Maine. At sites further west and north we captured only Bruce spanworm. In 2006, we confirmed that both winter moth and Bruce spanworm are present in Nova Scotia and in coastal Maine, but only Bruce spanworm was recovered in coastal New Brunswick, Canada; Pennsylvania; Vermont; or Quebec City, Canada. In 2007, we collected Bruce spanworm, but no winter moths, in New Brunswick and the interior areas of Maine, New Hampshire, and New York. Winter moth and Bruce spanworm differed in the COI sequence by 7.45% of their nucleotides. The prevalence of intermediate genitalia in the zone of overlap suggested that **hybridization** between the two species may be occurring. To confirm the presence of **hybrids**, we sequenced the nuclear gene, glucose-6-phosphate dehydrogenase (G6PD). We identified six nucleotides that routinely distinguished winter moth and Bruce spanworm, of which three were always diagnostic. We showed that eggs produced by **hybridizing** the two species in the laboratory contained copies of both species at these six sites. We found that most of the moths collected in the field with intermediate genitalia had winter moth COI and GOD sequences and thus were not **hybrids** (or at least F1 **hybrids**). We found three **hybrids** out of 158 moths with intermediate genitalia in the region both species were caught. We conclude that **hybrids** occur in nature, but are not as common as previously reported. Introgression of genes between the two species may still be significant.

Secondary contact between *Lycaeides idas* and *L. melissa* in the Rocky Mountains: extensive admixture and a patchy hybrid zone

Gompert, Z; Lucas, LK; Fordyce, JA; Forister, ML; Nice, CC. 2010

Studies of **hybridization** have increased our understanding of the nature of species boundaries, the process of speciation, and the effects of **hybridization** on the evolution of populations and species. In the present study we use genetic and morphological data to determine the outcome and consequences of secondary contact and **hybridization** between the butterfly species *Lycaeides idas* and *L. melissa* in the Rocky Mountains. Admixture proportions estimated from structure and geographical cline analysis indicate *L. idas* and *L. melissa* have **hybridized** extensively in the Rocky Mountains and that reproductive isolation was insufficient to prevent introgression for much of the genome. Geographical patterns of admixture suggest that **hybridization** between *L. idas* and *L. melissa* has led to the formation of a **hybrid** zone. The **hybrid** zone is relatively wide, given estimates of dispersal for *Lycaeides* butterflies, and does not show strong evidence of cline concordance among characters. We believe the structure of the *Lycaeides* **hybrid** zone might be best explained by the patchy distribution of *Lycaeides*, local extinction and colonization of habitat patches, environmental variation and weak overall selection against **hybrids**. We found no evidence that **hybridization** in the Rocky Mountains has resulted in the formation of independent **hybrid** species, in contrast to the outcome of **hybridization** between *L. idas* and *L. melissa* in the Sierra Nevada. Finally, our results suggest that differences in male morphology between *L. idas* and *L. melissa* might contribute to isolation, or perhaps even that selection has favoured the spread of *L. melissa* male genitalia alleles.

Upslope movements and large scale expansions: the taxonomy and biogeography of the *Coenonympha arcania*-*C. d. arwiniana*-*C. gardetta* butterfly species complex

Schmitt, T; Besold, J. 2010

Sibling species groups are suitable models for the understanding of inter and intraspecific processes in taxonomy and biogeography. We analysed 262 individuals from the Alps of the *Coenonympha arcania* *lgardetta* species complex by allozyme electrophoresis. These taxa showed high variance amongst populations (F_{st} 0.391) and strong intertaxon genetic differentiation (F_{CT}: 0.376). Although morphologically similar, *Coenonympha gardetta* and *Coenonympha arcania* clearly differ in their genetic characteristics; the morphologically intermediate taxa *Coenonympha darwiniana darwiniana* and *Coenonympha darwiniana macromma* are genetically well distinguished from each other and the two other taxa. *Coenonympha arcania* and *C. d. macromma* most probably share a common ancestor and evolved by cladogenesis, whereas the taxonomic situation of *C. d. darwiniana* is still unresolved: This taxon might be the result of **hybridization** between *C. arcania* and *C. gardetta* or it might have a common ancestor together with *C. gardetta*. We suggest species rank for all four taxa. The distribution of genetic diversity of these populations and the differentiation amongst populations suggest rather different biogeographical scenarios: *C. arcania* most probably is of Mediterranean origin with postglacial range expansion northwards; *C. gardetta* survived the last ice age in peripheral refugia of the Alps and has spread all over this high mountain system in the postglacial; *C. darwiniana* and *C. macromma* survived the Würm in geographic proximity to their actual distribution areas and only have performed moderate uphill translocations during postglacial warming. (C) 2010 The Linnean Society of London, Zoological Journal of the Linnean Society, 2010, 159, 890-904.

Effect of Planting Date and Hybrid Selection on *Helicoverpa zea* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Damage on Maize Ears in Northeastern Mexico

Rodriguez-del-Bosque, LA; Cantu-Almaguer, MA; Reyes-Mendez, CA. 2010

This study determined the impact of planting date and kernel color variety (yellow vs. white) on the incidence of the maize, *Zea mays* L., ear insects corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J.E. Smith), in northern Tamaulipas, Mexico, from 2006 to 2009. Eight **hybrids** from six to 11 planting dates from late December to early March were evaluated each year. Overall, corn earworm and fall armyworm comprised 89 and 11%, respectively, of all Lepidoptera larvae inspected at the maize milk stage. Damage at harvest by ear insects was greater during the earlier planting dates (December), diminished during the intermediate dates (January-early February), and increased again at later planting dates (late February-March). Damage to ears of yellow maize **hybrids** was 53%

greater than damage to white **hybrids**, probably because of a differential grain hardness and husk wrap. However, H-443A, a yellow **hybrid** with good grain and husk characteristics for the region was less damaged by corn earworm and fall armyworm than were yellow **hybrids** imported from the U.S.

Molecular phylogenetics of the neotropical butterfly subtribe Oleriina (Nymphalidae: Danainae: Ithomiini)

de-Silva, DL; Day, JJ; Elias, M; Willmott, K; Whinnett, A; Mallet, J. 2010

The Oleriina is one of the most speciose subtribes of the neotropical nymphalid butterfly tribe ithomiini. They are widely distributed across the Andes and Amazonian lowlands and like other ithomiines they are involved in complex mimicry rings. This subtribe is of particular interest because it contains the most diverse ithomiine genus, Oleria, as well as two genera, Megoleria and Hyposcada, that feed on hostplants not utilized elsewhere in the tribe. Here we present the first comprehensive species-level phylogeny for the Oleriina, representing 83% of recognised species in the group, and based on 6698 bp from eight mitochondrial (mt) and nuclear (nc) genes. Topologies are largely congruent for ncDNA and the concatenated dataset and the genera Oleria, Hyposcada and Megoleria are recovered and well-supported, although strongly discordant genealogy between mtDNA and ncDNA suggest possible introgression among Hyposcada and Megoleria. A fourth clade containing the type species of ollantaya is consistently recovered, and this recently synonymized name is resurrected. Clear subdivisions within Oleria separate the genus into four species groups, onega, amalda, makrena and aegle, which also correspond to differing biogeographic and elevation range characteristics. Unlike other ithomiine genera, the Oleriina show homogeneity in mimetic wing pattern, in sharp contrast to the emerging paradigm that mimetic shifts have enhanced diversification in the tribe. Our results show a potentially more important role for geographic isolation in the diversification of the Oleriina compared to other Ithomiini studied to date and provide a framework for more detailed biogeographical studies, in addition to a rare opportunity for comparative analyses with other neotropical groups. (C) 2010 Elsevier Inc. All rights reserved.

Life Table Studies of Elasmopalpus lignosellus (Lepidoptera: Pyralidae) on Sugarcane

Sandhu, HS; Nuessly, GS; Webb, SE; Cherry, RH; Gilbert, RA. 2010

The lesser cornstalk borer, Elasmopalpus lignosellus (Zeller) (Lepidoptera: Pyralidae) is an important pest of sugarcane (a complex **hybrid** of Saccharum spp.) in southern Florida. Reproductive and life table parameters for E. lignosellus were examined at nine constant temperatures from 13 to 36 degrees C with sugarcane as the larval food source. The pre- and postoviposition periods decreased with increasing temperatures and reached their minimums at 33 and 36 degrees C, respectively. The oviposition period was longest at 27 degrees C. The mean fecundity, stage-specific survival, stage-specific fecundity, intrinsic rate of increase, and finite rate of increase were greatest at 30 degrees C and decreased with increasing or decreasing temperature. The net reproductive rate was greatest at 27 degrees C. The Logan-6 model best described the relationship between temperature and intrinsic rate of increase. The generation and population doubling times were longest at 13 and shortest at 33 and 30 degrees C, respectively. The most favorable temperatures for E. lignosellus population growth were between 27 and 33 degrees C. Life table parameters for E. lignosellus reared on sugarcane were greater than for the Mexican rice borer [Eoreuma loftini (Dyar) (Lepidoptera: Crambidae)] reared on an artificial diet at 30 degrees C. The intrinsic rates of increase for the sugarcane borer [Diatraea saccharalis (F.) (Lepidoptera: Crambidae)] reared on sugarcane or corn were the same as for E. lignosellus reared on sugarcane at 27 degrees C, but the net reproductive rate was four times higher for the former than the latter borer species.

Effects of Bt Maize on Agrotis segetum (Lepidoptera: Noctuidae): A Pest of Maize Seedlings

Erasmus, A; Van Rensburg, JBJ; Van Den Berg, J. 2010

The lepidopteran stemborers Busseola fusca (Fuller) (Lepidoptera: Noctuidae), Sesamia calamistis (Rawson) (Lepidoptera: Noctuidae), and Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) are effectively controlled by Bt maize that expresses the CryIAb insecticidal protein. Another noctuid species, the cutworm Agrotis segetum (Denis and Schiffermüller) (Lepidoptera: Noctuidae), which is the most common and injurious pest of maize seedlings in South Africa, is exposed to Bt toxin for a part of its life cycle. The effect of this exposure to Bt maize has not been studied yet. The aims of this study were to determine the effects of Bt maize (events MON810 and Bt11) on larval mass, development time, survival, and fecundity of A. segetum. Laboratory studies were conducted with first- and fourth-instar larvae and moths. Results showed that the effect of CryIAb toxin on the biology of A. segetum larvae and moths were largely insignificant. The effects of the two Bt maize events on the different parameters measured in this study was not similar between the Bt events and their respective iso-**hybrids**. Compared with larvae that fed on conventional (non-Bt) maize, Bt maize did not affect survival of first-instar larvae. However, mean mass of larvae that fed on Bt maize (Bt11) was significantly lower. Feeding on Bt maize did not have a significant effect on development and survival of fourth-instar larvae or moth longevity. It did, however, delay the development period to pupa formation. Fewer eggs were laid by moths fed as larvae on maize event Bt11 compared with MON810. This study indicates that Bt maize will most likely not have any significant effect on the control of A. segetum under field conditions.

Differential introgression causes genealogical discordance in host races of Acrocercops transecta (Insecta: Lepidoptera)

Ohshima, I; Yoshizawa, K. 2010

Recently diverged populations often exhibit incomplete reproductive isolation, with a low level of gene flow continuing between populations. Previous studies have shown that, even under a low level of gene flow, genetic divergence between populations can proceed at the loci governing local adaptation and reproductive isolation but not at other neutral loci. A leaf-mining moth, Acrocercops transecta, consists of Juglans- and Lyonia-associated host races. The two host races differ in host preferences of ovipositing females and in larval adaptation to host plants but mate readily in the laboratory, producing fertile **hybrids**. The Juglans and Lyonia races are often sympatric in the wild, implying that gene introgression could occur in nature between the two host races. We tested this hypothesis by combining phylogenetic analyses with coalescent simulations, focusing on mitochondrial genes (COI and ND5) and the nuclear Tpi, Per and Ldh genes located on the Z-chromosome. The mitochondrial genes clearly distinguished the Lyonia race from the Juglans race, whereas the Tpi, Per and Ldh genealogies did not reflect the two host races. Coalescent simulations indicated gene flow at the three Z-linked genes in both directions, whereas there was no introgression in the mitochondrial genes. The lack of introgression in mitochondrial genes suggests that female host preference is the primary force leading to the bifurcation of maternally inherited loci. Thus, the results show that a low level of gene flow coupled with the inflexible female host preference differentiates histories of divergence between maternally and biparentally inherited genes in this host race system.

AFLP Linkage Map of Hybridizing Swallowtail Butterflies, Papilio glaucus and Papilio canadensis

Winter, CB; Porter, AH. 2010

High-density linkage maps provide powerful tools for studying the genetic basis of ecologically relevant adaptations and the genomic scope of introgression. We back-crossed an F-1 **hybrid** male Papilio glaucus/Papilio canadensis tiger swallowtail butterfly to a pure P. glaucus female and constructed amplified fragment length polymorphism linkage maps from the progeny. The paternal map contains 309 markers distributed among 29 linkage groups, with a corrected map distance of 1167 cM [logarithm of the odds [LOD] = 4.0]. The average linkage group contained 10.65 +/- 4.85 markers separated by 32.7 +/- 3.8 cM, with statistically significant clustering. The paternal **hybrid** map had 18.65% more markers than the maternal P. glaucus map, which provides a rough estimate of the extent of genetic

differentiation between the species. The maternal map contains 253 markers among 28 linkage groups, without the X and Y chromosomes. Segregation distortion from expected Mendelian ratios was observed for 94/1096 scored loci (8.6%, $P < 0.05$). The X chromosome map includes 7 markers spanning 29.3 cM (LOD = 3.0). These naturally **hybridizing**, female heterogametic species are used to study important questions in the maintenance of species boundaries, sex chromosome introgression, sex-limited mimicry, and host plant use. The map will facilitate research into the physiological, ecological, and evolutionary genetics of these phenomena.

ARE SPECIES REAL? THE SHAPE OF THE SPECIES BOUNDARY WITH EXPONENTIAL FAILURE, REINFORCEMENT, AND THE "MISSING SNOWBALL"

Gourbiere, S; Mallet, J. 2010

Under simple assumptions, the evolution of epistatic "Dobzhansky-Muller" incompatibilities between a pair of species should yield an accelerating decline of log overall reproductive compatibility-a "snowball" effect that might rapidly provide new species with "reality." Possible alternatives include: (1) simple exponential failure, giving a linear rate of log compatibility loss, and (2) "slow-down," likely during reinforcement in which mate choice evolves to prevent deleterious **hybridization**, yielding a decelerating log compatibility loss. In analyses of multiple datasets, we find little support for the snowball effect, except possibly in Lepidoptera **hybrid** viability. The snowball predicts a slow initial rate of incompatibility acquisition, with low initial variance; instead, highly variable compatibility is almost universally observed at low genetic distances. Another deviation from predictions is that reproductive isolation usually remains incomplete until long after speciation. These results do not disprove snowball compatibility decay, but can result if large deleterious effects are due to relatively few genetic changes, or if different types of incompatibility evolve at very different rates. On the other hand, data on *Bacillus* and *Saccharomyces*, as well as theories of chromosomal evolution, suggest that some kinds of incompatibility accumulate approximately linearly, without Dobzhansky-Muller effects. In microorganisms, linearity can result from direct negative effects of DNA sequence divergence on compatibility. Finally, a decelerating slowdown model is supported for sympatric *Leptasterias* starfish, and in *Drosophila* prezygotic isolation in sympatry but not allopatry, providing novel comparative evidence for reinforcement.

Host-associated pre-mating reproductive isolation between host races of *Acrocercops transecta*: mating site preferences and effect of host presence on mating

Ohshima, I. 2010

1. A correlation between habitat and mating site preference is crucial for reproductive isolation between populations with different ecological requirements in the absence of any other barriers to gene flow. In phytophagous insects, host-plant preference of ovipositing females has been postulated to directly influence the extent of assortative mating. 2. Although this pleiotropic effect of host preference is commonly postulated in both theoretical and empirical studies in phytophagous insects, few studies have assessed the mating site preferences of both sexes. In herbivorous insects, it is crucial whether males have the same host preference as ovipositing females. 3. This study examines whether males and females have preferences for mating sites and assesses the effects of host presence on mating activity in a leaf-mining moth, *Acrocercops transecta* Meyrick (Gracillariidae). This species consists of two host races, which are associated with *Juglans ailanthifolia* Carr. (Juglandaceae) and *Lyonia ovalifolia* (Wall.) Drude (Ericaceae). The two host races clearly differ in host preferences of ovipositing females. 4. In an experiment of mating site selection, *Lyonia*-associated host race females significantly preferred *Lyonia*, while males of the *Lyonia*-associated host race and both sexes of the *Juglans*-associated host race did not show a preference for either plant. The mating activity of the *Lyonia*-associated host race was not reduced in the absence of host plants. In the *Juglans*-associated host race, however, the proportion of successful matings was significantly reduced when the host was absent. 5. These laboratory experiments provide empirical evidence that the different host associations in *A. transecta* do not directly lead to host-induced assortative mating between the two host races as a pleiotropic effect. However, the present results suggest that the combined effects of the mating-site preference of *Lyonia*-associated females and the mating propensity of *Juglans*-associated females could contribute to reproductive isolation between the two host races.
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A re-evaluation of the *Aricoris constantius* group with the recognition of three species (Lepidoptera: Riodinidae)

Callaghan, C.J. 2010

Material currently classified under the name *Aricoris constantius* (Fabricius, 1793) is examined and found to consist of three distinct species inhabiting southeastern and central Brazil, from the Brazilian state of Bahia to Paraguay. They are *A. constantius*, *A. tutana* (Godart, [1824]), reinstated status and *A. monotona* (Stichel, 1910), reinstated status, the latter two removed from synonymy. Besides significant morphological differences in wing pattern and genitalia, their status as separate species is supported by their overlapping ranges where each phenotype is sympatric with at least one other, with no evidence of **hybridization**. Notes are provided on the habitat in open formations, range and distribution in south eastern Brasil, and biology, including evidence of myrmecophily.

COCOON PRODUCTION OF THE SILKWORM, *Bombyx mori* L. (LEPIDOPTERA: BOMBYCIDAE), FED ON LEAVES OF MULBERRY HYBRIDS

de Mendonca, GA; Marchini, LC; Macedo, LPM. 2010

Brazil is the fourth cocoon producer in the world. In Sao Paulo State there are mulberry some **hybrids** whose productivity are higher than the commonly cultivated varieties. The objective of this study was to evaluate the effect of mulberry **hybrids** (*Morus* spp.) on the cocoon production of silkworm (*Bombyx mori* L.). The experiment was conducted at the Unidade Regional de Pesquisa de Galia do Instituto de Zootecnia, SP. The caterpillars were fed on leaves of the **hybrids** IZ-3/2, IZ-13/6, IZ-15/7, IZ-19/13, IZ-56/4, IZ-57/2, IZ-40, IZ-64, in a rearing hut at 25 degrees C +/- 3 degrees C and 75% +/- 5% relative humidity. 'Korin' was used as standard. The **hybrids** affected the duration of the larval period and the weight of the caterpillars, prepupae and the silk glands as well. There was a reduction in the duration of larval development when the caterpillars had been fed with **hybrid** IZ-56/4 and the 'Korin' variety. Hybrids IZ-57/2, IZ-56/4 and IZ-15/7 presented the highest cocoon production.

Survey for Winter Moth (Lepidoptera: Geometridae) in Northeastern North America With Pheromone-Baited Traps and Hybridization With the Native Bruce Spanworm (Lepidoptera: Geometridae) (vol 103, pg 135, 2010)

Elkinton, JS; Boettner, GH; Sremac, M; Gwiazdowski, R; Hunkins, RR; Callahan, J; Scheufele, SB; Donahue, CP; Porter, AH; Khirmian, A; Whited, BM; Campbell, NK. 2010

Expression of immune-response genes in lepidopteran host is suppressed by venom from an endoparasitoid, *Pteromalus puparum*

Fang, Q; Wang, L; Zhu, JY; Li, YM; Song, QS; Stanley, DW; Akhtar, ZR; Ye, GY. 2010

Background: The relationships between parasitoids and their insect hosts have attracted attention at two levels. First, the basic biology of host-parasitoid interactions is of fundamental interest. Second, parasitoids are widely used as biological control agents in sustainable agricultural programs. Females of the gregarious endoparasitoid *Pteromalus puparum* (Hymenoptera: Pteromalidae) inject venom along with eggs into their hosts. *P. puparum* does not inject polydnaviruses during

oviposition. For this reason, *P. puparum* and its pupal host, the small white butterfly *Pieris rapae* (Lepidoptera: Pieridae), comprise an excellent model system for studying the influence of an endoparasitoid venom on the biology of the pupal host. *P. puparum* venom suppresses the immunity of its host, although the suppressive mechanisms are not fully understood. In this study, we tested our hypothesis that *P. puparum* venom influences host gene expression in the two main immunity-conferring tissues, hemocytes and fat body. Results: At 1 h post-venom injection, we recorded significant decreases in transcript levels of 217 EST clones (revealing 113 genes identified in silico, including 62 unknown contigs) derived from forward subtractive libraries of host hemocytes and in transcript levels of 288 EST clones (221 genes identified in silico, including 123 unknown contigs) from libraries of host fat body. These genes are related to insect immune response, cytoskeleton, cell cycle and apoptosis, metabolism, transport, stress response and transcriptional and translational regulation. We verified the reliability of the suppression subtractive **hybridization** (SSH) data with semi-quantitative RT-PCR analysis of a set of randomly selected genes. This analysis showed that most of the selected genes were down-regulated after venom injection. Conclusions: Our findings support our hypothesis that *P. puparum* venom influences gene expression in host hemocytes and fat body. Specifically, the venom treatments led to reductions in expression of a large number of genes. Many of the down-regulated genes act in immunity, although others act in non-immune areas of host biology. We conclude that the actions of venom on host gene expression influence immunity as well as other aspects of host biology in ways that benefit the development and emergence of the next generation of parasitoids.

Bromatological Characterization of Mulberry Cultivars, *Morus* spp., and Determination of Nutritional Indexes of *Bombyx mori* L. (Lepidoptera: Bombycidae)

Meneguim, AM; Lustri, C; de Oliveira, DD; Yada, IFU; Pasini, A. 2010

The objective of this work was to assess, through consumption and utilization of natural food measurements, whether mulberry cultivars, *Morus* spp., could be recommended to rear the silkworm, *Bombyx mori* L., in a commercial scale. The mulberry cultivars Miura (standard), Korin and Tailandesa and the **hybrids** FM 3/3, FM 86, SK I and SK 4 were tested. Seventy five fifth-instar commercial **hybrid** larvae were individualized in gerbox (R) unities and maintained in a rearing room (25 +/- 3 degrees C and 80 +/- 10% RH). The mulberry leaves of each of the five cultivars used to feed the silkworm larvae were submitted to bromatological analysis. The dry weight of larvae at the beginning and at the end of the fifth instar, the food consumed and the feces eliminated were recorded to determine the following indexes: relative consumption rate (RCR), relative metabolic rate (RMR), relative growth rate (RGR), approximate digestibility (AD), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), metabolic cost (MC). The bromatological composition analysis of mulberry leaf cultivars revealed that the **hybrid** SK 4 presented superior nutritional quality compared to the standard cultivar Miura, due to its higher content in crude protein and ethereal extract, and lower detergent fiber content. The **hybrid** SK 4 was the most adequate food to the silkworm larvae because it was ingested in small amounts, thus providing good digestibility to the larvae, low metabolic cost, good growth rate and one of the highest efficiencies in the conversion of the ingested food and ingested and digested in biomass.

Host Plant Association and Genetic Differentiation of Corn and Rice Strains of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Colombia

Saldamando, CI; Velez-Arango, AM. 2010

Spodoptera frugiperda (Smith) is a polyphagous insect of major economic impact in the western hemisphere and exhibits two strains (i.e., corn and rice) that are morphologically identical but differ in ecology, genetics and physiology. In this work we identified these strains and their respective **hybrids** by using a PCR-RFLP of the COI gene and PCR of the tandem region FR. Moreover, we performed a population structure analysis by using 253 larvae from Tolima, a region where *S. frugiperda* is a pest on corn, rice, sorghum and cotton. Corn strain was found on 42% in corn, 34% in cotton, 19% in sorghum and 0.04 % in rice and rice strain on 35% in corn, 0.06% in cotton, 0.06% in sorghum and 53% in rice, demonstrating that corn strain specificity is superior to rice strain. Hybrids between these strains were more abundant in corn. The distributions on their host plants reflect a population genetic differentiation in *S. frugiperda* with values of Φ_{IPT} (COI) = 0.31, $P < 0.0001$, Φ_{IPT} (FR) = 0.17, $P < 0.0001$ for all crops and Φ_{IPT} (COI) = 0.42, $P < 0.01$, Φ_{IPT} (FR) = 0.13, $P < 0.01$ for the sixteen sampled farms. The dendrograms showed two clusters representing both strains. The results obtained in this study suggest that the management of this insect must differ on each host plant, given the specialization that both strains present, particularly in corn and rice.

Speciation in Mediterranean refugia and post-glacial expansion of *Zerynthia polyxena* (Lepidoptera, Papilionidae)

Dapporto, L. 2010

Migration of populations to and from glacial refugia is responsible for various cases of speciation and subspeciation in Europe. The pattern of distribution and the degree of diversification between lineages originated by isolation in different glacial refugia usually depends on ecological traits, especially to their dispersal ability. *Zerynthia polyxena* is a philopatric species, scattered in small populations and rarely colonizing mountain areas. These characteristics probably caused repeated isolation during the Quaternary and may have favoured diversification. Actually two studies, based on both morphological and genetic data, suggest the existence of two highly distinct lineages in Europe having in Northern Italy their contact zone. In this study, I applied geometric morphometrics to male genitalia and demonstrated that (i) two morphotypes exist in Europe approximately facing on the two sides of the Po River; (ii) the two lineages probably survived glaciations in Italy and the Balkan Peninsula, respectively; then the Balkans lineage expanded to Central and Eastern Europe; (iii) no **hybrid** populations seem to exist in the contact area and, in one locality at least, the two lineages live in sympatry without any evidence of intermediates. These results suggest that (i) two sister species of *Zerynthia* exist in Europe. Accordingly, *Papilio cassandra* Geyer, 1828 is reinstated, as *Zerynthia cassandra* stat. rev., as the species to which the *Zerynthia* from Italy South of the Po River belong. Male genitalia differences with *Zerynthia polyxena* are described.

Shift happens! Shifting balance and the evolution of diversity in warning colour and mimicry

Mallet, J. 2010

2. Phase I, random local processes (including genetic drift and idiosyncratic selection), and phase III, interdemic selection are the most controversial phases of the shifting balance. Phase II consists of ordinary natural selection to a new adaptive peak within populations, and is uncontroversial. 3. *Heliconius* have bold patterns of iridescent blue, black, yellow, white, and red. These are clearly warning patterns, and near-perfect Mullerian mimicry has evolved among species, suggesting tight control by natural selection. Field experiments have also demonstrated strong selection (often $s > 0.1$ on single colour pattern loci), and the population structure of *Heliconius* is typically not conducive to phase I. Yet the colour patterns are clearly somewhat independent and incompatible signals of unpalatability. 4. There is empirical evidence in *Heliconius* for both controversial phases. For phase I, occasional and local polymorphisms of colour pattern in a number of species go against the generally expected (and generally observed) monomorphism for Mullerian mimics. 5. For phase III, one of the few colour pattern clines mapped in detail has been observed to move rapidly over a period of 20 years. There are also a number of curious 'leapfrog' geographical disjunctions in colour pattern races. Disjunctions are expected if successful races have spread from the centre of the range (e.g. the Amazonian rayed races) via phase III, in competition with earlier races that are now distributed in scattered places along the periphery of the range. 6. Evidence from the genomes of *Heliconius* may in the near future aid in understanding colour pattern 'supergenes' and to help test for origin and spread via shifting balance.
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Hybridisation and climate change: brown argus butterflies in Britain (*Polyommatus* subgenus *Aricia*)

Mallet, J; Wynne, IR; Thomas, CD. 2011

1. Distribution changes brought about by climate change are likely to alter levels of **hybridisation** between related taxa, and may threaten some species. 2. Nuclear (Tpi) and mitochondrial (cytB) DNA sequence data give evidence for introgression between two related *Polyommatus* (subgenus *Aricia*) butterfly species in a 150-200 km wide overlap zone in northern England and North Wales. A history of **hybridisation** is evident from the mixture of genotypes present within this region: some populations contain southern-origin (*Polyommatus agestis*) mtDNA and northern-origin (*Polyommatus artaxerxes*) Tpi alleles, and many populations contain mixtures of Tpi alleles. 3. The timing of the original **hybridisation** is unknown, but could be immediately post-glacial or much more recent in origin. 4. Both species are now beginning to shift northwards, associated with recent climatic warming. 5. It is thus expected that anthropogenic climate change will unleash a new spate of **hybridisation**, potentially threatening the long-term survival of the northern species in Britain.

Differences in leafminer (*Phyllonorycter*, *Gracillariidae*, *Lepidoptera*) and aphid (*Tuberculatus*, *Aphididae*, *Hemiptera*) composition among *Quercus dentata*, *Q. crispula*, *Q. serrata*, and their hybrids

Hata, Y; Hashiba, T; Nakamura, T; Kitamura, M; Ishida, TA; Akimoto, S; Sato, H; Kimura, MT. 2011

Leafminer (*Phyllonorycter*, *Gracillariidae*, *Lepidoptera*) and aphid (*Tuberculatus*, *Aphididae*, *Hemiptera*) composition were studied in three deciduous oak species, *Quercus dentata*, *Q. crispula*, and *Q. serrata*, and their **hybrids** in Tomakomai Experimental Forest of Hokkaido University, Hokkaido, northern Japan. Identification of trees in this forest was done mainly on the basis of discriminant analysis on leaf morphology with reference to trees in pure *Q. dentata* and *Q. crispula* stands and a *Q. serrata* stand mixed with *Q. crispula*. The results suggested that **hybridization** occurred in all combinations (i.e. *Q. dentata*-*Q. crispula*, *Q. crispula*-*Q. serrata*, and *Q. serrata*-*Q. dentata*) and the frequency of **hybrids** was approximately 10%. The composition of *Phyllonorycter* and *Tuberculatus* species differed between *Q. dentata* and *Q. crispula* or *Q. serrata*, but did not differ between *Q. crispula* and *Q. serrata*. Thus, *Q. dentata* could differ from *Q. crispula* and *Q. serrata* in chemical properties that determine herbivore host selection, survival, and performance, possibly reflecting eco-physiological differences or phylogenetic distances. The study insects were divided into three groups: species specialized to *Q. dentata* (three *Phyllonorycter* and one *Tuberculatus* species), those to *Q. crispula* and *Q. serrata* (six *Phyllonorycter* and two *Tuberculatus* species), and a species collected at least from *Q. dentata* and *Q. crispula* (one *Tuberculatus* species). Putative **hybrid** trees of *Q. dentata* and *Q. crispula* harbored both *Q. dentata*-specific and *Q. crispula*-specific insects.

Morphological reinforcement, ancient introgressive hybridization and species delimitation in African stem-borer species of the genus *Sesamia* Guenee (*Lepidoptera*: *Noctuidae*)

Moyal, P; Le Ru, B; Van den Berg, J; Ratnadass, A; Cugala, D; Matama-Kauma, T; Pallangyo, B; Conlong, D; Defabachew, B. 2011

Species delimitation, an issue central to systematics and biodiversity studies, is addressed in the epunctifera group of the stem borer genus *Sesamia* Guenee (*Lepidoptera*: *Noctuidae*). This group is composed of four sub-Saharan species: *Sesamia poephaga* Tams & Bowden; *Sesamia epunctifera* Hampson; *Sesamia penniseti* Tams & Bowden; and *Sesamia poebora* Tams & Bowden, the taxonomic status of which was unclear. The first species was considered a possible synonym of the second, and the third species was considered a possible synonym of the fourth. An analysis combining morphological, ecological and molecular data enables us to conclude that *S. epunctifera* and *S. poephaga* are different species, and that *S. poebora* is a synonym of *S. penniseti*. Two new species were discovered: *Sesamia firmata* sp.n. and *Sesamia veronica* sp.n. *Sesamia firmata* sp.n. has atypical genitalic morphology, suggesting a strong selection resulting in a reinforcement of pre-zygotic isolation. Some specimens previously identified as *S. penniseti* on the basis of morphology are sisters to *S. epunctifera* on the mitochondrial tree, and are connected to *S. penniseti* on the nuclear tree. The mitochondrial distance from *S. penniseti* and *S. epunctifera* is 7.6% and 3.9%, respectively, suggesting an ancient mitochondrial introgression from *S. epunctifera* into *S. penniseti*. The possible causes of the reinforcement and introgressive **hybridization** are discussed. This case of mitochondrial introgression, uncommon in *Lepidoptera*, in which females are the heterogametic sex, may be an exception to Haldane's rule. The **hybrid** is assigned the rank of species and named *Sesamia pennipuncta* sp.n.

Larval pattern morphotypes in the Western Palaearctic *Hyles euphorbiae* complex (*Lepidoptera*: *Sphingidae*: *Macroglossinae*)

Hundsdoerfer, AK; Mende, MB; Harbich, H; Pittaway, AR; Kitching, IJ. 2011

External morphological variation in larval pattern elements in the *Hyles euphorbiae* complex is documented and described from independent samples of numerous populations across Europe and North Africa. Variability in the distribution of black cuticle and other colours was found to be much higher within populations than previously believed and it proved difficult to characterize any species, subspecies and even local population unequivocally using this feature alone. A representative sample of larvae from the Canary Islands, Madeira, North Africa, Iberia, Italy, Germany, Eastern Europe, Malta, Greece and Yemen, is illustrated, supplemented by observations from photographs of larvae from the Middle East and Western Asia. Taxonomy-independent definitions of larval morphotypes demonstrate significant overlap between species. We discuss the results in the context of Western Palaearctic biogeography, postulating several areas of **hybridisation** between the more northern *Hyles euphorbiae* and more southern *H. tithymali* that have led to the mosaic distribution patterns observed, and consider the potential of these moths for tracking the future effects of climate change in the Mediterranean Basin.

Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (*Lepidoptera*: *Tortricidae*)

Lumley, LM; Sperling, FAH. 2011

Species identifications have been historically difficult in the *Choristoneura fumiferana* group, an important insect pest complex. We examined the utility of simple sequence repeats (SSRs, also referred to as microsatellites) and mitochondrial DNA (mtDNA) for delimiting and identifying eight currently recognized species sampled across North America. Four of these species formed discrete clusters using SSRs, while only two species were delimited with mtDNA. There was evidence for **hybridization** or incomplete lineage sorting between several species pairs. An integrative approach, using both phenotypic traits and molecular markers, allowed for the discrimination of more biologically relevant species units than did the use of molecular markers alone. As species are currently identified using putatively adaptive phenotypic traits, the differences observed between recognized species and neutral SSRs or mtDNA suggests that these species (or evolutionary significant units) have diverged via natural selection in spite of some gene flow. (C) 2010 Elsevier Inc. All rights reserved.

Reproductive Isolation Between Two Populations of *Spodoptera frugiperda* (*Lepidoptera*: *Noctuidae*) Collected in Corn and Rice Fields From Central Colombia

Velasquez-Velez, MI; Saldamando-Benjumea, CI; Rios-Diez, JD. 2011

Spodoptera frugiperda (J.E. Smith) (*Lepidoptera*: *Noctuidae*) is a Neotropical moth that has diverged into corn, *Zea mays* L., and rice, *Oryza sativa* L., host strains because these plants are their most frequently used hosts. The corn strain also has been found in cotton, *Gossypium hirsutum* L., and sorghum, *Sorghum bicolor* (L.) Moench, and the rice strain in small grasses and pasture grasses. Studies of the reproductive isolation between these two strains have provided ambiguous results from populations in the United States. In Colombia, we tested pre- and postzygotic isolation in these strains. Both strains showed postzygotic isolation for several life-history traits, including number of egg masses, number of larvae, number of females, pupal developmental time, female and male longevity, and female and male pupal weight. We observed a reduction of the number of **hybrid** females and a reduction in fertility in **hybrids** in *S. frugiperda*. These results suggest the possibility of

Haldane's rule. Heterosis in the F₁(2) and F₂(1) generations was observed for number of larvae and adult longevity. This line presented a high standard deviation, suggesting instability in this cross. A possible effect of the X chromosome may explain the reduction in viability and sterility in F₁ **hybrids** of host strains of *S. frugiperda*. No temporal isolation was observed between the corn and rice strains. Differences in longevity between corn and rice strains might be another form of temporal isolation between these strains, because differences in adulthood time might reduce the encounters between them and thus **hybridization**.

Genetics of diapause in the comma butterfly *Polygonia c-album*

Soderlind, L; Nylin, S. 2011

The processes of local adaptation and ecological speciation can be better understood by studying the genetic background of life-history decisions. The sex chromosomes host genes for many population differences in the Lepidoptera and therefore the inheritance of diapause determination in the butterfly *Polygonia c-album* may be hypothesized to be sex-linked. In the present study, *Polygonia c-album* (L.) from Spain and Sweden and **hybrid** offspring are raised under an LD 17 : 7 h photocycle that induces most pure Swedish individuals to develop into the diapausing dark morph and most pure Spanish individuals into the light and directly-developing morph. If inheritance of the daylength threshold for diapause is X-linked, as is known to be the case for host-plant preferences, females should follow the developmental path of their male parents' populations. However, female **hybrids** instead have a diapause propensity intermediate to that of their parental stocks and, consequentially, diapause determination is not X-linked. However, male **hybrids** eclose as the diapausing morph to a higher extent than females and, moreover, this pattern is more pronounced in the Spanish female x Swedish male cross than in the reciprocal cross. Hence, it is concluded that the genetic determination of the critical daylength for diapause is mainly autosomal but with some influence of sex-linked genes and/or parental effects, possibly as an effect of the importance of protandry for males. Such sex effects could provide a starting point for the evolution of population differences inherited on the sex chromosomes.

Genetic Variation and Inheritance of Diapause Induction in Two Distinct Voltine Ecotypes of *Ostrinia nubilalis* (Lepidoptera: Crambidae)

Ikten, C; Skoda, SR; Hunt, TE; Molina-Ochoa, J; Foster, JE. 2011

European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae), displays a larval diapause in response to short photoperiods and is adapted to a variety of local conditions throughout North America. Hence, the effective photoperiod inducing larval diapause will differ among geographic ecotypes. This study considers the inheritance of photoperiodic larval diapause induction by **hybridization** and backcrossing two latitudinally distinct ecotypes of the European corn borer collected between 41 degrees N, 96 degrees W and 48 degrees N, 96 degrees W and under a range of photoperiods representative of their respective locations: from 14:10 to 16:8 (L:D) h. The ecotype adapted to a bivoltine habitat (southeastern Nebraska) exhibited a shorter critical photoperiod (1480 h) than the ecotype (1533 h), originating from a univoltine habitat (northwestern Minnesota). Reciprocal F₁ crosses exhibited intermediate values with indication of sex-linked inheritance. In addition, the male parent had significantly more influence on diapause incidence of subsequent progeny than the female. The F₂ and backcross progeny further supported the supposition that diapause response is a sex-linked inheritance. The minimum number of genes estimates, and the response from backcross progeny, suggest that diapause response of European corn borer larva may be controlled by only a few loci. The overall results indicated that both ecotypes had adopted unique diapause responses, which ultimately lead to seasonal synchrony in their ecosystems.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and random amplified polymorphic DNA (RAPD) based genetic variation studies in eri silkworm (*Samia cynthia ricini* Lepidoptera: Saturniidae)

Singh, HR; Unni, BG; Neog, K; Bhattacharyya, M. 2011

Four strains of eri, *Samia cynthia ricini* Lepidoptera: Saturniidae that can be identified morphologically and maintained at North East Institute of Science and Technology, Jorhat were characterized based on their protein profile by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and DNA by random amplified polymorphic DNA (RAPD) technique. Fiber yield was highest in Gs strain. SDS-PAGE profile showed 11 prominent bands in the strains with molecular weight ranging from 35 to 200 kDa together with 28 minor bands with molecular weight ranging from 33 to 210 kDa. Two polypeptides of molecular weight 90 and 110 kDa were absent in both Y and Ys. Eight random primers and one universal primer used for RAPD analysis generated a total of 79 bands, of which 49 were polymorphic. In both SDS-PAGE and RAPD, the UPGMA based dendrogram showed two clusters: cluster 1 included Gs and G, whereas Y and Ys was grouped in cluster 2 by SDS-PAGE analysis but RAPD analysis grouped Ys and G in cluster 1 and Gs and Y in cluster 2. The range of genetic diversity observed among the strains affirms the potentiality of RAPD technique for identification and selection of distant parents for silkworm **hybridization** for high silk yield.

Hairpencil Volatiles Influence Interspecific Courtship and Mating Between Two Related Moth Species

Hillier, NK; Vickers, NJ. 2011

Reproductive isolation between sympatric, closely related species can be accomplished through a variety of pre-zygotic isolating mechanisms, including courtship-signaling behavior that involves pheromones. In the moths *Heliothis virescens* and *H. subflexa*, males display abdominal hairpencils (HP), which release volatile chemicals during courtship. In this study, we demonstrated that HP volatiles released by male *H. subflexa* function to improve mating success with conspecific females. Interspecific mating experiments were conducted to determine any influence of HP volatiles on species isolation. Female *H. virescens* and *H. subflexa* were observed during courtship with males of the other species, following either sham-operation or ablation of HPs, both with and without concurrent presentation of HP volatiles. Mating success was improved by co-presentation of HP extract from males of the same species during courtship. Ablation of HPs improved mating between *H. subflexa* females and *H. virescens* males. During interspecific matings, male *H. virescens* attempted copulation less frequently in the presence of *H. virescens* HP extract, though *H. subflexa* males were not affected by the presence of *H. subflexa* HP extract. This suggests that HP volatiles produced by males of each species may inhibit mating between species through effects on males (*H. virescens*) and females (*H. subflexa*).

Male death resulting from hybridization between subspecies of the gypsy moth, *Lymantria dispar*

Higashiura, Y; Yamaguchi, H; Ishihara, M; Ono, N; Tsukagoshi, H; Yokobori, S; Tokishita, S; Yamagata, H; Fukatsu, T. 2011

We explored the origin of all-female broods resulting from male death in a Hokkaido population of *Lymantria dispar* through genetic crosses based on the earlier experiments done by Goldschmidt and by testing for the presence of endosymbionts that are known to cause male killing in some insect species. The mitochondrial DNA haplotypes of the all-female broods in Hokkaido were different from those of normal Hokkaido females and were the same as those widely distributed in Asia, including Tokyo (TK). Goldschmidt obtained all-female broods through backcrossing, that is, F₁ females obtained by a cross between TK females (*L. dispar japonica*) and Hokkaido males (*L. dispar praeterea*) mated with Hokkaido males. He also obtained all-male broods by mating Hokkaido females with TK males. Goldschmidt inferred that female- and male-determining factors were weakest in the Hokkaido subspecies and stronger in the Honshu (TK) subspecies. According to his theory, the females of all-female broods mated with Honshu males should produce normal sex-ratio broods, whereas weaker Hokkaido sexes would be expected to disappear in F₁ or F₂ generations after crossing with the Honshu subspecies. We confirmed both of Goldschmidt's results: in the case of all-female broods mated with Honshu males, normal sex-ratio broods were produced, but we obtained only all-female broods in the Goldschmidt backcross and obtained an all-male brood in the F₁ generation of a Hokkaido female crossed with a TK male. We found no endosymbionts in all-female broods by 4,06-diamidino-2-phenylindole (DAPI) staining.

Lesser Peachtree Borer (Lepidoptera: Sesiidae) Oviposition on Prunus Germplasm

Cottrell, TE; Beckman, TG; Horton, DL. 2011

The lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson) (Lepidoptera: Sesiidae), is a serious pest of peach, *Prunus persica* (L.) Batsch, across the southeastern United States. We examined oviposition by *S. pictipes* on field-grown *Prunus* scion and rootstock cultivars and two endemic *Prunus* spp. when sawn limbs, not roots, were assayed in the laboratory. A choice test compared oviposition on the peach scion 'Harvester', peach rootstock 'Guardian', plum x peach **hybrid** rootstock 'MP-29', and the plum **hybrid** rootstock 'Sharpe'. A significantly lower percentage of eggs occurred on limbs of Sharpe rootstock than other choices. A choice test using two endemic hosts, black cherry (*P. serotina* Ehrh.) and Chickasaw plum (*P. angustifolia* Marsh.), along with Sharpe rootstock, found a lower percentage of eggs on limbs of Sharpe than either endemic host. However, when only limbs of Sharpe and a decoy were used, almost all eggs were laid on Sharpe. Interestingly, when Harvester and Sharpe limbs were paired side by side, a higher percentage of eggs were recovered from the Harvester limb than from the Sharpe limb. An analysis of volatiles from Sharpe may identify why fewer eggs were laid on it. Because *S. pictipes* attacks host trees above ground and Sharpe rootstock on grafted trees grows below ground, this rootstock might be a management option against the congeneric, root-attacking peachtree borer, *S. exitiosa* (Say). Our results suggest that high budding a peach scion onto Sharpe rootstock, thus allowing the rootstock to serve as the trunk, warrants further investigation against *S. exitiosa* under orchard conditions.

Incipient allochronic speciation in the pine processionary moth (Thaumetopoea pityocampa, Lepidoptera, Notodontidae)

Santos, H; Burban, C; Rousselet, J; Rossi, JP; Branco, M; Kerdelhue, C. 2011

A plausible case of allochronic differentiation, where barrier to gene flow is primarily attributable to a phenological shift, was recently discovered in Portugal for the pine processionary moth *Thaumetopoea pityocampa*. Previous results suggested that the observed 'summer population' (SP) originated from the sympatric winter population (WP). Our objectives were to finely analyse these patterns and test their stability in time, through field monitoring and genetic analyses of larvae and adults across different years. Reproductive activity never overlapped between SP and WP. Microsatellites showed a clear differentiation of the SP, consistent with a strong reduction in gene flow owing to the phenological shift. Assignment tests suggested that some individuals shift from the SP to the WP phenology, causing some **hybridization**. We discuss these patterns and their maintenance over time. This could be a first stage of allochronic speciation, and SP should be considered as a distinct phenological race.

Sublethal Effects of Cry 1F Bt Corn and Clothianidin on Black Cutworm (Lepidoptera: Noctuidae) Larval Development

Kullik, SA; Sears, MK; Schaafsma, AW. 2011

Black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is an occasional pest of maize (corn), *Zea mays* L., that may cause severe stand losses and injury to corn seedlings. The efficacy of the neonicotinoid seed treatment clothianidin at two commercially available rates and their interaction with a transgenic corn **hybrid** (Bt corn), trait expressing the *Bacillus thuringiensis* variety aizawai insecticidal toxin Cry 1Fa2, against black cutworm larvae was investigated. Clothianidin at a rate of 25 mg kernel⁻¹ on Bt corn increased larval mortality and reduced larval weight gains additively. In contrast, weights of larvae fed non-Bt corn seedlings treated with clothianidin at a rate of 25 mg kernel⁻¹ increased significantly, suggesting either compensatory overconsumption, hormesis, or hormoligosis. Both Bt corn alone and clothianidin at a rate of 125 mg kernel⁻¹ applied to non-Bt corn seedlings caused increased mortality and reduced larval weight gains. In two field trials, plots planted with Bt corn **hybrids** consistently had the highest plant populations and yields, regardless of whether they were treated with clothianidin at the lower commercial rate of 25 mg kernel⁻¹. The use of Bt corn alone or in combination with the low rate of clothianidin (25 mg kernel⁻¹) seems suitable as a means of suppressing black cutworm in no-tillage cornfields, although rescue treatments may still be necessary under severe infestations. Clothianidin alone at the low rate of 25 mg kernel⁻¹ is not recommended for black cutworm control until further studies of its effects on larval physiology and field performance have been completed.

Effects of Harvest Residue and Tillage on Lesser Cornstalk Borer (Lepidoptera: Pyralidae) Damage to Sugarcane

Sandhu, HS; Nuessly, GS; Cherry, RH; Gilbert, RA; Webb, SE. 2011

Lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae) is an important pest of sugarcane (a complex **hybrid** of *Saccharum* spp.) in southern Florida. Cultural controls for *E. lignosellus* were evaluated in preparation for the potential loss of effective insecticides. Field studies conducted in 2006 compared the effects of harvest residues from green-harvested sugarcane (no preharvest burning to remove leaf matter) on *E. lignosellus* stalk damage and yield. Damage by *E. lignosellus* was significantly lower in plant cane plots that were covered with harvest residues collected from a green-harvested field before shoot emergence compared with plots without harvest residue. There were no yield differences between plots with and without harvest residues in plant or ratoon sugarcane fields in the 2006 study. The effects of three postharvest tillage levels (conventional, intermediate, and no tillage) were evaluated in preharvest burned and green-harvested fields in 2008 and 2009. Significantly less *E. lignosellus* damage was observed in the green- versus preharvest burned fields in both years. Intermediate and no-tillage plots had very little damage in green-harvested field. Conventional tillage plots had the greatest damage in the green-harvested field and the lowest damage in the preharvest burned field. In 2008, biomass yield was greater in the intermediate than conventional tillage in the green-harvested field, but it was greater in the conventional than in other tillage levels in the preharvest burned field. These studies demonstrated that cultural controls could greatly reduce *E. lignosellus* damage in sugarcane without the use of insecticides.

A preliminary bibliographic survey of the insects found in poultry houses from the Neotropical Region, with remarks on selected taxa shared with native birds' nests

Di Iorio, O; Turienzo, P. 2011

Species of insects associated to the habitat of *Gallus gallus* (Aves: Phasianidae) in the Neotropical Region belong to 144 identified species (42 Coleoptera; 14 Diptera; 17 Hymenoptera; 9 Siphonaptera; 1 Lepidoptera; 56 Hemiptera (one **hybrid**); 5 Dermaptera); 33 identified to genus (21 Coleoptera; 3 Diptera; 7 Hymenoptera; 1 Hemiptera; 1 Blattaria); 37 identified to family (23 Coleoptera; 9 Diptera; 2 Hymenoptera; 1 Lepidoptera; 2 Hemiptera); and 6 to order (2 Coleoptera; 1 Hymenoptera; 1 Siphonaptera; 1 Psocoptera; 1 Dermaptera). Most of the insects are haematophagous ectoparasites (Hemiptera; Siphonaptera; Diptera), detritivores (Coleoptera; Lepidoptera; Diptera; Blattaria), predators (Coleoptera; Diptera; Hemiptera; Dermaptera; Hymenoptera), and parasitoids (Hymenoptera). A total of 46 native American species and/or subspecies of Triatominae (Hemiptera: Reduviidae) were found in chicken houses, from which 18 were also found in birds' nests. It was recently observed that other insects from poultry houses, generally exotic species, had colonized native American birds' nests. Comments and remarks of selected taxa also found in birds' nests from Argentina and other countries are provided.

Analysis of genetic divergence for classification and evaluation of 37 productive performances in 54 oval cocoon strains of Iran silkworm germplasm

Najafi, N; Seidavi, AR; Mirhosseini, SZ; Gharahveysi, S; Mavvajpour, M; Nezhad, MS. 2011

The purpose of this experiment was to analyse the genetic divergence for the classification and evaluation of 37 productive performances in all 54 oval cocoon strains of Iran silkworm *Bombyx mori* (Lepidoptera: Bombycidae) germplasm. The newly hatched larvae of all strains were reared for experiment under same standard conditions. Studied strains are ranked based on the average of evaluation index method and sub-ordinate function method. From the obtained results, it was clear that different strains of silkworm *B. mori* showed different performances based on productive traits. The analysis of variance regarding the studied traits showed that different strains have significant difference for traits ($P < 0.01$). Totally, W2-11-19-2[110] (2030.234), W2-11-19-3 (2025.089), BH-4 (1981.516), 104x110 (1963.656) and 17 (1935.046) showed higher evaluation index values. W2-11-19-3 (19.868), W2-11-19-2 [110] (2030.234), BH-4 (18.857), 104x110 (18.560) and 17 (17.917) showed higher sub-ordinate function values. All strains with the same origin did not group together because they had different biological and development performance. The main groups were also divided into separate sub-groups. Three sub-groups included various strains. Several strains were grouped together and far from other silkworm strains, indicating that they might be suitable for future crossings, maintenance of parental strains and **hybridizations** with peanut cocoon strains so as to maximize heterosis and to avoid depression inbreeding. After evaluation by two statistical methods (evaluation index method and sub-ordinate function method), some oval type strains that stood within high ranks were identified as potential parents for further breeding programme.

Effect of Rearing Temperature and Humidity on Fecundity and Fertility of Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

Hussain, M; Khan, SA; Naeem, M; Nasir, MF. 2011

The effect of temperature and humidity fluctuations on fecundity and fertility of the silkworm lines was investigated during autumn and spring by exposing 4(th) and 5(th) instar larvae of eleven inbred silkworm lines (M-101, M-103, M-104, M-107, Pak-1, Pak-2, Pak-3, Pak-4, PFI-1, PFI-2 and S-1) to 25 +/- 1 degrees C, 30 +/- 1 degrees C and 35 +/- 1 degrees C in combination with 55, 65 and 75% relative humidity (RH) for 3hr daily. Temperature and relative humidity during larval rearing affected the fecundity and fertility of inbred silkworm lines adversely. The maximum average number of eggs were laid at 25 +/- 1 degrees C with 75 +/- 5% RH (413.1) followed by 25 +/- 1 degrees C with 75% RH (400.4) and 25 +/- 1 degrees C with 65% RH (390.9). Significant difference in egg fertility was observed between all the treatments. Maximum average fertilized eggs were recorded at 25 +/- 1 degrees C with 75 +/- 5% RH (88.77) followed by 25 +/- 1 degrees C with 75% RH (86.88) and 25 +/- 1 degrees C with 65% RH (85.09) while lowest fertility was shown by 35 +/- 1 degrees C with 55% RH (67.52). The deleterious effect of temperature and humidity was observed on fecundity and fertility of inbred silkworm lines. Pak-4 was the best performer with maximum eggs (471.5) at 25 +/- 1 degrees C with 75 +/- 5% RH followed by PFI-1 (465.2) and M-101(464.1) in the same environment. The best temperature and humidity for rearing silkworm lines for seed cocoon production (to obtain higher number of eggs per moth with increased fertility) is 25 +/- 1 degrees C with 75 +/- 5% RH. Pak-2, Pak-4, Pak-3 and PFI-I were the better lines which may be utilized in seed cocoon production and **hybridization**. Investigations elucidated that temperature and humidity variations during larval rearing resulted in low fecundity and high incidence of unfertilized eggs. The study suggests that fecundity and egg fertility can be enhanced by avoiding temperature and humidity fluctuations during larval rearing.

Screening strains of the mulberry silkworm, *Bombyx mori*, for thermotolerance

Kumari, SS; Subbarao, SV; Misra, S; Murty, US. 2011

A tropical climate prevails in most of the sericultural areas in India, where temperature increases during the summer lead to adverse effects on temperate bivoltine silkworm rearing and cause crop losses. Screening for thermotolerance in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an essential prerequisite for the development of thermotolerant breeds/**hybrids**. In the current study, the aim was to identify potential bivoltine silkworm strains specific for tolerance to high temperature. The third day of fifth stage silkworm larvae of bivoltine strains were subjected to high temperature of 36 +/- 1 degrees C with RH of 50 +/- 5 % for six hours (10:00-16:00) every day until spinning for three consecutive generations. Highly significant differences were found among all genetic traits of bivoltine silkworm strains in the treated groups. Three groups of silkworm resulted including susceptible, moderately tolerant, and tolerant by utilizing pupation rate or survival rate with thermal stress as the index for thermotolerance. Furthermore, based on the overall silkworm rearing performance of nine quantitative genetic traits such as larval weight, cocoon yield by number and weight, pupation, single cocoon and shell weight, shell ratio, filament length and denier, three bivoltine silkworm strains, BD2-S, SOF-BR and BO2 were developed as having the potential for thermotolerance. The data from the present study enhance knowledge for the development of thermo tolerant silkworm breeds/**hybrids** and their effective commercial utilization in the sericulture industry.

Genetic Basis Regulating the Sex Pheromone Blend in *Ostrinia zealis* (Lepidoptera: Crambidae) and Its Allies Inferred From Crossing Experiments

Tabata, J; Ishikawa, Y. 2011

To gain insight into the genetic basis of the divergence of sex pheromone signals in the genus *Ostrinia*, we conducted crossing experiments between three congeners: *Ostrinia zealis* (Guenee), *Ostrinia zaguliaevi* Mutuura & Munroe, and *Ostrinia* sp. near *zaguliaevi*. Female sex pheromones of the three species are comprised of three common components (Z)-9-tetradecenyl acetate, (E)-11-tetradecenyl acetate, and (Z)-11-tetradecenyl acetate-blended at species-specific ratios: 70:24:6 in *O. zealis*, 45:5:50 in *O. zaguliaevi*, and 19:6:75 in *O. sp. near zaguliaevi*. The differences between *O. zealis* and *O. zaguliaevi* blends, and *O. zealis* and *O. sp. near zaguliaevi* blends, could be explained by a single autosomal locus with three alleles. However, sex chromosomal as well as autosomal genes should be taken into consideration to explain the difference between *O. zaguliaevi* and *O. sp. near zaguliaevi* blends. From analyses of the fatty acyl precursors of pheromone components, it seems that these genetic factors are involved in the last steps of pheromone biosynthesis including reduction. The process of divergence of pheromone components in the genus *Ostrinia* is discussed with reference to the findings of previous studies.

Analysis of heterosis and recombination loss for fitness and productivity traits in different hybrids of mulberry silk moth *Bombyx mori*

Subramanya, G; Bishop, SC. 2011

Three different races of lepidopteron silk moth *Bombyx mori* were used in reciprocal and inter se crosses to determine heterosis effects at F(1) and recombination loss at the F(2) generation for three fitness traits (fecundity, larval duration, survival rate) and four productivity traits (larval weight, cocoon weight, shell weight, filament length). Eleven mating types were represented in the present study, including three pure breeds and a variety of F(1) and F(2) populations arising from regular and reciprocal crosses, respectively. Equations were derived to evaluate heterosis, maternal and overdominance effects for the above traits. Estimates of heterosis and overdominance effects revealed significant heterosis effects for all the traits, but overdominance was only seen for larval duration (favorable effect) and survival rate (unfavorable effect). Maternal effects were significant for the majority of the traits under study. The results revealed significant reduction for all the quantitative traits from F(1) to F(2), except for larval duration. The most obvious explanation for the reduction of fitness parameters and productive traits is the reduction in heterozygosity from F(1) to F(2) (it is expected that one half of the heterozygosity of F(1) is lost in F(2)). For larval duration this explanation seems insufficient and breakdown of epistatic gene effects (i.e. recombination loss) has been suggested.

Haplotype Identification within *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) Corn and Rice Strains from Colombia

Salinas-Hernandez, H; Saldamando-Benjumea, CI. 2011

The fall army worm *Spodoptera frugiperda* (Smith) is a migratory important pest of corn, sorghum, rice, grass and bermudagrass in North and South America. This species has diverged into two genetically differentiated but morphologically identical strains, "the rice" and "the corn". They have been analyzed by sequencing the genes cytochrome oxidase I, II and ITS1 from populations from the United States and Brazil. However, no such studies were performed in Colombia. In here, we identified 43 haplotypes by sequencing a fragment of the COI gene from 102 individuals, of which 40 had already been identified as the "corn" and "rice" strains or to their **hybrids** from Tolima, and the rest were collected from corn, cotton, sorghum, grass and rice fields in other regions of Colombia. The corn strain haplotype H1 was the most frequently found in this country, representing the main target for FAW monitoring programs. AMOVA analysis confirmed the population structure between Colombian and North American *S. frugiperda* haplotypes ($F_{ST} = 0.76812$, $P < 0.001$), but not within the different Colombian regions, suggesting high gene flow within the country. The ML trees obtained for Tolima and for Colombia as a whole did not generate clustering amongst *S. frugiperda* sequences, neither via host-plant association nor by geographical areas. The minimum spanning network for Colombia corroborated our finding that the haplotype H1 has the highest frequency in the country. Our data suggest that haplotype frequency determination will be useful in the establishment of a monitoring system for this species.

DNA barcodes and morphology reveal a hybrid hawkmoth in Tahiti (Lepidoptera: Sphingidae)

Rougerie, R; Haxaire, J; Kitching, IJ; Hebert, PDN. 2012

Interspecific **hybridisation** is a rare but widespread phenomenon identified as a potential complicating factor for the identification of species through DNA barcoding. Hybrids can, however, also deceive morphology-based taxonomy, resulting in the description of invalid species based on **hybrid** specimens. As the result of an unexpected case of discordance between barcoding results and current morphology-based taxonomy, we discovered an example of such a **hybrid** 'species' in hawkmoths. By combining barcodes, morphology and a nuclear marker, we show that *Gnathothlibus collardi* Haxaire, 2002 is actually an F1 **hybrid** between two closely related species that co-occur on Tahiti. In accordance with the International Code of Zoological Nomenclature, the taxon *G. collardi* is thus invalid as a species. This study demonstrates the potential of DNA barcodes to detect overlooked **hybrid** taxa. With the growth of sequence libraries, we anticipate that more unsuspected **hybrid** species will be detected, particularly among those taxa that are very rare, such as those known from only the type specimen.

To hybridize or not to hybridize: what separates two genetic lineages of the Chalk-hill Blue *Polyommatus coridon* (Lycaenidae, Lepidoptera) along their secondary contact zone throughout eastern Central Europe?

Schmitt, T; Zimmermann, M. 2012

As a consequence of postglacial range expansion, **hybrid** zones evolved where different genetic lineages met. In this study, we analysed the Chalk-hill Blue *Polyommatus coridon* all along the contact zone of two expansive lineages. This zone stretches from the sandy areas of north-eastern Germany, along the mountain ranges of the German-Czech border and throughout the eastern Alps. We studied allozymes (19 loci) of 38 populations (1542 individuals) and compared these data sets against 15 populations of the western and 15 populations of the eastern lineages and found different degrees of **hybridization**. Thus, the calcareous regions of Thuringia and Sachsen-Anhalt were mostly colonized by the western lineage. The middle mountain ranges between Bavaria and Bohemia represented a strong barrier blocking further expansion and thus completely impeding **hybridization** in this region. More intense **hybridization** was detected in the populations of the eastern Alps, especially in the north-eastern part, where the Danube most probably acted as an expansion corridor for both lineages followed by intensive **hybridization**. In the south-eastern Alps, **hybrid** populations were mostly detected in the easternmost parts and along the larger river valley of Drava and Mur; pure western populations dominated in the other areas of this region. These results show that the degree of **hybridization** along a contact zone is correlated with the ecological demands of a species and the regional physical geographic circumstances. This finding was proved for the Chalk-hill Blue in our study but is also the most likely scenario in most animal and plant species.

Butterfly genome reveals promiscuous exchange of mimicry adaptations among species

Dasmahapatra, KK; Walters, JR; Briscoe, AD; Davey, JW; Whibley, A; Nadeau, NJ; Zimin, AV; Hughes, DST; Ferguson, LC; Martin, SH; Salazar, C; Lewis, JJ; Adler, S; Ahn, SJ; Baker, DA; Baxter, SW; Chamberlain, NL; Chauhan, R; Counterman, BA; Dalmay, T; Gilbert, LE; Gordon, K; Heckel, DG; Hines, HM; Hoff, KJ; Holland, PWH; Jacquini-Joly, E; Jiggins, FM; Jones, RT; Kapan, DD; Kersey, P; Lamas, G; Lawson, D; Mapleson, D; Maroja, LS; Martin, A; Moxon, S; Palmer, WJ; Papa, R; Papanicolaou, A; Pauchet, Y; Ray, DA; Rosser, N; Salzberg, SL; Supple, MA; Surridge, A; Tenger-Trolander, A; Vogel, H; Wilkinson, PA; Wilson, D; Yorke, JA; Yuan, FR; Balmuth, AL; Eland, C; Gharbi, K; Thomson, M; Gibbs, RA; Han, Y; Jayaseelan, JC; Kovar, C; Mathew, T; Muzny, DM; Ogeri, F; Pu, LL; Qu, JX; Thornton, RL; Worley, KC; Wu, YQ; Linares, M; Blaxter, ML; French-Constant, RH; Joron, M; Kronforst, MR; Mullen, SP; Reed, RD; Scherer, SE; Richards, S; Mallet, J; McMillan, WO; Jiggins, CD. 2012

The evolutionary importance of **hybridization** and introgression has long been debated(1). Hybrids are usually rare and unfit, but even infrequent **hybridization** can aid adaptation by transferring beneficial traits between species. Here we use genomic tools to investigate introgression in *Heliconius*, a rapidly radiating genus of neotropical butterflies widely used in studies of ecology, behaviour, mimicry and speciation(2-5). We sequenced the genome of *Heliconius melpomene* and compared it with other taxa to investigate chromosomal evolution in Lepidoptera and gene flow among multiple *Heliconius* species and races. Among 12,669 predicted genes, biologically important expansions of families of chemosensory and Hox genes are particularly noteworthy. Chromosomal organization has remained broadly conserved since the Cretaceous period, when butterflies split from the *Bombyx* (silkworm) lineage. Using genomic resequencing, we show **hybrid** exchange of genes between three co-mimics, *Heliconius melpomene*, *Heliconius timareta* and *Heliconius elevatus*, especially at two genomic regions that control mimicry pattern. We infer that closely related *Heliconius* species exchange protective colour-pattern genes promiscuously, implying that **hybridization** has an important role in adaptive radiation.

Steinernema changbaiense sp. n. (Rhabditida: Steinernematidae), a new species of entomopathogenic nematodes from Northeast China

Ma, J; Chen, SL; De Clercq, P; Han, R; Moens, M. 2012

During an extensive survey for entomopathogenic nematodes in north China, a new species was recovered from soil samples; it is described herein as *Steinernema changbaiense* sp. n. This nematode was obtained from soil samples by the insect-baiting technique using last instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae). *Steinernema changbaiense* sp. n. is closely related to species in the 'feltiae-kraussei-oregonense' group. It is characterised by morphometrics of the infective juvenile (IJ) with a body length of 757 (730-790) µm, distance from head to excretory pore of 61 (58-68) µm, tail length of 77 (71-85) µm, E % = 80 (73-88), and a lateral field pattern 2, 5, 6, 4, 2. The male of the first generation can be recognised by a spicule of 63 (56-70) µm long and a gubernaculum of 40 (33-46) µm long; the spicule is slender, the velum short. The first generation female has a symmetrical, slightly protruding vulva and a ratio D (%) of 49 (35-55). The new species differs distinctly from related species such as *S. ashiunense*, *S. robustispiculum* and *S. monticolum* by a different number of ridges in the lateral fields and the distance from the head to excretory pore of the IJ, and the body length, morphology of spicules and gubernaculum of the male. Cross **hybridisation** tests showed that these species are reproductively isolated. The sequences analyses of the internal transcribed spacers (ITS) of rDNA and D2-D3 domain of the LSU regions confirmed the new species identity.

Natural Biological Control of *Diaphania* spp. (Lepidoptera: Crambidae) by Social Wasps

Santana, PA; Gonring, AHR; Picanco, MC; Ramos, RS; Martins, JC; Ferreira, DD. 2012

The social wasps (Hymenoptera: Vespidae) are important agents of biological control for agricultural pests. *Diaphania hyalinata* L. and *Diaphania nitidalis* Cramer (Lepidoptera: Crambidae) are among the main pests of plants in the Cucurbitaceae family. Although the importance of social wasps is acknowledged, little is known about their activity as biological control agents in *Diaphania* spp. Thus, this work aimed to study the natural biological control of the caterpillars *D. hyalinata* and *D. nitidalis* by social wasps. We studied the natural biological control of caterpillars of *D. hyalinata* and *D. nitidalis* on cucumber **hybrids** Sprint 440 II and Vlasstar. The main predators of *Diaphania* caterpillars were the social wasps, followed by Diptera: Syrphidae; Hemiptera: Anthocoridae; Coleoptera: Coccinellidae, Anthicidae; Neuroptera: Chrysopidae and Arachnida: Araneae. Predation of *D. hyalinata* caterpillars by social wasps was high from the second to fifth instar. The predation of *D. nitidalis* caterpillars by social wasps was high from the second to fourth instar. There was no predation by social wasps on the first instar larvae of *Diaphania* spp. The cucumber **hybrids** did not influence the predation of *Diaphania* spp. by social wasps. The main social wasp predator of *Diaphania* spp. was *Polybia ignobilis* (Haliday). Also, we observed the social wasp *Polybia scutellaris* (White) preying on *D. hyalinata* but at low intensity.

A new entomopathogenic nematode, *Steinernema tielingense* n. sp. (Rhabditida: Steinernematidae), from north China

Ma, J; Chen, SL; Li, XH; Han, RC; Khatri-Chhetri, HB; De Clercq, P; Moens, M. 2012

A new species of *Steinernema* was isolated from shrub soils in Liaoning province during a survey for entomopathogenic nematodes in north China. This nematode was obtained by the insect-baiting technique using last instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae). This new species is described herein as *S. tielingense* n. sp. It belongs to the 'feltiae-kraussei-oregonense' group and is characterised by infective third-stage juvenile (IJ) with a body length of 915 (824-979) μ m, distance from head to excretory pore of 69 (64-73) μ m, tail length of 81 (74-85) μ m, ratio E = 88 (85-94)%, lateral field with eight ridges at mid-body, first generation male spicule 88 (79-98) μ m and gubernaculum 61 (49-70) μ m long, and first generation female with a vulval protrusion and ratio D = 41 (32-49)%. The new species distinctly differs from the related species *S. kraussei*, *S. silvaticum*, *S. oregonense* and *S. cholashanense* in the different number of ridges in the lateral fields and hyaline tail length as % of total tail length of 11 and male body length and distance from head to excretory pore. Cross **hybridisation** tests showed that these species were reproductively isolated. The sequences analyses of the internal transcribed spacers (ITS) and D2-D3 regions of the ribosomal DNA confirm this to be a new species.

Seasonal variation in food consumption, assimilation, and conversion efficiency of Indian bivoltine hybrid silkworm, *Bombyx mori*

Rahmathulla, VK; Suresh, HM. 2012

Food consumption and utilization is influenced by various biotic and abiotic factors. Under different environmental, feeding, and nutritional conditions, and with ingestion of the same amount of mulberry leaves, the silkworm shows significant difference in its ability to digest, absorb, and convert food to body matter. Here, influences of season, temperature, and humidity on food intake, assimilation, and conversion efficiency of the Indian bivoltine **hybrid** (CSR2 x CSR4) *Bombyx mori* L. (Lepidoptera: Bombycidae) were studied. The results indicated that food ingestion and assimilation were significantly higher among silkworm batches where optimum temperature and humidity were maintained compared with silkworm batches exposed to natural climatic conditions of the respective season. However, during summer the nutritional efficiency parameters were significantly higher among silkworms reared under natural temperature and humidity conditions when compared with the control. During the winter and rainy season, the nutritional efficiency parameters were significantly higher in control batches, where optimum temperature and humidity were maintained. Ingesta and digesta required to produce one gram of cocoon/shell were also lower in control batches for all seasons except summer. This may be due to the physiological adaptation of silkworms to overcome stress during the summer season.

A Comparison of Artificial Diet and Hybrid Sweet Corn for the Rearing of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) Based on Life Table Characteristics

Jha, RK; Chi, H; Tang, LC. 2012

The demographic characteristics of *Helicoverpa armigera* (Hubner) reared on **hybrid** sweet corn (*Zea mays* L. variety *saccharata*) (**hybrid** super sweet corn KY bright jean) and on an artificial diet were compared by using the age-stage, two-sex life table. Because the hatch rate of eggs varies with maternal age, age-specific fecundity was calculated based on the numbers of hatched eggs to reveal the biological characteristics of *H. armigera* accurately. The intrinsic rate of increase (r), finite rate (λ) and mean generation time (T) of *H. armigera* were 0.0853 d⁻¹, 1.0890 d⁻¹ and 46.6 d, respectively, on *Z. mays* and 0.1015 d⁻¹, 1.1068d⁻¹, and 46.3 d, respectively, on the artificial diet. There were significant differences in the intrinsic rate of increase and finite rate between two treatments. The age-stage life expectancy and reproductive value also were calculated. The relationships among the net reproductive rate, the mean female fecundity, the number of emerged females, and the total number of individuals used in the life table study are consistent with theoretical expectations. We recommend the age-stage, two-sex life table for use in insect demographic studies to incorporate both sexes and the variation in developmental rate among individuals and to obtain accurate population parameters. The artificial diet is more suitable for the mass rearing of *H. armigera*.

DNA barcoding of the leaf-mining moth subgenus *Ectoedemia* s. str. (Lepidoptera: Nepticulidae) with COI and EF1-alpha: two are better than one in recognising cryptic species

van Nieukerken, EJ; Doorenweerd, C; Stokvis, FR; Groenenberg, DSJ. 2012

We sequenced 665bp of the Cytochrome C Oxidase I (COI) barcoding marker for 257 specimens and 482bp of Elongation Factor 1-alpha. (EF1-alpha) for 237 specimens belonging to the leaf-mining subgenus *Ectoedemia* (*Ectoedemia*) in the basal Lepidopteran family Nepticulidae. The dataset includes 45 out of 48 West Palearctic *Ectoedemia* s. str. species and several species from Africa, North America and Asia. Both COI and EF1-alpha proved reliable as an alternative to conventional species identification for the majority of species and the combination of both markers can aid in species validation. A clear barcode gap is not present, and in some species large K2P intraspecific pairwise differences are found, up to 6.85% in COI and 2.9% in EF1-alpha. In the *Ectoedemia rubivora* species complex, the species *E. rubivora*, *E. arcuatella* and *E. atricollis* share COI barcodes and could only be distinguished by EF1-alpha. Diagnostic base positions, usually third codon positions, are in this and other cases a useful addition to species delimitation, in addition to distance methods. *Ectoedemia albifasciella* COI barcodes fall into two distinct clusters not related to other characters, whereas these clusters are absent in EF1-alpha, possibly caused by mtDNA anomalies or **hybridisation**. In the *Ectoedemia subbimaculella* complex, both sequences fail to unequivocally distinguish the species *E. heringi*, *E. liechtensteini*, *E. phyllotomella* and one population of *E. subbimaculella*. DNA barcodes confirm that North American *Ectoedemia argyropeza* are derived from a European introduction. We strongly advocate the use of a nuclear marker in addition to the universal COI barcode marker for better identifying species, including cryptic ones.

Irradiation of Adult *Epiphyas postvittana* (Lepidoptera: Tortricidae): Egg Sterility in Parental and F1 Generations

Jang, EB; McInnis, DO; Kurashima, R; Woods, B; Suckling, DM. 2012

Adult *Epiphyas postvittana* Walker were irradiated using a Cobalt 60 source to determine the dose needed to achieve complete egg sterility of mated female moths, and egg sterility of female moths mated to F1 generation males. Adult male and female *E. postvittana* were irradiated at 100, 200, 250, and 300 Gy and their fertility

(when crossed with normal moths) was compared with nonirradiated moths. Viable progeny (determined by egg hatch) were found at doses of 100 and 200 Gy, but very little at 250 and 300 Gy. In particular, there was no survival of female progeny into the F1 generation. Males irradiated at 250 and 300 Gy had very low egg eclosion rates (2.25 and 1.86% at 250 and 300 Gy, respectively) when mated with normal females. The F2 generation from those male progeny had a mean percent hatched of <1.02%. Based on our results, a dose of 250-300 Gy is recommended for irradiation of *E. postvittana* adults used for sterile insect technique (SIT) if sterility of parental moths is the desired outcome. Our data also suggests that inclusion of F1 **hybrid** sterility rather than parental generation sterility into programs using the SIT may allow for doses lower than what we have reported, especially during initial phases of an eradication program where increase fitness of moths might be desirable. Further research is needed to verify the use of F1 **hybrid** sterility in light brown apple moth SIT programs.

Nutrigenetic screening strains of the mulberry silkworm, *Bombyx mori*, for nutritional efficiency

Ramesha, C; Lakshmi, H; Kumari, SS; Anuradha, CM; Kumar, CS. 2012

The activity of sericulture is declining due the reduction of mulberry production area in sericulture practicing countries lead to adverse effects on silkworm rearing and cocoon production. Screening for nutrigenetic traits in silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an essential prerequisite for better understanding and development of nutritionally efficient breeds/**hybrids**, which show less food consumption with higher efficiency conversion. The aim of this study was to identify nutritionally efficient polyvoltine silkworm strains using the germplasm breeds RMW2, RMW3, RMW4, RMG(3), RMG(1), RMG(4), RMG(5), RMG(6) and APM(1) as the control. The 1st day of 5th stage silkworm larvae of polyvoltine strains were subjected to standard gravimetric analysis until spinning for three consecutive generations covering 3 different seasons on 19 nutrigenetic traits. Highly significant ($p \leq 0.001$) differences were found among all nutrigenetic traits of polyvoltine silkworm strains in the experimental groups. The nutritionally efficient polvoltine silkworm strains were resulted by utilizing nutrition consumption index and efficiency of conversion of ingesta/cocoon traits as the index. Higher nutritional efficiency conversions were found in the polyvoltine silkworm strains on efficiency of conversion of ingesta to cocoon and shell than control. Comparatively smaller consumption index, respiration, metabolic rate with superior relative growth rate, and quantum of food ingesta and digesta requisite per gram of cocoon and shell were found; the lowest amount was in new polyvoltine strains compared to the control. Furthermore, based on the overall nutrigenetic traits utilized as index or 'biomarkers', three polyvoltine silkworm strains (RMG(4), RMW2, and RMW3) were identified as having the potential for nutrition efficiency conversion. The data from the present study advances our knowledge for the development of nutritionally efficient silkworm breeds/**hybrids** and their effective commercial utilization in the sericulture industry.

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Evaluation of the impact of the toxic protein Cry1Ab expressed by the genetically modified cultivar MON810 on honey bee (*Apis mellifera* L.) behavior

Grabowski, M; Dabrowski, ZT. 2012

Pollinators play a crucial role in natural ecosystems by maintaining both the biodiversity of wild plants and agricultural activity. In modern agriculture health problems affecting honey bee populations have resulted in an increased exploitation of alternative pollinators such as bumblebees. When Bt products are used, either as sprays or transgenic crops, non-target organisms such as pollinators may be exposed to the Cry proteins via contact or through ingestion of contaminated pollen and nectar. The cultivation of transgenic crops, e.g. maize, raises concerns among different social groups. Even in scientific bee journals there have appeared articles saying that bees might avoid collecting pollen and/or nectar from such plants. Therefore, the aim of our research was to assess the potential negative influence of the genetically modified maize MON810 expressing Cry1Ab - a protein toxic to the most serious lepidopteran pest, the European corn borer (*Ostrinia nubilalis*) [Hubner] (Lepidoptera: Crambidae) - on the behavior of the honey bee in conditions of the limited possible choice of a host plant. The insect material was one honey bee (*Apis mellifera* L.) family in a hive, which included a queen and approximately 800 worker bees. The honey bees were from the Apiculture Division, Faculty of Animal Sciences, Warsaw University of Life Sciences. The plant material consisted of transgenic maize (*Zea mays* L.) plants DKC3421 Yield Gard (event MON810) from the Monsanto Company (St. Louis, MO, USA), expressing a gene encoding a truncated form of the Cry1Ab protein derived from *B. thuringiensis* Berliner, and the corresponding near-isogenic **hybrid** (DKC 3420). This research has not revealed any significant differences in the number of honey bees visiting the two maize cultivars that would be caused by the potential negative impact of the genetically modified maize MON810.

A TRANSECT FOR REPRODUCTIVE COMPATIBILITY AND EVIDENCE FOR A "HYBRID SINK" IN A HYBRID ZONE OF *HYALOPHORA* (INSECTA: LEPIDOPTERA: SATURNIIDAE)

Collins, MM; Rawlins, JE. 2013

Egg fertility, embryo viability, and fecundity were measured, using experimental **hybridization**, to produce a profile of reproductive compatibility across a **hybrid** zone between *Hyalophora ezyalus* (Boisduval, 1855) and *H. columbia gloveri* (Strecker, 1872) (Lepidoptera: Saturniidae) in the Sierra Nevada mountains of California. Three aspects of the genetic structure of the **hybrid** zone are discussed: 1) **hybrid** zone females have normal fecundity, in contrast to typically barren laboratory-reared F-1 **hybrid** females from crosses between widely allopatric parental taxa; 2) **hybrid** zone females from defined sites along the transect, in comparison to allopatric species matings, showed no reduction in fertility or viability in matings with males from the same site; and 3) a "**hybrid** sink" was discovered, the moths from which were the least compatible among various inter-site crosses. Topography affects the genetic structure of the **hybrid** zone, especially by reducing gene flow in relation to rain shadows and associated ecological features. Within the **hybrid** zone, natural selection has apparently modified postzygotic isolation by favoring compatible genotypes regulating oogenesis. The long-term structure and stability of the **hybrid** zone is discussed in terms of ecological heterogeneity in complex montane landscapes. The suitability of the "tension zone" model, which depicts an equilibrium between the effects of selection balanced against dispersal, is discussed for the *Hyalophora* **hybrid** zone.

Identification of immune response-related genes in the Chinese oak silkworm, *Antheraea pernyi* by suppression subtractive hybridization

Liu, QN; Zhu, BJ; Wang, L; Wei, GQ; Dai, LS; Lin, KZ; Sun, Y; Qiu, JF; Fu, WW; Liu, CL. 2013

Insects possess an innate immune system that responds to invading microorganisms. In this study, a subtractive cDNA library was constructed to screen for immune response-related genes in the fat bodies of *Antheraea pernyi* (Lepidoptera: Saturniidae) pupa challenged with *Escherichia coli*. Four hundred putative EST clones were identified by suppression subtractive **hybridization** (SSH), including 50 immune response-related genes, three cytoskeleton genes, eight cell cycle and apoptosis genes, five respiration and energy metabolism genes, five transport genes, 40 metabolism genes, ten stress response genes, four transcription and translation regulation genes and 77 unknown genes. To verify the reliability of the SSH data, the transcription of a set of randomly selected immune response-related genes were confirmed by semi-quantitative reverse transcription-PCR (RT-PCR) and real-time quantitative reverse transcription-PCR (qRT-PCR). These identified immune response-related genes provide insight into understanding the innate immunity in *A. pernyi*. (C) 2013 Elsevier Inc. All rights reserved.

Erebia serotina Descimon & de Lesse 1953 (Lepidoptera: Nymphalidae), a recurrent hybrid between two distantly related species

Michel, F; Meglecz, E; Martin, JF; Descimon, H. 2013

Erebia serotina was described in 1953 as a scarce, low-elevation endemic Pyrenean species flying late in the season. At least 34 individuals are known from various locations. However, the absence of females suggests a **hybrid** origin, and *E. epiphron* and either *E. pronoe* or *E. manto* have been proposed as possible parents. Electrophoretic analysis of five allozyme loci and sequencing of three mitochondrial DNA segments and one nuclear gene now demonstrate that *E. serotina* results from the cross between *E. epiphron* females and *E. pronoe* males. We have used our and previously published sequence data to generate a molecular phylogenetic tree of the genus *Erebia* which shows that these two species are only distantly related. The question of why they happen to **hybridize** on a seemingly routine basis is thus raised.

Phylogeography of Fischer's blue, *Tongeia fischeri*, in Japan: Evidence for introgressive hybridization

Jeratthitikul, E; Hara, T; Yago, M; Itoh, T; Wang, M; Usami, S; Hikida, T. 2013

The widespread lycaenid butterfly *Tongeia fischeri* is distributed from eastern Europe to northeastern Asia and represented by three geographically isolated populations in Japan. In order to clarify the phylogeographic history of the species, we used sequences of three mitochondrial (COI, Cyt b and ND5) and two nuclear (Rp15 and Ldh) genes of 207 individuals collected from 55 sites throughout Japan and five sites on the Asian continent. Phylogenetic trees and the median-joining network revealed six evolutionary mitochondrial haplotype clades, which corresponded to the geographic distribution of the species. Common ancestors of Japanese *T. fischeri* might have come to Japan during the mid-Pleistocene by multiple dispersals of continental populations, probably via a land bridge or narrow channel between western Japan and the Korean Peninsula. The geographical patterns of variation of mitochondrial and nuclear markers are discordant in northeastern Kyushu, possibly as a result of introgressive **hybridization** during the ancient contact between the Kyushu and Shikoku populations in the last glacial maximum. The phylogeographic pattern of *T. fischeri* in Japan are probably related to the geological history, Pleistocene climatic oscillations and distribution of the host plant. (C) 2012 Elsevier Inc. All rights reserved.

In the shadow of phylogenetic uncertainty: The recent diversification of *Lysandra* butterflies through chromosomal change

Talavera, G; Lukhtanov, VA; Rieppel, L; Pierce, NE; Vila, R. 2013

The phylogeny of the butterfly genus *Lysandra* (Lycaenidae, Polyommatainae) has been intractable using both molecular and morphological characters, which could be a result of speciation due to karyotype instability. Here we reconstruct the phylogeny of the group using multi-locus coalescent-based methods on seven independent genetic markers. While the genus is ca. 4.9 Mya old, the diversification of the extant lineages was extremely recent (ca. 1.5 Mya) and involved multiple chromosomal rearrangements. We find that relationships are uncertain due to both incomplete lineage sorting and **hybridization**. Minimizing the impact of reticulation in inferring the species tree by testing for mitochondrial introgression events yields a partially resolved tree with three main supported clades: *L. punctifera* + *L. bellargus*, the corydonius taxa, and *L. coridon* + the Iberian taxa, plus three independent lineages without apparently close relatives (*L. ossmar*, *L. syriaca* and *L. dezina*). Based on these results and new karyotypic data, we propose a rearrangement recognizing ten species within the genus. Finally, we hypothesize that chromosomal instability may have played a crucial role in the *Lysandra* recent diversification. New chromosome rearrangements might be fixed in populations after severe bottlenecks, which at the same time might promote rapid sorting of neutral molecular markers. We argue that population bottlenecks might be a prerequisite for chromosomal speciation in this group. (C) 2013 Elsevier Inc. All rights reserved.

Investigating the origin of parthenogenesis and ploidy level in *Dahlica fennicella* (Lepidoptera: Psychidae)

Chevasco, V; Elzinga, JA; Galarza, JA; Mappes, J; Grapputo, A. 2013

The theories for the predominance of sexual reproduction predict that parthenogens should have no long-term evolutionary potential due to the lack of genetic recombination, despite short term advantages. Although parthenogenesis is rare among high order animals, true parthenogens can be found in various taxa. The intriguing question of the proliferation and persistence of parthenogenetic species needs investigation. An ideal species for such research is a parthenogenetic moth *Dahlica fennicella* that appears to be as equally successful as other coexisting sexual species. We investigated whether high ploidy level of *D. fennicella* is due to **hybridization** between closely related sexual species. The results from flow cytometry measurements confirmed that *D. fennicella* is exclusively tetraploid whereas all sexual species of the genera *Dahlica* and *Siederia* are diploid. Our phylogenetic results showed the non-**hybrid** origin of the parthenogenetic *D. fennicella* in this group of Lepidoptera. Most likely, the parthenogenetic *D. fennicella* originated by autopolyploidization of *D. lazuri*, which is one of its closely related sexual species. We suggest that the apparent evolutionary success of *D. fennicella* might be due to polyploidy that could mask deleterious mutations and provide greater levels of genetic variation.

Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae)

Sanudo-Restrepo, CP; Dinca, V; Talavera, G; Vila, R. 2013

Butterflies of the *Aricia* species group represent a paradigm of unresolved taxonomy, both at the genus and species levels. We studied phylogenetic relationships, biogeography, and systematics based on genetic - nuclear and mitochondrial - and morphometric - external (wings) and internal (genitalia) data. We show that *Aricia* is a monophyletic genus comprising the taxa *Pseudoaricia*, *Ultraaricia* and *Umpria*, which are here considered junior synonyms of *Aricia*. The taxa *allous*, *inhonora*, *issekutzi*, *mandzhuriana*, *myrmecias* and *transalaica*, which have often been raised to species rank, are shown to probably represent subspecies or synonyms. We show that *montensis* is likely a good species that is sister to all *A. artaxerxes* populations across the Palearctic region. The species *A. anteros* and *A. morronensis* are shown to display deep intraspecific divergences and they may harbor cryptic species. We also discovered that *A. cramera* and *A. agestis* exhibit a pattern of mutual exclusion on islands, and a parapatric distribution in mainland with a narrow contact zone where potential **hybrids** were detected. The lack of a prezygotic barrier that

prevents their coexistence could explain this phenomenon. This study will hopefully contribute to the stability of the systematics of *Aricia*, a group with potential for the study of the link between speciation and biogeography. (C) 2012 Elsevier Inc. All rights reserved.

Genetic differentiation without mimicry shift in a pair of hybridizing *Heliconius* species (Lepidoptera: Nymphalidae)

Merot, C; Mavarez, J; Evin, A; Dasmahapatra, KK; Mallet, J; Lamas, G; Joron, M. 2013

Butterflies in the genus *Heliconius* have undergone rapid adaptive radiation for warning patterns and mimicry, and are excellent models to study the mechanisms underlying diversification. In *Heliconius*, mimicry rings typically involve distantly related species, whereas closely related species often join different mimicry rings. Genetic and behavioural studies have now shown reproductive isolation in many pairs of *Heliconius* taxa is largely mediated by natural and sexual selection on wing colour patterns. However, recent studies have uncovered new cases in which pairs of closely related species are near-perfect mimics of each other. Here, we provide morphometric and genetic evidence for the coexistence of two closely related, **hybridizing** co-mimetic species on the eastern slopes of the Andes, *H. melpomene amaryllis* and *H. timareta* sp. nov., which is described here as *H. timareta thelxinoe*. A joint analysis of multilocus genotyping and geometric morphometrics of wing shape shows a high level of differentiation between the two species, with only limited gene flow and mixing. Some degree of genetic mixing can be detected, but putative **hybrids** were rare, only one of 175 specimens being a clear **hybrid**. In contrast, we found phenotypic differentiation between populations of *H. timareta thelxinoe*, possibly indicative of strong selection for local mimicry in different communities. In this pair of species, the absence of breakdown of genetic isolation despite near-identical wing patterns implies that factors other than wing patterns keep the two taxa apart, such as chemical or behavioural signals, or ecological adaptation along a strong altitudinal gradient. (c) 2013 The Linnean Society of London, Biological Journal of the Linnean Society, 2013, 109, 830-847.

Identification and ecological characterisation of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) populations in southwestern Alberta, Canada

Brunet, BMT; Hundsdoerfer, AE; Sperling, FAH. 2013

The western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), is an important pest of western North American coniferous forests, where its principal larval host is Rocky Mountain Douglas-fir, *Pseudotsuga menziesii* subspecies *glauca* (Beissner) Murray (Pinaceae). In southwestern Alberta, Canada, populations were recently discovered feeding on an unconventional host, an Engelmann spruce, *Picea engelmannii* Parry ex. Engelmann x white spruce, *Picea glauca* (Moench) Voss **hybrid** (Pinaceae), in a transition zone between the two major forest types characterised by these conifer species. We use molecular evidence to verify the species identity of outbreeding *Choristoneura* populations collected from Douglas-fir and **hybrid** spruces in southwestern Alberta in 2009, and characterise the larval and male moth phenology. Both mitochondrial and microsatellite markers confirmed these populations as *C. occidentalis*, but admixture with *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) was detected in some individuals. Few differences associated with separate host trees were evident in the phenology of larvae and the flight period of male moths.

Incongruence between morphological and molecular markers in the butterfly genus *Zizina* (Lepidoptera: Lycaenidae) in New Zealand

Gillespie, M; Wratten, SD; Cruickshank, R; Wiseman, BH; Gibbs, GW. 2013

The butterfly genus *Zizina* in New Zealand has a complex taxonomic history due to the presence of morphological intermediates between the two species, the endemic *Z. oxleyi* and the introduced *Z. labradus*, in a putative **hybrid** zone on the east coast of the South Island of New Zealand. This makes species identification in the field problematic, particularly as the presence of **hybrids** has not been confirmed. We address this uncertainty through morphological and molecular analyses. Specimens were collected from a range of locations in New Zealand, as well as from Australia, and measurements were made of male genitalia and ventral wing coloration. Two mitochondrial genes (COI, ND5) and three nuclear gene fragments (28S, ITS2 and wingless) were also sequenced for a selection of individuals, and the presence of *Wolbachia* species in genomic DNA was tested. The two species were separable in morphological space, although there was some overlap, and the contact zone appeared to be around Kaikoura on the east coast of the South Island. Furthermore, specimens from the putative **hybrid** zone could be classified as *Z. oxleyi* using morphological characters individually, but not when these were used in a principal component analysis. Molecular analysis showed that there was a mean sequence divergence of 2.0% between two clades for COI, and 4.1% for ND5, but suggested that the contact zone between them was in the north-west of the South Island. However, there was only a single clade for each of the three nuclear markers. It is thought that this incongruence between morphological and molecular markers is indicative of **hybridization** which is more extensive than previously thought. However, the possibility that recent speciation has occurred or is occurring is not ruled out.

Differentially expressed genes in the cuticle and hemolymph of the silkworm, *Bombyx mori*, injected with the fungus *Beauveria bassiana*

Hou, CX; Qin, GX; Liu, T; Mei, XL; Li, B; Shen, ZY; Guo, XJ. 2013

The most important pathogenic fungus of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), is *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Clavicipitaceae), which causes significant damage to sericulture production. Therefore, diagnosing fungal disease and developing new control measures are crucial to silk production. To better understand the responsive and interactive mechanisms between the host silkworm and this fungus, variations in silkworm gene expression were investigated using the suppression subtractive **hybridization** method following the injection of *B. bassiana* conidia. Two cDNA libraries were constructed, and 140 cDNA clones were isolated. Of the 50 differentially expressed genes identified, 45 (112 clones) were identified in the forward library, and 5 (28 clones) were identified in the reverse library. Expression profiling of six of these genes by quantitative polymerase chain reaction (qPCR) verified that they were induced by the fungal challenge. The present study provides insight into the interaction between lepidopteran insects and pathogenic fungi.

Corn Earworm (Lepidoptera: Noctuidae) in Northeastern Field Corn: Infestation Levels and the Value of Transgenic Hybrids

Bohnenblust, E; Breining, J; Fleischer, S; Roth, G; Tooker, J. 2013

Corn earworm, *Helioverpa zea* (Boddie), is a polyphagous noctuid pest of agricultural crops across the United States that is gaining attention as a pest of field corn. Before the introduction of transgenic insect-resistant **hybrids**, this pest was largely ignored in field corn, but now many *Bacillus thuringiensis* (Bt) corn **hybrids** have activity against corn earworm. However, the value of control in the northeastern United States is unclear because the risk posed by corn earworm to field corn has not been well characterized. To understand the threat from corn earworm and the value of Bt **hybrids** in field corn, we assessed corn earworm injury in Bt and non-Bt **hybrids** at 16 sites across four maturity zones throughout Pennsylvania in 2010, and 10 sites in 2011. We also used corn earworm captures from the PestWatch pheromone trapping network to relate moth activity to larval damage in field corn. Corn earworm damage was less than one kernel per ear at 21 of 26 sites over both years, and the percentage of ears damaged was generally <15%, much lower than in the southern United States where damage can be up to 30 kernels per ear. At sites with the highest damage levels, Bt **hybrids** suppressed corn earworm damage relative to non-Bt **hybrids**, but we found no differences among Bt traits. Cumulative moth captures through July effectively predicted damage at the end of the season. Currently, the additional benefit of corn earworm control provided by Bt **hybrids** is typically less than US\$4.00/ha in northeastern field corn.

Nitrogen Fertilizer Application in Maize and Its Impact on the Development of *Chilo partellus* (Lepidoptera: Pyralidae)

Arshad, MJ; Freed, S; Akbar, S; Akmal, M; Gul, HT. 2013

The current experiment was conducted to check the efficacy of different nitrogen doses on the bionomics of maize stem borer *Chilo partellus* (Lepidoptera: Pyralidae) under laboratory and field conditions applied on three maize **hybrids** i.e., KK-8711, KWS-55 and KS-85. The results revealed that the body weight (mg) and frass weight (mg) of larvae positively correlated with nitrogen levels. There was a significant difference in weight gained by larval instars at various nitrogen applications with highest weight (0.085mg/l) at N4 (150KgN/ha) and minimum (0.078mg/l) at N0 (0KgN/ha). Identical results were observed for the frass weight of different larval instars while maximum frass weight i.e., 0.018 mg was recorded for fifth instar fed on KWS-55 with the application of N4, while minimum frass weight of fifth instar i.e., 0.012 mg fed on KK-8711 applied with N0 and N1. Significant difference for weight gained by the larvae on all three **hybrids** was recorded but 5th instar maximum larval weight 0.078 mg was recorded by feeding on KWS-55 while minimum 0.066 mg on KK-8711. Cumulative field data analysis showed significant difference among the average maize borer/plant at different nitrogen doses (N0-N4). Maximum number of *C. partellus* (2 larvae/ plant) was found on KWS-55 at (N4) while minimum (0 larvae / plant) was found on KK-8711. The results suggest that the population development of maize borer is dependent on the application of nitrogen doses.

TEMPERATURE-DEPENDENT REPRODUCTIVE AND LIFE TABLE PARAMETERS OF *ELASMOPALPUS LIGNOSELLUS* (LEPIDOPTERA: PYRALIDAE) ON SUGARCANE

Sandhu, HS; Nuessly, GS; Webb, SE; Cherry, RH; Gilbert, RA. 2013

The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae) is an important pest of sugarcane (a complex **hybrid** of *Saccharum* spp.) in southern Florida. Reproductive and life table parameters for *E. lignosellus* were examined at nine constant temperatures from 13 to 36 degrees C with sugarcane as the larval food source. The pre- and post-oviposition periods decreased with increasing temperatures and reached their minimums at 33 and 36 degrees C, respectively. The oviposition period was longest at 27 degrees C. The mean fecundity, intrinsic rate of increase (r), and finite rate of increase (λ) were greatest at 30 degrees C and decreased with increasing or decreasing temperature. The net reproductive rate (R_0) was greatest at 27 degrees C. The Briere-1 model best described the relationship between temperature and r . The generation (T) and population doubling times (DT) were shortest at 33 and 30 degrees C, respectively. The temperatures around 27 to 30 degrees C are most favorable for *E. lignosellus* population growth and significant damage can be expected under these climatic conditions. This data in combination with population monitoring of *E. lignosellus* under field conditions can be effectively used in population predictions of this pest in field and severe damage can be prevented by timely implementation of control measures.

Chromosome evolution in Neotropical butterflies

Saura, A; Von Schoultz, B; Saura, AO; Brown, KS. 2013

We list the chromosome numbers for 65 species of Neotropical Hesperidae and 104 species or subspecies of Pieridae. In Hesperidae the tribe Pyrrhopygini have a modal $n = 28$, Eudaminae and Pyrgini a modal $n = 31$, while Hesperinae have $n =$ around 29. Among Pieridae, Coliadinae have a strong modal $n = 31$ and among Pierinae Anthocharidini are almost fixed for $n = 15$ while Pierini vary with $n = 26$ as the most common chromosome number. Dismorphiinae show wide variation. We discuss these results in the context of chromosome numbers of over 1400 Neotropical butterfly species and subspecies derived from about 3000 populations published here and in earlier papers of a series. The overall results show that many Neotropical groups are characterized by karyotype instability with several derived modal numbers or none at all, while almost all taxa of Lepidoptera studied from the other parts of the world have one of $n = 29-31$ as modal numbers. Possibly chromosome number changes become fixed in the course of speciation driven by biotic interactions. Population subdivision and structuring facilitate karyotype change. Factors that stabilize chromosome numbers include **hybridization** among species sharing the same number, migration, sexual selection and possibly the distribution of chromosomes within the nucleus.

First Report of *Endoclitia signifer* (Lepidoptera: Hepialidae) as a New Pest on Eucalyptus

Yang, XH; Yu, YH; Wu, YJ; Qin, JL; Luo, YQ. 2013

Endoclitia signifer Walker (Lepidoptera: Hepialidae) has become a new wood borer pest in Eucalyptus plantations in southern China. This article documents survey results of its geographic distribution and host plant range in Guangxi and its morphological measurements, life cycle and behavior. In total, 83 Eucalyptus growing counties were surveyed. *E. signifer* was found in 59 counties. Host plants included 31 species in 16 families and 24 genera. Four Eucalyptus **hybrid** species were recorded as its host plant with *E. grandis* x *E. urophylla* and *E. urophylla* x *E. grandis* infested the heaviest. The infestation of Eucalyptus trees 1-2 yr old was heavier than that of older trees. Most individuals of *E. signifer* took 1 yr to complete a generation, overwintering as larvae in tunnels in wooden stems, and pupating in February of the following year. Adults emerge, mate, and lay eggs in April, and the eggs hatch in late April or early May. Adult emergence peaks between 17:00-18:59 hours. Mating flights last under 30 min at dusk and the copulation duration was 24 h. Moths were large, weighting and average of 3.4 g. Eggs and newly hatched larvae were very small, weighing only 0.127 +/- 0.001 mg and 0.093 +/- 0.017 mg, respectively. The larvae have two distinct development stages. One stage spends 1-2 mo living in the forest litter, the second stage then moves to woody stems where it feeds for approximate to 10 mo. Larvae start boring into hosts between June and November, mainly in July and August. This study indicated that *E. signifer*, a highly polyphagous native species, has shifted host to exotic Eucalyptus and can cause significant damage to plantations.

The influence of planting date, transgenic Bt maize and hybrid relative maturity on European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae) ovipositional patterns

Obopile, M; Hammond, RB. 2013

A three year study was carried out at Hoytville and at Wooster, Ohio, USA from 2006 to 2008 to investigate the influence of planting date, transgenic maize and **hybrid** maturity on *Ostrinia nubilalis* (Hubner) population dynamics and oviposition patterns. Maize plants were planted in late April or early May, mid-May and early June during each year. The moth flight pattern showed bivoltine generations during the three years. The first moth flight peaked in June, with the populations declining during July. The second moth flight peaked in August and declined towards the end of September or early October. Egg mass density did not differ significantly between transgenic and non-transgenic maize of different maturities. Significant differences were observed, however, among planting dates, sampling dates, and sampling date x planting date interactions. Generally higher numbers of egg masses from second generation moths were deposited on late planted maize than middle and early plantings.

SURVIVAL OF A LEPIDOPTERAN DEFOLIATOR OF EUCALYPTUS IS INFLUENCED BY LOCAL HILLSIDE AND FOREST REMNANTS IN BRAZIL

Macedo-Reis, LE; Soares, LGS; De Faria, ML; Santo, MMD; Zanuncio, JC. 2013

We tested the hypothesis that *Euselasia eucerus* (Lepidoptera: Riodinidae) the intensities of mortality factors in eucalyptus plantations in xeric environments are

greater near fragments of native forest and they diminish with increasing distance from the latter. Samples were collected along a transect crossing a 70-ha planting of *Eucalyptus urophylla* x *Eucalyptus grandis* **hybrid** extending from adjacent native vegetation to a hilltop. Sampling was completed along an elevation and distance gradient from the native forest in three environments, near the native forest (base), intermediate (mid way between the native forest and the hilltop) and distant from the native forest (hilltop) ones. Fungi, parasites and predators caused mortality of *E. eucerus* pupae. Damage to *E. urophylla* x *E. grandis* **hybrid** by *E. eucerus* was greater at the more distant location possibly because of plant water deficits and/or increased *E. eucerus* survival. Mortality of *E. eucerus* in all ontogenetic stages was greater in areas near the native forest. Proximity to native vegetation appeared to be a key factor that influenced survival of local *E. eucerus* populations in *E. urophylla* x *E. grandis* **hybrid** plantations. The results presented here reinforce the concept that native vegetation near eucalyptus plantations exerts a local effect supporting the maintenance of natural enemies of *E. eucerus*, and favoring infections by entomopathogenic fungi during the pupal stage of this insect.

Ecological and genetic factors influencing the transition between host-use strategies in sympatric *Heliconius* butterflies

Merrill, RM; Naisbit, RE; Mallet, J; Jiggins, CD. 2013

Shifts in host-plant use by phytophagous insects have played a central role in their diversification. Evolving host-use strategies will reflect a trade-off between selection pressures. The ecological niche of herbivorous insects is partitioned along several dimensions, and if populations remain in contact, recombination will break down associations between relevant loci. As such, genetic architecture can profoundly affect the coordinated divergence of traits and subsequently the ability to exploit novel habitats. The closely related species *Heliconius cydno* and *H. melpomene* differ in mimetic colour pattern, habitat and host-plant use. We investigate the selection pressures and genetic basis underlying host-use differences in these two species. Host-plant surveys reveal that *H. melpomene* specializes on a single species of *Passiflora*. This is also true for the majority of other *Heliconius* species in secondary growth forest at our study site, as expected under a model of interspecific competition. In contrast, *H. cydno*, which uses closed-forest habitats where both *Heliconius* and *Passiflora* are less common, appears not to be restricted by competition and uses a broad selection of the available *Passiflora*. However, other selection pressures are likely involved, and field experiments reveal that early larval survival of both butterfly species is highest on *Passiflora menispermifolia*, but most markedly so for *H. melpomene*, the specialist on that host. Finally, we demonstrate an association between host-plant acceptance and colour pattern amongst interspecific **hybrids**, suggesting that major loci underlying these important ecological traits are physically linked in the genome. Together, our results reveal ecological and genetic associations between shifts in habitat, host use and mimetic colour pattern that have likely facilitated both speciation and coexistence.

Invasion spread of *Operophtera brumata* in northeastern United States and hybridization with *O. bruceata*

Elkinton, JS; Liebhold, A; Boettner, GH; Sremac, M. 2014

We used five methods to estimate the rate of spread of the winter moth, *Operophtera brumata* L., a European Lepidoptera, invading the northeastern USA and occasionally **hybridizing** with the closely related *O. bruceata*. These two species utilize the same sex attractant and pheromone traps capture both species. We estimated spread based on the ratio of the two species captured in pheromone-baited traps. Population boundaries were derived from captures in a grid of traps and spread was estimated as 6.6 km/year based on displacement of population boundaries between 2005 and 2008. Radial spread rate was also estimated as 6.9 km/year from the displacement of boundaries using logistic regression of trap captures along a single east-west transect of traps deployed yearly from 2007 to 2011. We also estimated the rate of spread from the expansion of defoliation mapped during aerial surveys. Based on the displacement of defoliation boundaries from 2005 to 2008, spread rate was estimated as 6.0 km/year. Based on the year of first defoliation, spread was estimated as 4.8 km/year and regression of the square-root of the cumulative area/pi versus time yielded an estimate of 4.7 km/year. All five estimates were similar, and differences reflect the nuances of the data from which they were derived. We discuss here how the occasional **hybridization** with *O. bruceata* may be either retarding or enhancing *O. brumata* spread.

Comparison of virulence of hybridized entomopathogenic nematode *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) strains and their parents

Kongu, Y; Susurluk, IA. 2014

Efficacy of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) species can be changed on targeted insect pests in different climatic zone. In this study, six different *H. bacteriophora* strains, which isolated in different cities [Antalya (H. b. 6), Canakkale (H. b. 876), Kirklareli (H. b. 17), Izmir (H. b. HIZ) and Adana (H. b. 10)] from Turkey, were **hybridized** and their virulence were determined on the last instar of *Galleria mellonella* (Lepidoptera: Pyralidae) at six different dosages. Male and female individuals of having lowest virulence were crossbred with other strain females and males in vitro conditions. Ten different strains were obtained by **hybridization** and their virulence was compared with their parent and each other on the last instar larvae of *G. mellonella*. The results showed that 70% of **hybridized** strains were more effective than their parents. The results indicated that male and female individuals have different effect on the virulence of the **hybridized** strains. The **hybridized** strains, which were more effective than their parents, were influenced from female with 42.9% and from male with 57.1%.

Behavioural studies on Shoot and Fruit Borer, *Conogethes punctiferalis*, Guenee, (Crambidae: Lepidoptera) host-associated populations reveal occurrence of cryptic species

Pathour, SR; Chakravarthy, AK; Chandrashekharaiyah, R; Bhanu, KRM. 2014

Conogethes punctiferalis, Guenee, reared on castor (*Ricinus*) and cardamom (*Elettaria*) was observed for moth emergence pattern, calling and mating behaviour and effect of interbreeding on offsprings. Females *C. punctiferalis* reared on castor emerged four hour (17.78 %) after lights off (ALO), those reared on cardamom emerged an hour (23.46 %) ALO. The calling frequency was more pronounced in female *C. punctiferalis* reared on castor compared to that on cardamom. *C. punctiferalis* moths reared on cardamom showed peak mating between 4 to 6 hours ALO, while 6 to 9 hours in females reared on castor. Failure of **hybridization** between *C. punctiferalis* reared on castor and cardamom suggest that the two *C. punctiferalis* populations segregated into two species. *C. punctiferalis* is a complex of species and observations, experiments, history and ecology support separation of *C. punctiferalis* on castor and cardamom into two.

Pre- and post-mating reproductive barriers drive divergence of five sympatric species of Naryciinae moths (Lepidoptera: Psychidae)

Elzinga, JA; Mappes, J; Kaila, L. 2014

The biological species concept suggests that species can be separated on the basis of reproductive isolation. However, because natural interbreeding capabilities are often unknown, differences in morphology are generally used to separate species. Alternatively, genetic dissimilarity is used to separate morphologically similar species. Many genetic markers, including the maternally inherited mitochondrial cytochrome oxidase I sequence, cannot show interbreeding and therefore species status of groups may remain unresolved. In species of the genera *Dahlica* and *Siederia* (Lepidoptera: Psychidae: Naryciinae) the lack of morphological distinction and unknown interbreeding has led to unclear and unresolved taxonomic status. Mitochondrial DNA sequences suggest five sexual species to occur in Finland. However, their species status remains unconfirmed, due to a lack of knowledge on interbreeding, unclear morphological distinction and the limited variation in mitochondrial DNA. We combine three methods, a cross-mating experiment, an analysis of mitochondrial and nuclear DNA, and a detailed male genital morphological examination, to establish the species status of the five suspected species. All suspected species exhibit intraspecific mating preference, although several interspecies pairs readily produce offspring. The genetic analysis, however, fails to show **hybrids** or introgression, suggesting that both pre- and post-copulation mechanisms isolate the

species reproductively. Morphological analysis of the male genitalia confirms that the species have diverged. Our results highlight the need of combining behavioural, morphological and genetic methods to determine species status in challenging taxonomic groups.(c) 2014 The Linnean Society of London, Biological Journal of the Linnean Society, 2014, 112, 584-605.

More evidence for an admixture of the Hyles euphorbiae complex's main lineages in Mediterranean Europe (Lepidoptera: Sphingidae)

Mende, MB; Hundsdoerfer, AK. 2014

The populations of the Hyles euphorbiae complex on Crete and the Dodecanese Islands have been described as an endemic species, Hyles "cretica". Alternatively, they are thought to be of **hybrid** origin from the complex's two main lineages, the European H. euphorbiae and African H. tithymali, because of their intermediate morphology. That they belong to a discrete taxon is supported by the fact that all the recent samples analyzed belong to an endemic mitochondrial haplotype cluster; however, this cluster is so close to the tithymali cluster that it might also be regarded as a sublineage of H. tithymali. Similarly, southern Italy is currently dominated by another distinct tithymali-related cluster, named "italica". However, sequencing of historic museum specimens, which were collected in Italy during the past century, revealed that mitochondrial haplotypes of H. euphorbiae were once present throughout the entire range of "italica" thus contradicting a long-term isolated evolution of a southern Italian refugial entity. In the present study we likewise sequenced the few available historic specimens from the Aegean Islands and also found a specimen from Crete which belonged to the euphorbiae cluster. In conclusion, southern Italian and southern Aegean populations both appear to be dynamic **hybrid** mixtures of the European H. euphorbiae and African H. tithymali, rather than separately evolved taxa.

Testing DNA Barcode Performance in 1000 Species of European Lepidoptera: Large Geographic Distances Have Small Genetic Impacts

Huemer, P; Mutanen, M; Sefc, KM; Hebert, PDN. 2014

This study examines the performance of DNA barcodes (mt cytochrome c oxidase 1 gene) in the identification of 1004 species of Lepidoptera shared by two localities (Finland, Austria) that are 1600 km apart. Maximum intraspecific distances for the pooled data were less than 2% for 880 species (87.6%), while deeper divergence was detected in 124 species. Despite such variation, the overall DNA barcode library possessed diagnostic COI sequences for 98.8% of the taxa. Because a reference library based on Finnish specimens was highly effective in identifying specimens from Austria, we conclude that barcode libraries based on regional sampling can often be effective for a much larger area. Moreover, dispersal ability (poor, good) and distribution patterns (disjunct, fragmented, continuous, migratory) had little impact on levels of intraspecific geographic divergence. Furthermore, the present study revealed that, despite the intensity of past taxonomic work on European Lepidoptera, nearly 20% of the species shared by Austria and Finland require further work to clarify their status. Particularly discordant BIN (Barcode Index Number) cases should be checked to ascertain possible explanatory factors such as incorrect taxonomy, **hybridization**, introgression, and Wolbachia infections.

Morphological and chemical analysis of male scent organs in the butterfly genus Pyrgus (Lepidoptera: Hesperidae)

Hernandez-Roldan, JL; Bofill, R; Dapporto, L; Munguira, ML; Vila, R. 2014

Chemical communication in the family Hesperidae (Lepidoptera) is practically unstudied, even though this group includes approximately 4,000 species and represents a fifth of the world's butterfly fauna. We present the first comparative morphological and chemical analysis of scent organs for nine species in the genus Pyrgus, the most species-rich hesperiid genus in the Palearctic region. Our results show that the morphology of the two main male scent organs-the costal fold and the tibial tufts-does not differ between species. The chemical analyses detected a total of 125 different compounds exclusively present in these organs. We document great interspecific differences and much narrower intraspecific variability in the chemical profiles. The dynamics of chemical versus genetic distances indicate two different phases: a faster (but more variable) initial chemical divergence at lower genetic divergences (probably related to speciation) and a slower but more constant differentiation (drift). As a result most species can be identified based on their chemical profiles, except for a closely related species pair (P. malvae/P. malvoides) for which **hybridisation** is common in the contact zone. Our results suggest that the Hesperidae is a group with great potential for the study of chemical communication that deserves further attention.

Host plant-related variation in thermal tolerance of Eldana saccharina

Kleynhans, E; Conlong, DE; Terblanche, JS. 2014

Understanding tolerance of thermal extremes by pest insects is essential for developing integrated management strategies, as tolerance traits can provide insights into constraints on activity and survival. A major question in thermal biology is whether thermal limits vary systematically with microclimate variation, or whether other biotic or abiotic factors can influence these limits in a predictable manner. Here, we report the results of experiments determining thermal limits to activity and survival at extreme temperatures in the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae), collected from either *Saccharum* spp. **hybrids** (sugarcane) (Poaceae) or *Cyperus papyrus* L. (Cyperaceae) and then reared under standard conditions in the laboratory for 1-2 generations. Chill-coma temperature (CTmin), critical thermal maximum (CTmax), lower lethal temperatures (LLT), and freezing temperature between *E.saccharina* collected from the two host plants were compared. CTmin and CTmax of *E.saccharina* moths collected from sugarcane were significantly lower than those from *C.papyrus* (CTmin=2.8 +/- 0.4 vs. 3.9 +/- 0.4 degrees C; CTmax=44.6 +/- 0.1 vs. 44.9 +/- 0.2 degrees C). By contrast, LLT of moths and freezing temperatures of pupae did not vary with host plant [LLT for 50% (LT50) of the moth population, when collected from sugarcane: -3.2 +/- 0.5 degrees C, from *C.papyrus*: -3.9 +/- 0.8 degrees C]. Freezing temperatures of pupae collected from *C.papyrus* were -18.0 +/- 1.0 degrees C and of those from sugarcane -17.5 +/- 1.8 degrees C. The *E.saccharina* which experienced the lowest minimum temperature (in *C.papyrus*) did not have the lowest CTmin, although the highest estimate of CTmax was found in *E.saccharina* collected from *C.papyrus* and this was also the microsite which reported the highest maximum temperatures. These results therefore suggest that host plant may strongly mediate lower critical thermal limits, but not necessarily LLT or freezing temperatures. These results have significant implications for ongoing pest management and thermal biology of these and other insects.

Genetic diversity in Pareuchaetes insulata and its implications for biological control of Chromolaena odorata

Dube, N; Assefa, Y; Zachariades, C; Olckers, T; Conlong, D. 2014

Chromolaena odorata (L.) King & H.E. Robins. (Asteraceae) threatens several economic and environmental activities in the eastern subtropical regions of South Africa and is a target for biological control. Three populations of *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebidae) originating from Florida (USA), Cuba and Jamaica were released at 30 separate sites in KwaZulu-Natal, South Africa. Subsequent overlapping of two of these populations in the field and the likelihood of interbreeding posed a potential risk of establishment failure. To explore the genetic diversity of the different *P. insulata* populations and test for the existence of pre- and post-zygotic reproductive isolation between them, molecular analyses and cross-mating experiments were conducted. Mitochondrial DNA sequences revealed significant genetic similarity between them. Cross-mating trials between Floridian and Jamaican populations of *P. insulata* revealed no significant pre- and post-zygotic isolation, with no demonstrable differences in measured parameters between the 'parental' populations, the F-1 '**hybrids**' and the backcrosses. These results suggest that *P. insulata* populations released in South Africa are part of the same 'parental' population. Genetic incompatibility is, therefore, relatively unlikely to be the cause of any failure in establishment of *P. insulata* at any of the release sites.

Associations between host plant concentrations of selected biochemical nutrients and Mexican rice borer, *Eoreuma loftini*, infestation

Showler, AT; Moran, PJ. 2014

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), is an economic pest of sugarcane (*Saccharum* spp. **hybrids**) and other graminaceous host crops, and it attacks grassy weeds. Oviposition preference has been known to be for plants with leaves that form folds. This study is the first to associate the nutritional quality of crop and forage plant hosts with Mexican rice borer injury. Three experiments were conducted to determine the levels of selected biochemical nutrients, including free amino acids and sugars, in four grass weeds [barnyardgrass, *Echinochloa crus-galli* (L.); broadleaf signalgrass, *Urochloa platyphylla* (Munro ex C. Wright); johnsongrass, *Sorghum halepense* (L.) Pers.; and Vasey's grass, *Paspalum urvillei* Steud.], a forage grass [sudangrass, *Sorghum bicolor* (L.) Moench spp. *drummondii* (Nees ex Steud.) de Wet & Harlan], three crop species [maize, *Zea mays* L.; sorghum, *Sorghum bicolor* (L.) Moench; and sugarcane, *Saccharum* spp.], and a sorghum-sudangrass forage **hybrid** (all Poaceae). Of 16 free amino acids detected among plants in the first two experiments, only high accumulations of free histidine in sudangrass and maize were associated with increased infestations by Mexican rice borer larvae. In all three experiments, high levels of fructose were associated with heightened Mexican rice borer infestation. Ramifications of these findings on the potential dispersal of this invasive pest in the USA and possible applications of fructose in baits are discussed.

Genetic Characterization of *Bombyx mori* (Lepidoptera: Bombycidae) Breeding and Hybrid Lines With Different Geographic Origins

Furdui, EM; Marghitas, LA; Dezmirean, DS; Pasca, I; Pop, IF; Erler, S; Schluns, EA. 2014

The domesticated silkworm *Bombyx mori* L. comprises a large number of geographical breeds and **hybrid** lines. Knowing the genetic structure of those may provide information to improve the conservation of commercial lines by estimating inbreeding over generations and the consequences of excessive use of those lineages. Here, we analyzed the genetic diversity of seven breeds and eight **hybrid** lines from Eastern Europe and Asia using highly polymorphic microsatellites markers to determine its genetical impact on their use in global breeding programs. No consistent pattern of deviation from Hardy-Weinberg equilibrium was found for most breed and **hybrids**; and the absence of a linkage disequilibrium also suggests that the strains are in equilibrium. A principal coordinate analysis revealed a clear separation of two silkworm breeds from the rest: one (IBV) originated from India and the other one (RG(90)) from Romania/Japan. The tendency of the other breeds from different geographic origins to cluster together in a general mix might be due to similar selection pressures (climate and anthropogenic factors) in different geographic locations. Phylogenetic analyses grouped the different silkworm breeds but not the **hybrids** according to their geographic origin and confirmed the pattern found in the principal coordinate analysis.

Mate recognition and reproductive isolation in the sibling species *Spodoptera littoralis* and *Spodoptera litura*

Saveer, AM; Becher, PG; Birgersson, G; Hansson, BS; Witzgall, P; Bengtsson, M. 2014

Mate recognition is crucial for reproductive isolation and for maintaining species integrity. Chemosensory-mediated sexual communication with pheromones is an essential component of mate recognition in moths. Confronted with sex pheromone stimuli released from conspecific and closely related heterospecific females, which partially overlap in chemical composition, male moths are under strong selection to recognize compatible mates. Here, we investigated the role of pheromone signals in premating communication in the sibling species *Spodoptera littoralis* and *S. litura* (Lepidoptera, Noctuidae). Further, we measured the reproductive consequence of conspecific vs. heterospecific matings. Both species use Z9,E11-14:Ac as the major pheromone compound, and the 11-component blend found in pheromone glands of *S. littoralis* comprises the compounds found in *S. litura*. Accordingly, *S. littoralis* and *S. litura* males readily responded to conspecific and heterospecific calling females in no-choice behavioral tests. In contrast, in a dual-choice test, *S. littoralis* males choose conspecific calling females, whereas *S. litura* males did not discriminate between conspecific and heterospecific females. In *S. littoralis* females, heterospecific matings had a negative fitness effect as compared to conspecific matings. Female longevity, egg-laying and hatching of larvae were significantly reduced by matings with heterospecific males. Reciprocal crossings, between *S. litura* females and *S. littoralis* males, were prevented by genital morphology, which is consistent with reduced heterospecific attraction of *S. littoralis* males in a dual-choice assay. On the other hand, matings between *S. littoralis* females and *S. litura* males, under a no-choice situation, show that interspecific matings occur in zones of geographical overlap and corroborate the idea that mate quality, in these closely related species, is a continuous and not a categorical trait.

Arthrobacter pityocampae sp. nov., isolated from *Thaumetopoea pityocampa* (Lep., Thaumetopoeidae)

Ince, IA; Demirbag, Z; Kati, H. 2014

A bacterium (strain Tp2(T)) was isolated from a caterpillar of the pine processionary moth, *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Thaumetopoeidae), a destructive pine forest pest. The bacterium is a Gram-stain-positive, red-pigmented coccus, oxidase-negative, nitrate-reducing, non-motile and non-spore-forming. Strain Tp2(T) was subjected to a taxonomic study using polyphasic approach that included morphological and biochemical characterizations, 16S rRNA gene sequence analysis, DNA DNA **hybridization**, DNA G + C content analysis, comparative fatty acid profiles, and analyses of quinones and polar lipids. The 16S rRNA gene sequence of strain Tp2(T) revealed that *Arthrobacter agilis* DSM 20550(T) was the closest known strain (98% 16S rRNA gene sequence similarity). DNA DNA **hybridization** of *A. agilis* DSM 20550(T) and strain Tp2(T) resulted in a DNA DNA relatedness value of 11.9% (20.2% reciprocal). The DNA base composition of strain Tp2(T) was 69.5 mol%, which is consistent with the other recognized members of Actinobacteria that have a high G+C content in their genome. The polar lipid pattern of strain Tp2(T) consisted of diphosphatidylglycerol (major), phosphatidylglycerol and phosphatidylinositol and unknown glycolipids. The cellular fatty acids were anteiso C-15:0 and anteiso 0170 and the major menaquinone was MK-9(IH-2). The peptidoglycan type was A3 alpha with an L-Lys L-Thr L-Ala(3) interpeptide bridge. The above-mentioned characterization qualifies strain Tp2(T) as genotypically and phenotypically distinct from closely related species of the genus *Arthrobacter* with validly published names. Strain Tp2(T) is therefore proposed to represent a novel species of the genus *Arthrobacter*, described as *Arthrobacter pityocampae* sp. nov. The type strain is Tp2(T) (=DSM 21719(T)=NCCB 100254(T)).

A Comparison of Bt Transgene, Hybrid Background, Water Stress, and Insect Stress Effects on Corn Leaf and Ear Injury and Subsequent Yield

Brewer, MJ; Odvody, GN; Anderson, DJ; Remmers, JC. 2014

Experimentally manipulated water and insect stresses were applied to field-grown corn with different *Bacillus thuringiensis* (Bt) transgenes and no Bt transgenes, and different nontransgenic **hybrid** backgrounds (2011 and 2012, Corpus Christi, TX). Differences in leaf injury, ear injury, and yield were detected among experimental factors and their interactions. Under high and low water stress, injury from noctuid larvae (Lepidoptera: Noctuidae) on leaves during vegetative growth (primarily from fall armyworm, *Spodoptera frugiperda* J. E. Smith) and on developing ears (primarily from corn earworm, *Helioverpa zea* [Boddie]) was lowest on more recent releases of Bt **hybrids** (newer Bt **hybrids**) expressing Cry1A. 105+Cry2Ab2 and Cry 3Bb1, compared with earlier Bt **hybrids** (older Bt **hybrids**) expressing Cry1Ab and Cry3Bb1 and non-Bt **hybrids**. High water stress led to increased leaf injury under substantial fall armyworm feeding pressure in 2011 (as high as 8.7 on a 1-9 scale of increasing injury). In contrast, ear injury by corn earworm (as high as 20 cm² of surface area of injury) was greater in low water stress conditions. Six **hybrid** backgrounds did not influence leaf injury, while ear injury differences across **hybrid** backgrounds were detected for non-Bt and older Bt **hybrid** versions. While newer Bt **hybrids** expressing Cry1A. 105+Cry2Ab2 and Cry 3Bb1 had consistent low leaf injury and high yield and low but less consistent ear injury across six **hybrid** backgrounds, water stress was a key factor that influenced yield. Bt transgenes played a more variable and lesser role when interacting with water stress to affect

yield. These results exemplify the interplay of water and insect stress with plant injury and yield, their interactions with Bt transgenes, and the importance of these interactions in considering strategies for Bt transgene use where water stress is common.

INHERITANCE OF ELECTROPHYSIOLOGICAL RESPONSES TO LEAF SAPS OF HOST- AND NONHOST PLANTS IN TWO *Helicoverpa* SPECIES AND THEIR HYBRIDS

Tang, QB; Huang, LQ; Wang, CZ; Tang, QB; Zhan, H; van Loon, JJA. 2014

The polyphagous cotton bollworm *Helicoverpa armigera* (Hubner) and the oligophagous oriental tobacco budworm *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) display contrasting heritable feeding preferences for cotton and pepper leaves. In this study, electrophysiological response patterns to cotton and pepper leaf saps in gustatory sensilla styloconica on the maxillae of these two species, their reciprocal F1 **hybrids**, and backcrossed lines were investigated using the tip recording technique. The identity of the neurons responding to the two leaf saps has been established using action potential waveform analysis. The two plant leaf saps elicited neural activity in at least six of the eight taste neurons innervating the lateral and medial sensilla styloconica of the parental species and crosses. Discriminant analysis of this multineural input predicted that correct classification occurred in 87 - 92% of the cases. Differences in taste neuron responses between insect lines to the two plant saps were consistent with differences in feeding preference behaviors. Comparisons of taste neuron response patterns of parental species, F1 **hybrids** and backcrosses indicate that autosomal loci contributed to the difference in gustatory response patterns between the two *Helicoverpa* species with the *H. armigera* derived alleles being partly dominant to those carried by *H. assulta*. These findings contribute to the understanding of gustatory codes for preference and provide insight into taste evolution of lepidopteran insects.

Building-Up of a DNA Barcode Library for True Bugs (Insecta: Hemiptera: Heteroptera) of Germany Reveals Taxonomic Uncertainties and Surprises

Raupach, MJ; Hendrich, L; Kuechler, SM; Deister, F; Moriniere, J; Gossner, MM. 2014

During the last few years, DNA barcoding has become an efficient method for the identification of species. In the case of insects, most published DNA barcoding studies focus on species of the Ephemeroptera, Trichoptera, Hymenoptera and especially Lepidoptera. In this study we test the efficiency of DNA barcoding for true bugs (Hemiptera: Heteroptera), an ecological and economical highly important as well as morphologically diverse insect taxon. As part of our study we analyzed DNA barcodes for 1742 specimens of 457 species, comprising 39 families of the Heteroptera. We found low nucleotide distances with a minimum pairwise K2P distance <2.2% within 21 species pairs (39 species). For ten of these species pairs (18 species), minimum pairwise distances were zero. In contrast to this, deep intraspecific sequence divergences with maximum pairwise distances >2.2% were detected for 16 traditionally recognized and valid species. With a successful identification rate of 91.5% (418 species) our study emphasizes the use of DNA barcodes for the identification of true bugs and represents an important step in building-up a comprehensive barcode library for true bugs in Germany and Central Europe as well. Our study also highlights the urgent necessity of taxonomic revisions for various taxa of the Heteroptera, with a special focus on various species of the Miridae. In this context we found evidence for on-going **hybridization** events within various taxonomically challenging genera (e.g. *Nabis* Latreille, 1802 (Nabidae), *Lygus* Hahn, 1833 (Miridae), *Phytocoris* Fallen, 1814 (Miridae)) as well as the putative existence of cryptic species (e.g. *Aneurus avenius* (Duffour, 1833) (Aradidae) or *Orius niger* (Wolff, 1811) (Anthracoridae)).

Bombyx mori pylorus infection by Alphabaculovirus

Baggio, MPD; Ribeiro, LFC; Vessaro-Silva, SA; Brancalhão, RMC. 2014

Alphabaculovirus is an entomopathogenic virus genus that infects *Bombyx mori*, which is known as the *Bombyx mori* multiple nucleopolyedrovirus (BmMNPV). This virus is polyorganotrophic, and a series of tissues are known as targets; however, there is currently no information regarding infection in the pylorus, the segment of the hindgut that is present in the midgut transition and is responsible for food passage control. Thus, in the present study, we aimed to analyze infection of the *B. mori* pylorus by BmMNPV. To do so, **hybrid** *B. mori* larvae were inoculated with a viral suspension of BmMNPV, and segments of the intestine containing the pylorus and its subdivisions, the posterior interstitial ring (PIR), pyloric cone, and pyloric valve, were dissected and processed for light microscopy on different days post inoculation. The results showed that *B. mori* pylorus subdivisions respond differently to infection, and the anterior area of the PIR is susceptible with these cells being the secondary infection targets. Cytological analysis revealed the presence of viroplasm in the hypertrophic nucleus, followed by the formation and development of viral polyhedra. Cytolysis occurred at the end of the infectious cycle, thereby releasing polyhedra and enabling the spread of the disease. There was no evidence of BmMNPV infection in the posterior area of the PIR, cone, or pyloric valve. These results will contribute to greater understanding of the virus infectious cycle, whose consequent epizootic disease can negatively impact this economically important insect that is used in silk production in Brazil.

Morphology of the Pylorus of *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae)

Baggio, MPD; Vessaro-Silva, SA; Ribeiro, LFC; Brancalhão, RMC. 2014

The pylorus is a segment of the hindgut of great morphological diversity, which is subdivided into the posterior interstitial ring, pyloric cone, and pyloric valve. There is no morphological information on *Bombyx mori* (L.) **hybrids**, which is an insect of economic interest to Brazil. Consequently, this study aimed to analyze the morphology of the pylorus of **hybrid** larvae of fifth instars of *B. mori* from crossbreeding of Chinese and Japanese races. These were anesthetized, and the pylorus was fixed and processed by light microscopy and scanning electron microscopy. The pylorus presented as a separate compartment, consisting of a simple epithelium, with large variation in cell morphology, covered on the luminal side by an intima with specializations in some areas, the spicules. The musculature was formed by bundles of striated muscle fibers in longitudinal and circular arrangement. These fibers were multinucleated with central and peripheral nuclei, operating in peristalsis. The morphology of the pylorus is a reflection of functional dynamism, and this study provided a better understanding of its physiology, which promotes the regulated movement of the bolus, preventing reflux and directing it towards the ileum in the formation of fecal pellets.

'Becoming a species by becoming a pest' or how two maize pests of the genus *Ostrinia* possibly evolved through parallel ecological speciation events

Bourguet, D; Ponsard, S; Streiff, R; Meusnier, S; Audiot, P; Li, J; Wang, ZY. 2014

New agricultural pest species attacking introduced crops may evolve from pre-existing local herbivores by ecological speciation, thereby becoming a species by becoming a pest. We compare the evolutionary pathways by which two maize pests (the Asian and the European corn borers, ACB and ECB) in the genus *Ostrinia* (Lepidoptera, Crambidae) probably diverged from an ancestral species close to the current Adzuki bean borer (ABB). We typed larval *Ostrinia* populations collected on maize and dicotyledons across China and eastern Siberia, at microsatellite and mitochondrial loci. We found only two clusters: one on maize (as expected) and a single one on dicotyledons despite differences in male mid-tibia morphology, suggesting that all individuals from dicotyledons belonged to the ABB. We found evidence for migrants and **hybrids** on both host plant types. Hybrids suggest that field reproductive isolation is incomplete between ACB and ABB. Interestingly, a few individuals with an ABB-like' microsatellite profile collected on dicotyledons had ACB' mtDNA rather than ABB-like' mtDNA, whereas the reverse was never found on maize. This suggests asymmetrical gene flow directed from the ACB towards the ABB. Hybrids and backcrosses in all directions were obtained in no-choice tests. In laboratory conditions, they survived as well as parental strain individuals. In Xinjiang, we found ACB and ECB in sympatry, but no **hybrids**. Altogether, our results suggest that reproductive isolation between ACB and ABB is incomplete and mostly prezygotic. This points to ecological speciation as a possible evolutionary scenario, as previously found for ECB and ABB in Europe.

Progress Towards RNAi-Mediated Insect Pest Management

Baum, JA; Roberts, JK. 2014

Gene suppression via RNA interference (RNAi) provides an alternative strategy for insect pest management. The ingestion by insects of double-stranded RNAs targeting essential insect genes can trigger RNAi and lead to growth inhibition, developmental aberrations, reduced fecundity, and mortality. This RNAi response is particularly acute in certain coleopteran species, most notably the western corn rootworm, a devastating pest impacting corn production in the United States. The development of next-generation rootworm-protected corn **hybrids** includes an RNAi-based trait that provides a mode of action distinct from those of *Bacillus thuringiensis* insecticidal protein-based traits currently used for rootworm pest management. Unfortunately, many insect species including important lepidoptera and hemiptera pests appear largely recalcitrant in their response to environmental RNA, suggesting biological barriers that thus far limit the utility of RNAi for agricultural pest management. This review will highlight recent efforts to understand the barriers to RNA delivery in recalcitrant insect species, describe recent advances in the commercial development of insect-protected crops and biological insecticides utilizing RNAi, and discuss this strategy in the context of an integrated pest management approach.

Life table and consumption capacity of corn earworm, *Helicoverpa armigera*, fed asparagus, *Asparagus officinalis*

Jha, RK; Tuan, SJ; Chi, H; Tang, LC. 2014

The life table and consumption rate of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) reared on asparagus, *Asparagus officinalis* L. (Asparagales: Asparagaceae) were studied under laboratory conditions to assess their interaction. Development, survival, fecundity, and consumption data were analyzed by the age-stage, two-sex life table. This study indicated that asparagus is a natural host of *H. armigera*. However, the poor nutritional content in asparagus foliage and the poor fitness of *H. armigera* that fed on asparagus indicated that asparagus is a suboptimal host in comparison to **hybrid** sweet corn. The uncertainty associated with life table parameters was estimated by using jackknife and bootstrap techniques, and the results were compared for statistical inference. The intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T) were estimated by the jackknife technique to be 0.0780 day⁻¹, 1.0811 day⁻¹, 67.4 offspring, and 54.8 days, respectively, while those estimated by the bootstrap technique were 0.0752 day⁻¹, 1.0781 day⁻¹, 68.0 offspring, and 55.3 days, respectively. The net consumption rate of *H. armigera*, as estimated by the jackknife and bootstrap technique, was 1183.02 and 1132.9 mg per individual, respectively. The frequency distribution of sample means obtained by the jackknife technique failed the normality test, while the bootstrap results fit the normal distribution well. By contrast, the relationship between the mean fecundity and the net reproductive rate, as estimated by the bootstrap technique, was slightly inconsistent with the relationship found by mathematical proof. The application of the jackknife and bootstrap techniques in estimating population parameters requires further examination.

GLYPHOSATE TOLERANT VOLUNTEER CORN CONTROL AT TWO DEVELOPMENT STAGES

Costa, NV; Zobiolo, LHS; Scariot, CA; Pereira, GR; Moratelli, G. 2014

The loss of grains during the harvest of glyphosate tolerant corn may generate volunteer plants, which can interfere in the conventional or glyphosate crop in succession. The current work aim to evaluate the control of the volunteer corn glyphosate tolerant under two weed stages. Aimed to evaluate the control of volunteer glyphosate tolerant corn in two stages of development. There were conducted two experiments with **hybrid** 2B688 HR (lepidoptera and glyphosate tolerant), the application were at V-5 and V-8 stage. The experiment was randomized block design with four replicates, using the treatments: haloxyfop at 25, 50 and 62 g ha⁻¹ alone and associated with 2,4-D at 670 g ha⁻¹ or fluroxypyr at 200 g ha⁻¹. The standard was clethodim at 84 g ha⁻¹ with 2,4-D and fluroxypyr at same rates. The applications of haloxyfop and clethodim both isolated or in a mixture with 2,4-D and fluroxypyr at V5 stage showed total control (100%) at 32 and 39 days after the application, except for haloxyfop + 2,4-D (25 + 670 g ha⁻¹) mixture, which did not provided adequate control. At V8 stage, haloxyfop + 2,4-D (50 + 670 g ha⁻¹) and haloxyfop + 2,4-D (62 + 670 g ha⁻¹) mixtures took up to 6 and 10 days or longer to reach adequate to excellent control, when compared to haloxyfop isolated applications in the same doses, respectively. Either isolated clethodim or mixed with 2, 4-D and fluroxypyr did not show adequate control. The treatments showed efficient control on volunteer corn plants at V-5 stage, except for haloxyfop + 2, 4-D (25 + 670 g ha⁻¹) mixture. At V-8 stage applications, haloxyfop either isolated or mixture with fluroxypyr demonstrated excellent control on every evaluated dose. The mixture with 2, 4-D can reduce haloxyfop efficiency at low doses. Clethodim alone or mixed with 2,4-D or fluroxypyr did not provide acceptable level of control.

Molecular evidence for hybridization in *Colias* (Lepidoptera: Pieridae): are *Colias* hybrids really hybrids?

Dwyer, HE; Jasieniuk, M; Okada, M; Shapiro, AM. 2015

Gene flow and **hybridization** among species dramatically affect our understanding of the species as a biological unit, species relationships, and species adaptations. In North American *Colias eurytheme* and *Colias eriphyle*, there has been historical debate over the extent of **hybridization** occurring and the identity of phenotypically intermediate individuals as genetic **hybrids**. This study assesses the population structure of these two species to measure the extent of **hybridization** and the genetic identity of phenotypic intermediates as **hybrids**. Amplified fragment length polymorphism (AFLP) marker analysis was performed on 378 specimens collected from northern California and Nevada. Population structure was inferred using a Bayesian/Markov chain Monte Carlo method, which probabilistically assigns individuals to genetic clusters. Three genetic clusters provided the best fit for the data. *C.eurytheme* individuals were primarily assigned to two closely related clusters, and *C.eriphyle* individuals were mostly assigned to a third, more distantly related cluster. There appeared to be significant **hybridization** between the two species. Individuals of intermediate phenotype (putative **hybrids**) were found to be genetically indistinguishable from *C.eriphyle*, indicating that previous work based on the assumption that these intermediate forms are **hybrids** may warrant reconsideration.

Maternal effect and speciation: maternal effect contributes to the evolution of hybrid inviability between *Drosophila simulans* and *Drosophila mauritiana*

Eizadshenass, S; Singh, RS. 2015

Haldane's rule has been the basis of speciation research during the last 30 years. Most studies have focused on the nature of incompatibilities in the **hybrid** male, but not much attention has been given to the genetic basis of fertility and inviability in **hybrid** females. Hybridizations between *Drosophila simulans* and *Drosophila mauritiana* produce fertile females and sterile males. Here, we re-examined the level of fertility in reciprocal F1 females of these two species and looked for the presence of maternal effects. Our results show that the reciprocal F1 females of *D. simulans* and *D. mauritiana* **hybridizations** are fully fertile and in fact show a significant level of heterosis in the rate of oviposition but display reduced egg hatching in one direction. Reduced egg hatching was observed in the progenies of F1 **hybrid** females with *D. mauritiana* as mother, the same cross that showed a stronger negative effect on F1 male fertility. A review of the literature on the **hybridizations** in Lepidoptera also showed a maternal effect on inviability when reciprocal crosses produced asymmetric results. Our findings point to the importance of maternal effects in the evolution of embryo inviability and thus enhancing the process of speciation through the evolution of **hybrid** inviability.

Hybridization promotes speciation in *Coenonympha* butterflies

Capblancq, T; Despres, L; Rioux, D; Mavarez, J. 2015

Hybridization has become a central element in theories of animal evolution during the last decade. New methods in population genomics and statistical model testing now allow the disentangling of the complexity that **hybridization** brings into key evolutionary processes such as local adaptation, colonization of new environments, species diversification and extinction. We evaluated the consequences of **hybridization** in a complex of three alpine butterflies in the genus *Coenonympha*, by combining morphological, genetic and ecological analyses. A series of approximate Bayesian computation procedures based on a large SNP data set strongly suggest that the Darwin's Heath (*Coenonympha darwiniana*) originated through **hybridization** between the Pearly Heath (*Coenonympha arcania*) and the Alpine Heath (*Coenonympha gardetta*) with different parental contributions. As a result of **hybridization**, the Darwin's Heath presents an intermediate morphology between the parental species, while its climatic niche seems more similar to the Alpine Heath. Our results also reveal a substantial genetic and morphologic differentiation between the two geographically disjoint Darwin's Heath lineages leading us to propose the splitting of this taxon into two different species.

Genetic structure, admixture and invasion success in a Holarctic defoliator, the gypsy moth (*Lymantria dispar*, Lepidoptera: Erebiidae)

Wu, YK; Molongoski, JJ; Winograd, DF; Bogdanowicz, SM; Louyakis, AS; Lance, DR; Mastro, VC; Harrison, RG. 2015

Characterizing the current population structure of potentially invasive species provides a critical context for identifying source populations and for understanding why invasions are successful. Non-native populations inevitably lose genetic diversity during initial colonization events, but subsequent admixture among independently introduced lineages may increase both genetic variation and adaptive potential. Here we characterize the population structure of the gypsy moth (*Lymantria dispar* Linnaeus), one of the world's most destructive forest pests. Native to Eurasia and recently introduced to North America, the current distribution of gypsy moth includes forests throughout the temperate region of the northern hemisphere. Analyses of microsatellite loci and mitochondrial DNA sequences for 1738 individuals identified four genetic clusters within *L. dispar*. Three of these clusters correspond to the three named subspecies; North American populations represent a distinct fourth cluster, presumably a consequence of the population bottleneck and allele frequency change that accompanied introduction. We find no evidence that admixture has been an important catalyst of the successful invasion and range expansion in North America. However, we do find evidence of ongoing **hybridization** between subspecies and increased genetic variation in gypsy moth populations from Eastern Asia, populations that now pose a threat of further human-mediated introductions. Finally, we show that current patterns of variation can be explained in terms of climate and habitat changes during the Pleistocene, a time when temperate forests expanded and contracted. Deeply diverged matrilineal lineages in Europe imply that gypsy moths have been there for a long time and are not recent arrivals from Asia.

Comparative Feeding Performance and Digestive Physiology of *Helicoverpa armigera* (Lepidoptera: Noctuidae) Larvae Fed 11 Corn Hybrids

Hosseininejad, AS; Naseri, B; Razmjou, J. 2015

This study aimed to evaluate the feeding responses and digestive proteolytic and amylolytic activity of *Helicoverpa armigera* (Hubner) on 11 corn (*Zea mays* L.) **hybrids** at 25 +/- 1 degrees C, 65 +/- 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. The fourth- and fifth-instar larvae fed on **hybrid K47K19** had the highest weight of food consumption and those reared on **hybrid KSC705** had the lowest value of food consumption. The highest weight gain of the larvae was observed when *H. armigera* were fed **hybrid KLM78M017** and lowest when they were fed **hybrids K36 * M017, KSC705, and K35 * K36**. Pupal weight of *H. armigera* was heaviest when larvae were fed **hybrid K47K19** and lightest when they were fed **hybrid KSC705**. The highest proteolytic activity of the fourth-instar larvae was observed when they were fed **hybrid KSC705**, and the lowest activity was observed when they were fed **hybrid K47A67**. Fifth-instar larvae that fed on **hybrid K47K19** showed the highest proteolytic activity. Fourth-instar larvae that fed on **hybrid K36M017** showed the highest amylase activity. The fifth-instar larvae fed on **hybrid K47A67** showed the maximum amylase activity and those reared on the **K48K18** showed the minimum activity. Our results indicated that **K36 * M017, KSC705, and K48 * K18** were the most unsuitable **hybrids** for feeding *H. armigera*.

Thermal Requirements, Fertility, and Number of Generations of *Neoleucinodes elegantalis* (Guenee) (Lepidoptera: Crambidae)

Moraes, CP; Foerster, LA. 2015

The small tomato borer *Neoleucinodes elegantalis* (Guenee) (Lepidoptera: Crambidae) is one of the major pests of solanaceous in Central and South America. Little is known about the role of temperature on the biological traits of this species. Development time of the immature stages and longevity and reproduction of adults of *N. elegantalis* at constant temperatures (15, 20, 25, 27, and 30 +/- 1 degrees C) were determined when larvae were fed on fruits of the tomato **hybrid "Paronset"**. *Neoleucinodes elegantalis* completed its life cycle at temperatures between 15 degrees C and 27 degrees C, indicating that it can remain active throughout the year in the subtropical region of Brazil. The temperature threshold (T-t) was 8.8 degrees C for the egg stage and 7.7 degrees C for the larval and pupal stages. The small tomato borer required 588.2 degrees-day for its full development. At 30 degrees C, the eggs were infertile, indicating that temperatures above 27 degrees C are deleterious to the fertilization/embryonic development of the studied population of *N. elegantalis*. The number of generations of *N. elegantalis* per year in the Brazilian subtropical region is lower than for populations developing in warmer tropical areas. A relationship was found between the number of generations and the increase in latitude.

Analysis of transcripts of heat shock protein genes in silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)

Chandrakanth, N; Ponnuvel, KM; Moorthy, SM; Sasibhushan, S; Sivaprasad, V. 2015

Silkworm is a poikilothermic insect, whose growth and development is significantly affected by high temperatures. The current study aimed to identify bivoltine breeds tolerant of the high temperature conditions that occur in the tropics. Percentage pupation at high temperatures and heat shock responses of silkworms were used as measures of thermotolerance. Thermotolerance of 20 silkworm breeds was assessed by rearing them at 36 degrees C. Based on percentage pupation, three breeds, namely Nistari (multivoltine), SK4C (bivoltine) and CSR2 (bivoltine) were designated tolerant, moderately tolerant and susceptible, respectively. To understand the heat shock responses and the molecular mechanisms underlying thermotolerance, the tissue specific expression profiles of the nine heat shock protein (Hsp) genes were determined in the three breeds after a heat shock of 1 h at 36 degrees C and a 2 h recovery period by performing real-time qPCR. The level of expression of Hsp genes was significantly increased in heat shocked tissues and gradually decreased during the recovery period. The greatest increase in the expression of Hsp genes was recorded in fat body followed by mid gut and silk gland. Of the three breeds, Nistari showed the highest expression of Hsp genes and SK4C a moderate expression relative to CSR2. The qPCR results showed that the transcript levels of sHsp20.4 and 20.1, and Hsp70 were increased by 10.3, 9.7 and 2.3 times, respectively, in Nistari compared to CSR2. Similarly the expression of sHsp20.4 and 20.1, and Hsp70 were increased by 3.5, 2.3 and 1.5 times, respectively in SK4C compared to CSR2. The expression levels of Hsps during heat shock corresponded to the percentage pupation recorded for the three breeds at a high temperature. It is suggested that the Hsps and their levels of expression may play an important role in increasing the survival of silkworm larvae at high temperatures. This study identified SK4C as a bivoltine breed, which is highly tolerant of high temperature measured in terms of percentage pupation (of the bivoltine breeds) and higher levels of expression of Hsp genes compared to CSR2. The importance of SK4C as a thermotolerant bivoltine parent for breeding new bivoltine **hybrids** tolerant of high temperatures is discussed.

Beyond magic traits: Multimodal mating cues in *Heliconius* butterflies

Merot, C; Frerot, B; Leppik, E; Joron, M. 2015

Species coexistence involves the evolution of reproductive barriers opposing gene flow. *Heliconius* butterflies display colorful patterns affecting mate choice and survival through warning signaling and mimicry. These patterns are called "magic traits" for speciation because divergent natural selection may promote mimicry shifts in pattern whose role as mating cue facilitates reproductive isolation. By contrast, between comimetic species, natural selection promotes pattern convergence. We addressed whether visual convergence interferes with reproductive isolation by testing for sexual isolation between two closely related species with similar patterns, *H. timareta thelxinoe* and *H. melpomene amaryllis*. Experiments with models confirmed visual attraction based on wing phenotype, leading to indiscriminate approach. Nevertheless, mate choice experiments showed assortative mating. Monitoring male behavior toward live females revealed asymmetry in male preference, *H. melpomene* males courting both species equally while *H. timareta* males strongly preferred conspecifics. Experiments with **hybrid** males suggested an important genetic component for such asymmetry. Behavioral observations support a key role for short-distance cues in determining male choice in *H. timareta*. Scents extracts from wings and genitalia revealed interspecific divergence in chemical signatures, and **hybrid** female scent composition was significantly associated with courtship intensity by *H. timareta* males, providing candidate chemical mating cues involved in sexual isolation.

Hybrid Sex Pheromones of the Hibiscus Flower-bud Borer, *Rehimena surusalis*

Honda, H; Yamasaki, R; Sumiuchi, Y; Uehara, T; Matsuyama, S; Ando, T; Naka, H. 2015

The sex pheromone of the hibiscus flower borer *Rehimena surusalis* (Walker) (Lepidoptera: Crambidae) was analyzed by gas chromatography with electroantennographic detection (GC-EAD) and GC-mass spectrometry (GC/MS). Three EAD-active components were found in crude pheromone gland extracts of calling females. GC/MS and GC analyses using synthetic chemicals and derivatization of the extracts identified three components as (10E,12Z)-hexadeca-10,12-dienal (E10,Z12-16:Ald), (10E,12E)-hexadeca-10,12-dienyl acetate (E10,Z12-16:OAc), and (3Z,6Z,9Z)-tricos-3,6,9-triene (Z3,Z6,Z9-23:HC). In field tests, male moths were strongly attracted to a ternary blend of E10,Z12-16:Ald, E10,Z12-16:OAc, and Z3,Z6,Z9-23:HC at a ratio of 1:5:14, but single and binary blends showed only weak or no attraction.

Insect Visitors and Abundance of Four Species of *Apis* on Sunflower *Helianthus annuus* L. in Pakistan

Ali, H; Owayss, AA; Khan, KA; Alqarni, AS. 2015

Sunflower (*Helianthus annuus* L.) is an open pollinated crop and the world's fourth important source of edible oil. Two fields, one of them adjacent to an apiary with 40 hives, were sampled weekly for two months. A total of fifteen species of insect pollinators were recorded. Species of the order Hymenoptera were the most abundant (91%), followed by those of Lepidoptera (6%) and Diptera (3%). The relative abundance of four *Apis* species, i.e. *Apis mellifera*, *A. dorsata*, *A. cerana* and *A. florea*, varied on hourly and weekly basis. The peak densities of all honeybee species were recorded at 12:00 pm and 02:00 pm while minimum densities were recorded at 08:00 am and 04:00 pm. *Apis mellifera* was the most recorded honeybee species compared to other *Apis* spp. during the study. In both fields the differences were significant.

Spodoptera frugiperda (Lepidoptera: Noctuidae) host-plant variants: two host strains or two distinct species?

Dumas, P; Legeai, F; Lemaitre, C; Scaon, E; Orsucci, M; Labadie, K; Gimenez, S; Clamens, AL; Henri, H; Vavre, F; Aury, JM; Fournier, P; Kergoat, GJ; d'Alencon, E. 2015

The moth *Spodoptera frugiperda* is a well-known pest of crops throughout the Americas, which consists of two strains adapted to different host-plants: the first feeds preferentially on corn, cotton and sorghum whereas the second is more associated with rice and several pasture grasses. Though morphologically indistinguishable, they exhibit differences in their mating behavior, pheromone compositions, and show development variability according to the host-plant. Though the latter suggest that both strains are different species, this issue is still highly controversial because **hybrids** naturally occur in the wild, not to mention the discrepancies among published results concerning mating success between the two strains. In order to clarify the status of the two host-plant strains of *S. frugiperda*, we analyze features that possibly reflect the level of post-zygotic isolation: (1) first generation (F1) **hybrid** lethality and sterility; (2) patterns of meiotic segregation of **hybrids** in reciprocal second generation (F2), as compared to the meiosis of the two parental strains. We found a significant reduction of mating success in F1 in one direction of the cross and a high level of microsatellite markers showing transmission ratio distortion in the F2 progeny. Our results support the existence of post-zygotic reproductive isolation between the two laboratory strains and are in accordance with the marked level of genetic differentiation that was recovered between individuals of the two strains collected from the field. Altogether these results provide additional evidence in favor of a sibling species status for the two strains.

Preference for isolated host plants facilitates invasion of *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) by a bacterial male-killer *Spiroplasma*

Gordon, IJ; Ileri, P; Smith, DAS. 2015

Matrilineally inherited endosymbiotic bacteria are estimated to occur in a third of all terrestrial arthropods. They include male killers, which are typically associated with organisms that lay their eggs in batches. Batch laying favours male-killing bacteria because infected female larvae can cannibalise dead brothers and do not have to compete with them for larval resources. This paper tackles the paradox of high male-killer infection rates in *Danaus chrysippus* (Linnaeus, 1758), a butterfly that lays its eggs singly rather than in batches, making the selective advantage of male killing for the bacterium hard to understand. The study was conducted in the Nairobi region of Kenya where two substantially allopatric but seasonally migratory semispecies of *Danaus chrysippus* overlap and **hybridise**. Within this **hybrid** zone, but apparently not outside it, *D. chrysippus* hosts the endosymbiotic male killer, *Spiroplasma ixodetis*. In Nairobi, this bacterium can infect up to 95% of females. Semispecies *D. chrysippus chrysippus* and its **hybrid** with *D. chrysippus dorippus* (form transiens) are heavily infected, unlike the parental pure *D. chrysippus dorippus* semispecies, which may be protected by a suppressor gene. We find that: (1) adult females oviposit preferentially on isolated host plants; (2) female larvae on isolated plants survive better than on plants growing in clumps; (3) female larvae survive in greater numbers on plants removed to the laboratory compared to plants left outside; (4) high *Spiroplasma* infection favours female survival; and (5) high egg/larval density adversely affects adult body size. We conclude that: (1) the butterflies' preference for isolated host plants creates high egg densities and hence enables invasion by *Spiroplasma*; (2) male killing favours female survival through cannibalism of male eggs and the extra food-plant resources so released; and (3) the butterfly's preference for isolated host plants may provide an enemy-free space that protects eggs and larvae from predators and parasitoids.

Functional characterization of two paralogous JH receptors, methoprene-tolerant 1 and 2, in the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)

Kayukawa, T; Shinoda, T. 2015

Methoprene-tolerant (Met) is a bHLH-PAS transcription factor that functions as a core component of juvenile hormone (JH) receptors in insects. The genome of the silkworm, *Bombyx mori* (L.), encodes two Met paralogs, BmMet1 and BmMet2. We have previously proposed that BmMet2 forms a functional complex with steroid receptor co-activator (BmSRC) in the presence of JH and induces Kruppel-homolog 1 (BmKr-h1). In this study, we characterized BmMet2 domains and examined the

role of BmMet1. A series of reporter and two-**hybrid** assays on BmMet2 revealed that the bHLH domain played a critical role in the binding to the JH response element of BmKr-h1 (kJHRE), the PAS-A in the transcriptional activation, and the PAS-B in the JH recognition. Furthermore, we confirmed that the BmMet2/BmSRC complex recognizes the core kJHRE (GGCCTCCACGTG). BmMet1 was found to interact with BmSRC and weakly activated the kJHRE reporter in a JH-dependent manner. The BmMet1 transcript level was much higher than the BmMet2 transcript level in the epidermis during the larval and pupal stages. By contrast, the BmMet2 transcript was hardly detected during the larval stage, but was abundant in the adult stage. These results suggest that BmMet1 is involved in the JH-dependent induction of BmKr-h1 in larvae, whereas BmMet2 is involved in adults.

Evidence for low-level hybridization between two allochronic populations of the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae)

Burban, C; Gautier, M; Leblais, R; Landes, J; Santos, H; Paiva, MR; Branco, M; Kerdelhue, C. 2016

Divergence between populations sharing the same habitat can be initiated by different reproductive times, leading to allochronic differentiation. A spatially localized allochronic summer population (SP) of the pine processionary moth *Thaumetopoea pityocampa*, recently discovered in Portugal, occurs in sympatry with the local winter population (WP). We examined the level of genetic differentiation between the two populations and estimated the current gene flow within the spatial framework of their co-occurrence. Mitochondrial data indicated that the two sympatric populations were genetically closer than other WP populations. Conversely, microsatellite genotyping uncovered greater differentiation between the two sympatric populations than between allopatric ones. While male trapping confirmed that reproduction of SP and WP occurred at distinct times, clustering approaches demonstrated the presence of a few LateSP individuals emerging within the WP flight period, although genetically identified as SP. We also identified rare recent **hybridization** events apparently occurring mainly in the margins of the current SP range. The ongoing gene flow detected between the ancestral and the emerging allochronic populations revealed an incomplete reproductive isolation, which must therefore be taken into account and integrated with studies focussed on ecological drivers, so that a complete understanding of the ongoing speciation process might be achieved. (c) 2016 The Linnean Society of London, Biological Journal of the Linnean Society, 2016, 119, 311-328.

Complete mitochondrial genome of a hybrid strain of the domesticated silkworm (Qiufeng x Baiyu)

Li, FB; Zhang, HX; Liu, PG; Wang, YQ; Meng, ZQ. 2016

The **hybrid** strain of the domesticated silkworm (Qiufeng x Baiyu) is one of the most popular commercial silkworm varieties in China. In this study, we reported its complete mitochondrial genome sequence for the first time. The 15,680 bp long genome contains 37 genes (13 protein-coding genes [PCGs], 2 rRNA genes, and 22 tRNA genes) and 1 major non-coding A + T-rich region, with the typical arrangement found in Lepidoptera. All PCGs started with typical ATN codons except for COI, which began with CGA. Eleven PCGs have complete stop codons, whereas COI and COII end with a single T. The 495 bp long A + T-rich region harbors the conserved sequence features typically found in lepidopteran insects. The complete mitochondrial genome sequence of Qiufeng x Baiyu provides an important data source for further study on the mechanism of silkworm domestication.

Genetic diversity among eight *Dendrolimus* species in Eurasia (Lepidoptera: Lasiocampidae) inferred from mitochondrial COI and COII, and nuclear ITS2 markers

Kononov, A; Ustyantsev, K; Wang, BD; Mastro, VC; Fet, V; Blinov, A; Baranchikov, Y. 2016

Background: Moths of genus *Dendrolimus* (Lepidoptera: Lasiocampidae) are among the major pests of coniferous forests worldwide. Taxonomy and nomenclature of this genus are not entirely established, and there are many species with a controversial taxonomic position. We present a comparative evolutionary analysis of the most economically important *Dendrolimus* species in Eurasia. Results: Our analysis was based on the nucleotide sequences of COI and COII mitochondrial genes and ITS2 spacer of nuclear ribosomal genes. All known sequences were extracted from GenBank. Additional 112 new sequences were identified for 28 specimens of *D. sibiricus*, *D. pini*, and *D. superans* from five regions of Siberia and the Russian Far East to be able to compare the disparate data from all previous studies. In total, 528 sequences were used in phylogenetic analysis. Two clusters of closely related species in *Dendrolimus* were found. The first cluster includes *D. pini*, *D. sibiricus*, and *D. superans*; and the second, *D. spectabilis*, *D. punctatus*, and *D. tabulaeformis*. Species *D. houi* and *D. kikuchii* appear to be the most basal in the genus. Conclusion: Genetic difference among the second cluster species is very low in contrast to the first cluster species. Phylogenetic position *D. tabulaeformis* as a subspecies was supported. It was found that *D. sibiricus* recently separated from *D. superans*. Integration of *D. sibiricus* mitochondrial DNA sequences and the spread of this species to the west of Eurasia have been established as the cause of the unjustified allocation of a new species: *D. kilmez*. Our study further clarifies taxonomic problems in the genus and gives more complete information on the genetic structure of *D. pini*, *D. sibiricus*, and *D. superans*.

Refining mimicry: phenotypic variation tracks the local optimum

Merot, C; Le Poul, Y; Thery, M; Joron, M. 2016

Mullerian mimicry between chemically defended preys is a textbook example of natural selection favouring phenotypic convergence onto a shared warning signal. Studies of mimicry have concentrated on deciphering the ecological and genetic underpinnings of dramatic switches in mimicry association, producing a well-known mosaic distribution of mimicry patterns across geography. However, little is known about the accuracy of resemblance between natural comimics when the local phenotypic optimum varies. In this study, using analyses of wing shape, pattern and hue, we quantify multimodal phenotypic similarity between butterfly comimics sharing the so-called postman pattern in different localities with varying species composition. We show that subtle but consistent variation between populations of the localized species, *Heliconius timareta thelxinoe*, enhance resemblance to the abundant comimics which drive the mimicry in each locality. Those results suggest that rarer comimics track the changes in the phenotypic optimum caused by gradual changes in the composition of the mimicry community, providing insights into the process by which intraspecific diversity of mimetic pattern may arise. Furthermore, our results suggest a multimodal evolution of similarity, with coordinated convergence in different features of the phenotype such as wing outline, pattern and hue. Finally, multilocus genotyping allows estimating local **hybridization** rates between *H. timareta* and comimic *H. melpomene* in different populations, raising the hypothesis that mimicry refinement between closely related comimics may be enhanced by adaptive introgression at loci modifying the accuracy of resemblance.

Combining Tpi and CO1 Genetic Markers to Discriminate Invasive *Helicoverpa armigera* From Local *Helicoverpa zea* (Lepidoptera: Noctuidae) Populations in the Southeastern United States

Nagoshi, RN; Gilligan, TM; Brambila, J. 2016

The recent establishment of the Old World pest *Helicoverpa armigera* (Hubner) into South America has had significant economic consequences and places the rest of the hemisphere at risk, emphasizing the need for improved methods of monitoring. A major complication is that a sibling species endemic to the New World, *Helicoverpa zea* (Boddie), is morphologically very similar, with the two species capable of producing fertile **hybrids** in the laboratory. The consequences of such **hybridization** in the field are uncertain, but could result in significant and unpredictable changes in the timing, range, and pesticide susceptibilities of *Helicoverpa* infestations. The objective here is to provide new genetic resources applicable to *Helicoverpa* populations in northern Florida and neighboring states (a region at risk for *H. armigera*) that can distinguish the two species and possible **hybrids**. The genetic variability in segments of the mitochondrial cytochrome oxidase 1 (CO1) and the Z-linked triosephosphate isomerase (Tpi) genes were determined for *H. zea* from the southeastern United States. These were compared to DNA sequences from

H. armigera specimens from Morocco, Australia, and Europe. Phylogenetic network analysis showed a clear demarcation between the two species for all gene segments. These results extend earlier studies establishing CO1 as marker for discriminating the *Helicoverpa* species complex and introduce a new sex-linked genomic marker. The CO1 and Tpi markers in combination provide a more accurate and sensitive method than existing techniques for identifying **hybridization** between *H. zea* and *H. armigera* and could potentially be used to extrapolate the likely source of invasive *H. armigera* populations.

Comparison of the ability to catabolize DIMBOA, a maize antibiotic, between *Ostrinia furnacalis* and *Ostrinia scapularis* (Lepidoptera: Crambidae), with reference to their hybrids

Phuong, TTT; Yamamoto, M; Fujii, T; Kojima, W; Matsuo, T; Ishikawa, Y. 2016

Maize contains 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which functions as a feeding deterrent, growth inhibitor, and toxin against many herbivorous insects. The Asian corn borer *Ostrinia furnacalis* (Guené) (Lepidoptera: Crambidae) feeds on maize, whereas the sympatric congener *O. scapularis* (Walker) does not. In laboratory assays, the addition of 0.3 mg/g of DIMBOA to an artificial diet markedly affected the survival of *O. scapularis* larvae but not that of *O. furnacalis* larvae. Hybrids of *O. furnacalis* and *O. scapularis*, crossed in both directions, tolerated DIMBOA to the same extent as *O. furnacalis*, indicating that this tolerance was conferred by a single or a few autosomal genes that are dominant to those of *O. scapularis*. In vitro, DIMBOA was rapidly catabolized when incubated with the homogenate of the digestive tract of *O. furnacalis* in the presence of uridine diphosphate (UDP)-glucose. The UDP-glucose-dependent DIMBOA-catabolizing activities of the homogenate of the digestive tracts of *O. scapularis* and **hybrids** correlated with their tolerance; low in *O. scapularis* and high in the **hybrids**. These results reconfirmed that UDP-glucosyltransferase (UGT) or other UDP-dependent enzymes are involved in the catabolism of DIMBOA in *O. furnacalis*; however, consistent with our previous findings, DIMBOA-glucoside, the expected product of UGT, was not detected in the products of in vitro assays.

A Z-linked sterility locus causes sexual abstinence in hybrid females and facilitates speciation in *Spodoptera frugiperda*

Kost, S; Heckel, DG; Yoshido, A; Marec, F; Groot, AT. 2016

In the fall armyworm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae), two sympatric strains have been recognized that have been termed corn strain (C) and rice strain (R), referring to their most common host plants. Both strains are reproductively isolated via a distinct prezygotic barrier as well as via an intriguing postzygotic phenomenon: when R females have mated with C males, the resulting RC **hybrid** females exhibit dramatically reduced fertility independent of their mating partner. Here, we demonstrate that the reduced fertility is caused by the fact that these females refrain from mating, that is, females are behaviorally sterile. We identified a Z-chromosomally linked sterility locus that is most likely incompatible with yet to be identified autosomal (or cytoplasmic) factors, leading to the observed sexual abstinence. Within-chromosome mapping revealed the sterility locus to be located in an area of strongly reduced interstrain recombination.

Impact of transgenic Bt cotton on nutritional indices of spotted bollworm, *Earias vittella* (Fabricius) (Lepidoptera: Nolidae)

Shera, PS; Arora, R. 2016

The quality of food consumed by the insect is the key factor determining the utilization of food, thus having great impact on its survival, development, and longevity. Genetically modified plants with genes from soil inhabiting spore forming bacterium, *Bacillus thuringiensis* Berliner produces delta-endotoxin, which target the gut of insect, so food intake and its utilization are likely to be affected. Four Bt cotton **hybrids**, each with one of four events, viz. MRC 6301 Bt (cry1Ac gene), JKCH 1947 Bt (modified cry1Ac gene), NCEH 6R Bt (cry1Ab/cry1Ac fused gene) and MRC 7017 BG II (cry1Ac and cry2Ab genes) were compared for nutritional indices of spotted bollworm, *Earias vittella* (Fabricius) (Lepidoptera: Nolidae) along their isogenic non-Bt genotypes. Various consumption and utilization indices, viz. consumption index (CI), relative growth rate (RGR), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were worked out. The data show that all the Bt **hybrids** significantly reduced CI, RGR, ECI and ECD of 4th instar *E. vittella* larvae as compared to their isogenic non-Bt genotypes when fed on squares and bolls of 90, 120 and 150 days crop age. Further, the Bollgard II genotype MRC 7017 BG II was found to be more lethal and recorded significantly lower values for all the above given indices. Despite the fact that, the differences between Bt and their isogenic non-Bt genotypes decreased with increasing age of the crop but still Bt cotton **hybrids** were significantly inferior as insect food as compared to the non-Bt ones even at the later stages of the crop growth. The amount of Cry1Ac toxin in squares and bolls of Bt cotton had a significant negative correlation with food consumption and utilization indices of *E. vittella* larvae. The studies thus indicated that Bt toxin has a substantial impact on the nutritional performance of *E. vittella*.

Genetic and phenotypic comparisons of viral genotypes from two nucleopolyhedroviruses interacting with a common host species, *Spodoptera litura* (Lepidoptera: Noctuidae)

Takatsuka, J; Okuno, S; Nakai, M; Kunimi, Y. 2016

Nucleopolyhedroviruses (NPVs) are known to be highly variable, both genetically and phenotypically, at several scales such as different geographic locations or a single host. A previous study using several geographic isolates indicated that two types of NPV, *Spodoptera littoralis* NPV (SpliNPV) and *S. litura* NPV (SpltNPV) types, were isolated from the common cutworm, *Spodoptera litura* (Fabricius), a polyphagous insect that causes serious damage to many forage crops and vegetables. That study also indicated that the SpliNPV type was widely distributed in Japan. Here, we investigated the genotypic and phenotypic variation of cloned NPVs that infect *S. litura*; such variation is an important resource for biological control agents, and may represent the genetic diversity of an NPV species. Eighteen genotypically distinct NPVs were cloned from four field-collected NPV isolates using an in vivo cloning technique. They were divided into two virus types according to the similarity of banding patterns of DNA fragments generated by restriction endonucleases, and Southern **hybridization** analysis. Partial polyhedrin gene sequences revealed that the two types corresponded to SpliNPV and SpltNPV. Bioassays seem to suggest that the SpliNPV virus type was, overall, more infectious and killed *S. litura* larvae faster, but yielded fewer viral occlusion bodies, than the SpltNPV type. These data provide a basis for explaining the distribution pattern of SpliNPV and SpltNPV types in *S. litura* populations in Japan. (C) 2016 Elsevier Inc. All rights reserved.

High regional genetic diversity and lack of host-specificity in *Ostrinia nubilalis* (Lepidoptera: Crambidae) as revealed by mtDNA variation

Piwczynski, M; Pabijan, M; Grzywacz, A; Glinkowski, W; Beres, PK; Buszko, J. 2016

The European corn borer (*Ostrinia nubilalis*) infests a wide array of host plants and is considered one of the most serious pests of maize in Europe. Recent studies suggest that individuals feeding on maize in Europe should be referred to *O. nubilalis* (sensu nov.), while those infesting dicots as *Ostrinia scapularis* (sensu nov.). We test if the clear genetic distinctiveness among individuals of *O. nubilalis* living on maize vs. dicots is tracked by mitochondrial DNA (mtDNA). We used fragments of COI and COII genes of 32 individuals traditionally recognized as *O. nubilalis* collected on three host plants, maize, mugwort and hop, growing in different parts of Poland. In addition, we reconstructed the mtDNA phylogeny of *Ostrinia* species based on our data and sequences retrieved from GenBank to assess host and/or biogeographic patterns. We also compared haplotype variation found in Poland (east-central Europe) with other regions (Anatolia, Eastern Europe, Balkans, Far East, North America). Our study showed high mtDNA diversity of *O. nubilalis* in Poland in comparison with other regions and revealed rare haplotypes likely of Asian origin. We did not find distinct mtDNA haplotypes in larvae feeding on maize vs. dicotyledonous plants. Phylogenetic analyses showed an apparent lack of mtDNA divergence among putatively distinct lineages belonging to the *O. nubilalis* group as identical haplotypes are shared by Asian and European individuals. We argue that

human-mediated dispersal, **hybridization** and sporadic host jumps are likely responsible for the lack of a geographic pattern in mtDNA variation.

Inheritance and World Variation in Thermal Requirements for Egg Hatch in *Lymantria dispar* (Lepidoptera: Erebidae)

Keena, MA. 2016

Mode of inheritance of hatch traits in *Lymantria dispar* L. was determined by crossing populations nearly fixed for the phenotypic extremes. The nondiapausing phenotype was inherited via a single recessive gene and the phenotype with reduced low temperature exposure requirements before hatch was inherited via a single dominant gene. There was no evidence for sex-linkage or cytoplasmic effects with either gene. Eggs from 43 geographic populations were evaluated for hatch characteristics after being held for 60d at 5 degrees C followed by incubation at 25 degrees C. There was considerable variation both within and among the populations in the proportion able to hatch, time to first hatch, and average time to hatch. Egg masses with reduced requirement for low temperatures before the eggs were ready to hatch were present in all subspecies of *L. dispar* and the phenotype was not fixed in most populations. The populations clustered into three distinct groups, and climatic variables were found to be rough predictors of those groups. Variation in hatch phenotypes between populations is likely an adaptation to local climate and within a population provides a bet-hedging strategy to ensure that at least some hatch synchronizes with host leaf-out. Continued vigilance to prevent movement of populations both within and between countries is warranted, because some of the alleles that confer nondiapause or reduced low temperature requirements before egg hatch are not present in all populations and their introduction would increase variation in egg hatch within a population.

Survival and development of spotted bollworm, *Earias vittella* (Fabricius) (Lepidoptera: Nolidae) on different transgenic Bt and isogenic non-Bt cotton genotypes

Shera, PS; Arora, R. 2016

Four Bt cotton **hybrids**, each with one of four different events, viz., MRC 6301 Bt (cry1Ac gene), JKCH 1947 Bt (modified cry1Ac gene), NCEH 6R Bt (fusion cry1Ac/cry1Ab gene) and MRC 7017 Bollgard II (cry1Ac and cry2Ab genes) were compared for survival and development of *Earias vittella* (Fabricius) along with their isogenic non-Bt genotypes. None of the neonates were able to complete the larval period and reach pupal stage on squares of 90, 120 and 150 days old crop of all Bt **hybrids**. Likewise, on bolls also, zero per cent larval survival was observed in all Bt **hybrids** except JKCH 1947 Bt where 0.67 per cent larvae could manage to reach pre-pupal stage at 120 and 150 days old crop but failed to form cocoon and enter pupal stage. The surviving larva took more development time (3.7 to 5.4 days) as compared to larvae fed on bolls of JKCH 1947 non-Bt. The average survival period (ASP) of larvae was in order of 150 > 120 > 90 days old crop among the crop ages; JKCH 1947 Bt > MRC 6301 Bt > NCEH 6 R Bt > MRC 7017 Bollgard II among Bt **hybrids**; and bolls > squares between fruiting bodies. However, reverse was true for speed index of toxic effect. The concentration of Cry toxin varied significantly in squares and bolls and also among the crop ages. The amount of Cry toxin in squares and bolls had significant negative correlation with ASP of the *E. vittella* larvae.

Modeling seasonal migration of fall armyworm moths

Westbrook, JK; Nagoshi, RN; Meagher, RL; Fleischer, SJ; Jairam, S. 2016

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a highly mobile insect pest of a wide range of host crops. However, this pest of tropical origin cannot survive extended periods of freezing temperature but must migrate northward each spring if it is to re-infest cropping areas in temperate regions. The northward limit of the winter-breeding region for North America extends to southern regions of Texas and Florida, but infestations are regularly reported as far north as Quebec and Ontario provinces in Canada by the end of summer. Recent genetic analyses have characterized migratory pathways from these winter-breeding regions, but knowledge is lacking on the atmosphere's role in influencing the timing, distance, and direction of migratory flights. The Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model was used to simulate migratory flight of fall armyworm moths from distinct winter-breeding source areas. Model simulations identified regions of dominant immigration from the Florida and Texas source areas and overlapping immigrant populations in the Alabama-Georgia and Pennsylvania-Mid-Atlantic regions. This simulated migratory pattern corroborates a previous migratory map based on the distribution of fall armyworm haplotype profiles. We found a significant regression between the simulated first week of moth immigration and first week of moth capture (for locations which captured a parts per thousand yen 10 moths), which on average indicated that the model simulated first immigration 2 weeks before first captures in pheromone traps. The results contribute to knowledge of fall armyworm population ecology on a continental scale and will aid in the prediction and interpretation of inter-annual variability of insect migration patterns including those in response to climatic change and adoption rates of transgenic cultivars.

A comprehensive phylogeography of the *Hyles euphorbiae* complex (Lepidoptera: Sphingidae) indicates a 'glacial refuge belt'

Mende, MB; Bartel, M; Hundsdoerfer, AK. 2016

We test the morphology based hypothesis that the Western Palaearctic spurge hawkmoths represent two species, the Eurasian *H. euphorbiae* and Afro-Macaronesian *H. tithymali*. It has been suggested that these species merged into several **hybrid** swarm populations, although a mitochondrial phylogeography revealed substructure with local differentiation. We analysed a three-gene mt-dataset (889 individuals) and 12 microsatellite loci (892 individuals). Microsatellite analyses revealed an overall weak differentiation and corroborated the superordinate division into two clusters. The data indicate that the populations studied belong to only one species according to the biological species concept, refuting the opening hypothesis. A future taxonomic revision appears necessary to reflect the division into two subgroups. Ancestral mitochondrial polymorphisms are retained in *H. euphorbiae*, indicating gene flow within a broad 'glacial refuge belt' and ongoing postglacial gene flow. Diverse patterns of extensive mito-nuclear discordance in the Mediterranean and the Middle East presumably evolved by more recent processes. This discordance indicates introgression of *H. tithymali*-related mitochondrial haplogroups, accompanied (to a lesser degree) by nuclear alleles, into Italian and Aegean *H. euphorbiae* populations as recently as the late Holocene. The complex mosaic of divergence and reintegration is assumed to have been influenced by locally differing environmental barriers to gene flow.

Natural enemies of *Stenoma cecropia* (Lepidoptera: Elachistidae) in oil palm, in the southwest of Colombia

Sendoya-Corrales, CA; Bustillo-Pardey, AE. 2016

Stenoma cecropia infests new plantations of **hybrid** oil palm (*Elaeis oleifera* x *E. guineensis*) in Tumaco (Narino). These infestations are causing defoliation of economic importance to the oil palm. For this reason, the aim of this study was to determine the biotic and abiotic factors affecting populations through fortnightly sequential sampling, following a 2 x 2 moving displacement system, which sampled every two palms and every other line in a one hectare oil palm plantation, counting the number of live and dead individuals present in the 17th and 25th *S. cecropia* leaves of each palm evaluated. Results indicate that *S. cecropia* is attacked by a variety of predators, parasitoids and entomopathogens. Among the former are the spiders (Araneae), bugs (Reduviidae), wasps (Vespidae), and ants (Formicidae), with Crematogaster being the most important. Among the parasitoids, *Brachymeria* sp. and *Rhyssalus* sp. were most abundant. These species require the nectar of plants such as *Melanthera aspera*, *Solanum quitense*, *Emilia sonchifolia*, *Lantana camara*, *Cassia reticulata*, *Stachytarpheta cayennensis* and *Urena lobata*, for food until the adult stage. An entomopathogenic fungus was also detected infecting the larval stage and was identified as *Isaria* sp. *Crematogaster* sp. predation on larval stages of *S. cecropia* was estimated to be 42 %. Parasitism by *Brachymeria* sp. in pupal stages was 16.8 %, mortality caused by *Rhyssalus* sp. on larval stages was 3.5 % and mortality by *Isaria* sp. was 1.5 %. It is concluded that these biological control agents are important in the regulation of *S. cecropia*

populations in oil palm plantations in Colombia.

Asymmetric hybridization between non-native winter moth, *Operophtera brumata* (Lepidoptera: Geometridae), and native Bruce spanworm, *Operophtera bruceata*, in the Northeastern United States, assessed with novel microsatellites and SNPs

Havill, NP; Elkinton, J; Andersen, JC; Hagen, SB; Broadley, HJ; Boettner, GJ; Caccone, A. 2017

The European winter moth, *Operophtera brumata*, is a non-native pest in the Northeastern USA causing defoliation of forest trees and crops such as apples and blueberries. This species is known to **hybridize** with *O. bruceata*, the Bruce spanworm, a native species across North America, although it is not known if there are **hybrid** generations beyond F1. To study winter moth population genetics and **hybridization** with Bruce spanworm, we developed two sets of genetic markers, single nucleotide polymorphisms (SNPs) and microsatellites, using genomic approaches. Both types of markers were validated using samples from the two species and their **hybrids**. We identified 1216 SNPs and 24 variable microsatellite loci. From them we developed a subset of 95 species-diagnostic SNPs and ten microsatellite loci that could be used for **hybrid** identification. We further validated the ten microsatellite loci by screening field collected samples of both species and putative **hybrids**. In addition to confirming the presence of F1 **hybrids** reported in previous studies, we found evidence for multi-generation asymmetric **hybridization**, as suggested by the occurrence of **hybrid** backcrosses with the winter moth, but not with the Bruce spanworm. Laboratory crosses between winter moth females and Bruce spanworm males resulted in a higher proportion of viable eggs than the reciprocal cross, supporting this pattern. We discuss the possible roles of population demographics, sex chromosome genetic incompatibility, and bacterial symbionts as causes of this asymmetrical **hybridization** and the utility of the developed markers for future studies.

A new species of *Melitaea* from Israel, with notes on taxonomy, cytogenetics, phylogeography and interspecific hybridization in the *Melitaea perseae* complex (Lepidoptera, Nymphalidae)

Lukhtanov, VA. 2017

Specimens with intermediate morphology are often considered to be the result of ongoing interspecific **hybridization**; however, this conclusion is difficult to prove without analysis of chromosomal and/or molecular markers. In the butterfly genus *Melitaea*, such an intermediacy can be detected in male genitalia, and is more or less regularly observed in localities where two closely related, presumably parental species are found in sympatry. Here I analyze a high altitude *Melitaea* population from Mt. Hermon in north Israel and show that its male genitalia are clearly differentiated from those found in phenotypically similar *M. perseae* and *M. didyma*, but in some aspects intermediate between them. This **hybrid**-like population is unique because, although *M. didyma* is present on Mt. Hermon, the true, low-altitude *M. perseae* has never been reported from Israel. Cytogenetic analysis revealed no apomorphic chromosomal characters to distinguish the Mt. Hermon population from other known taxa of the *M. perseae* and *M. didyma* species groups. At the same time, DNA barcode-based phylogeographic study showed that this population is ancient. It was estimated to originate 1-1.6 million years ago in the Levantine refugium from a common ancestor with *M. perseae*. Generally, the data obtained are incompatible with interpretation of the studied population as a taxon conspecific with *M. perseae* or *M. didyma*, or a swarm of recent **hybrids** between *M. perseae* and *M. didyma*, although the possibility of ancient homoploid **hybrid** speciation cannot be ruled out. I also argue that the name *Melitaea montium* assigned to butterflies from north Lebanon cannot be applied to the studied taxon from Mt. Hermon. Here I describe this morphologically and ecologically distinct entity as a new species *Melitaea acentria* sp. n., and compare it with other taxa of the *M. perseae* complex.

Potential for interspecific hybridization between *Zizina emelina* and *Zizina otis* (Lepidoptera: Lycaenidae)

Sakamoto, Y; Yago, M. 2017

Environmental changes such as global warming and biological invasion caused by human activities raise the possibility of secondary contact between the endangered butterfly species *Zizina emelina* and its sibling species *Zizina otis* in Japan. To assess the possible risks from their habitats overlapping, we investigated the potential for **hybridization** and the development of F1 individuals. We observed successful mating of the two sibling species under artificial conditions. The presence of a postzygotic **hybridization** barrier was supported by the delay of larval development only in females; a delay did not occur in males. Existence of the barrier was also supported by a decreased egg hatching rate in one brood; this was likely associated with infection with *Wolbachia*, a bacterium manipulating the reproductive capability of its host. The size and wing markings of F1 **hybrid** individuals were intermediate between those of the two species. These results suggest that, if *Z. emelina* and *Z. otis* are distributed sympatrically in the future, there is a possibility of introgression and reproductive interference between the two species, which would increase the risk of decline of each species.

Mito-nuclear discordance helps to reveal the phylogeographic patterns of *Melitaea ornata* (Lepidoptera: Nymphalidae)

Toth, JP; Varga, Z; Verovnik, R; Wahlberg, N; Varadi, A; Bereczki, J. 2017

Periodical changes of glacial and interglacial conditions have influenced the distribution of most living organisms and shape the separation of different genetic lineages significantly. We investigated the phylogeography of a nymphalid butterfly *Melitaea ornata*. Our main aim was to explore the existence, the origin, and the variability of different genetic lineages based on a multilevel approach. *M. ornata* and its close relatives (with a focus on *M. phoebe*) from the Palearctic were analysed based on five gene regions (COI, EF-1 α , MDH, RPS5, and wingless) using Bayesian methods to infer the phylogeographic history. The DNA-based analyses have been complemented with species distribution modelling (SDM) and *Wolbachia* screening. The Bayesian inference analysis showed mito-nuclear discordance in *M. ornata*, which is split into an eastern and a western clade. Based on mitochondrial DNA, the western clade of *M. ornata* clusters together with *M. phoebe*, while the eastern clade is well-separated. In contrast to this, the combined nuDNA-based analysis revealed that *M. ornata* forms a monophyletic group which is clearly separated from *M. phoebe*. The timing of divergence analyses suggest that the split between *M. ornata* and *M. phoebe* is about 6 million years old based on both the COI and the concatenated nuclear genes. SDM predicted considerably larger area shifts for *M. phoebe* than for *M. ornata*. LGM refugia were predicted for both species to the Mediterranean peninsulas in Europe and several Middle-East and Asian localities. The prevalence of *Wolbachia* infection was 88.9% in *M. phoebe* and only 7% in *M. ornata*. Our results clearly indicate a lack of ongoing **hybridization** between *M. phoebe* and *M. ornata*, but argue for an ancient **hybridization** event in the Apennine Peninsula which strongly influenced the observed split between the two clades of *M. ornata*.

Hybrid deep learning for automated lepidopteran insect image classification

Zhu, LQ; Ma, MY; Zhang, Z; Zhang, PY; Wu, W; Wang, DD; Zhang, DX; Wang, X; Wang, HY. 2017

Lepidopterans play an important role in human economy, since some of them are harmful to vegetation in agriculture and some others produce useful materials such as silks, etc. To recognize lepidopteran species correctly is very meaningful to farmers, forest workers, or even insect researchers. This study proposed a cascade architecture which combines the methods of deep convolutional neural network (DCNNs) and Supported Vector Machines (SVMs) to identify Lepidoptera species from their images. The data-set used in this study consists of 1301 Lepidoptera images from 22 species. Since the data-set is not large enough to fine-tune an end-to-end DCNN, we propose a customized solution using part of the DCNN as feature extractor, followed by SVMs as the insect classifiers. The proposed cascade architecture can achieve an accuracy of 100% with our testing data-set and it takes only about 200ms to recognize insect species from an image, which suggests that

the proposed method can be potentially used as a real time classifier for the identification of the Lepidopterans.

Heterozygosity and Chain Multivalents during Meiosis Illustrate Ongoing Evolution as a Result of Multiple Holokinetic Chromosome Fusions in the Genus *Melinaea* (Lepidoptera, Nymphalidae)

McClure, M; Dutrillaux, B; Dutrillaux, AM; Lukhtanov, V; Elias, M. 2017

Mitotic and meiotic chromosomes from 2 taxa of the genus *Melinaea*, *M. satevis cydon* and *M. "satevis" tarapotensis* (Lepidoptera: Nymphalidae), and from **hybrids** produced in captivity were obtained using an improved spreading technique and were subsequently analyzed. In one of the taxa, the presence of trivalents and tetravalents at diakinesis/metaphase I is indicative of heterozygosity for multiple chromosome fusions or fissions, which might explain the highly variable number of chromosomes previously reported in this genus. Two large and complex multivalents were observed in the meiotic cells of the **hybrid** males (32 chromosomes) obtained from a cross between *M. "s."* *tarapotensis* (28 chromosomes) and *M.s. cydon* (40-43 chromosomes). The contribution of the 2 different haploid karyotypes to these complex figures during meiosis is discussed, and a taxonomic revision is proposed. We conclude that chromosome evolution is active and ongoing, that the karyotype of the common ancestor consisted of at least 48 chromosomes, and that evolution by chromosome fusion rather than fission is responsible for this pattern. Complex chromosome evolution in this genus may drive reproductive isolation and speciation, and highlights the difficulties inherent to the systematics of this group. We also show that *Melinaea* chromosomes, classically considered as holocentric, are attached to unique, rather than multiple, spindle fibers. (c) 2018 S. Karger AG, Basel

Sex-linked inheritance of diapause induction in the butterfly *Pieris napi*

Pruisscher, P; Larsdotter-Mellstrom, H; Stefanescu, C; Nylin, S; Wheat, CW; Gotthard, K. 2017

Many temperate insects survive harsh environmental conditions, such as winter, by entering a state of developmental arrest. This diapause state is predominantly induced by photoperiod. The photoperiod varies with latitude and has led to local adaptation in the photoperiodic induction of diapause in many insects. To understand the rapid evolution of the photoperiodic threshold, it is important to investigate and understand the underlying genetic mechanisms. In the present study, the genetic basis of photoperiodic diapause induction is investigated in the green-veined white butterfly *Pieris napi* (Lepidoptera, Pieridae) by assaying diapause induction in a range of conditions for a Swedish and Spanish population. Furthermore, the inheritance of diapause induction is assessed in reciprocal F1 **hybrids** and backcrosses between the two populations. The southern population shows a clear photoperiodic threshold determining diapause or direct development, whereas the northern populations show a high incidence of diapause, regardless of photoperiod. The **hybrid** crosses reveal that the inheritance of diapause induction is strongly sex-linked, and that diapause incidence in the genetic crosses is highly dependent on photoperiod. This emphasizes the importance of assaying a range of conditions in diapause inheritance studies. The results indicate a strongly heritable diapause induction with a major component on the Z-chromosome, as well as a minor effect of the autosomal background.

AMPULLAE INFECTION OF THE MALPIGHIAN TUBULES OF *Bombyx mori* BY Alphabaculovirus

Santorum, M; Beu, CCL; Ribeiro, LDC; dos Santos, JS; dos Santos, DC; Brancalhão, RMC. 2017

Alphabaculovirus is a genus of the entomopathogenic virus, whose species *Bombyx mori* nucleopolyhedrovirus (BmNPV) infects the silkworm, *Bombyx mori*, which is an important insect in the sericulture industry. A geographic isolate of BmNPV was identified in the state of Paraná, Brazil. It was infecting *B. mori* larvae and various organs and target tissues were identified, however, there was no information about the infection of Malpighian tubules (MT). The MT comprises the excretory system of *B. mori* and acts in the elimination of toxic substances and in hydroelectrolytic homeostasis. Thus, the present study examined the susceptibility and cytopathology of *B. mori* MT to BmNPV. To this end, **hybrid** fifth instar larvae were inoculated with a virus suspension at different days post-inoculation (dpi). MT segments were collected and divided into the ampullae, proximal, medial and distal regions. These were processed for light microscopy and transmission electron analysis. The MT regions revealed differences in susceptibility to BmNPV and the ampullae in its transition area was infected from the sixth dpi; the other regions did not reveal any evidence of infection. The transition area of the ampullae has not been previously described in Lepidoptera and its cytopathology revealed a hypertrophic nucleus with viroplasm, followed by the formation and development of viral polyhedra, which are common characteristics of infections by Alphabaculovirus. Thus, infection of the ampullae of the MT of *B. mori* by BmNPV, together with other known targets, compromises the metabolic balance of the insect, which results in consequences for silk production and damage to the sericulture sector.

What shapes the continuum of reproductive isolation? Lessons from *Heliconius* butterflies

Merot, C; Salazar, C; Merrill, RM; Jiggins, CD; Joron, M. 2017

The process by which species evolve can be illuminated by investigating barriers that limit gene flow between taxa. Recent radiations, such as *Heliconius* butterflies, offer the opportunity to compare isolation between pairs of taxa at different stages of ecological, geographical, and phylogenetic divergence. Here, we report a comparative analysis of existing and novel data in order to quantify the strength and direction of isolating barriers within a well-studied clade of *Heliconius*. Our results highlight that increased divergence is associated with the accumulation of stronger and more numerous barriers to gene flow. Wing pattern is both under natural selection for Mullerian mimicry and involved in mate choice, and therefore underlies several isolating barriers. However, pairs which share a similar wing pattern also display strong reproductive isolation mediated by traits other than wing pattern. This suggests that, while wing pattern is a key factor for early stages of divergence, it may become facultative at later stages of divergence. Additional factors including habitat partitioning, **hybrid** sterility, and chemically mediated mate choice are associated with complete speciation. Therefore, although most previous work has emphasized the role of wing pattern, our comparative results highlight that speciation is a multi-dimensional process, whose completion is stabilized by many factors.

The Spread of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and Coexistence with *Helicoverpa zea* in Southeastern Brazil

Pinto, FA; Mattos, MVV; Silva, FWS; Rocha, SL; Elliot, SL. 2017

Helicoverpa armigera, one of the world's most destructive crop pests, was first documented in Brazil in 2013. Within a few months, this polyphagous insect had spread over the Northeast and Central-West of Brazil, causing great agricultural losses. With several reports of populations resistant to pesticides and Bt crops around the world, there is great concern about the spread of this pest in Brazil. There is confusion about the actual distribution of this species due to the high morphological similarity with the native corn earworm *Helicoverpa zea*, which may also coexist with *H. armigera* in the field. Our aims here were (i) to confirm its presence in the State of Minas Gerais, one of the most important agricultural regions in the country; and (ii) to assess the co-occurrence of this pest with the congeneric corn earworm *H. zea*. Using molecular screening, we confirmed the presence of *H. armigera* in Bt-crops of soybean and cotton, and non-Bt-crops of soybean, cotton and maize. Mixed infestations of *H. armigera* with *H. zea* were found in non-Bt maize (Vicosa, Southeastern Minas Gerais). These results highlight the need for adequate control strategies for *H. armigera* in Brazil, to deal with its polyphagous feeding habits, high dispersal capacity and possible risks of **hybridization** with congeneric species.

The role of leaf cellulose content in determining host plant preferences of three defoliating insects present in the Andean-Patagonian forest

Pietrantuono, AL; Bruzzone, OA; Fernandez-Arhex, V. 2017

Phytophagous insects choose their feeding resources according to their own requirements in addition to properties of the host plants, such as biomechanical defences. The feeding preferences of the native folivorous insects of the Andean-Patagonian forest (Argentina) have rarely been studied. These environments present a wide diversity and abundance of insects associated with trees of the *Nothofagus* and *Lophozonia* (*Nothofagaceae*) genera, which represent the main tree species of the forests of the southern hemisphere. In particular, *Lophozonia alpina* and *Lophozonia obliqua* are of great interest because they have a wide distribution, a high capacity for **hybridization** and exhibit great phenotypic plasticity. This versatility causes substantial variation in the biomechanical properties of leaves, affecting the feeding preferences of insects. The purpose of this work was to study the food selection behaviour of three leaf-chewing insects (*Polydrusus nothofagii*, *Polydrusus roseus* (Coleoptera: Curculionidae) and *Perzelia arda* (Lepidoptera: Oecophoridae)) associated with *L. alpina* and *L. obliqua* as host plants. Based on their choices, our aim was to determine a preference scale for each insect species and the variables on which these preferences were based. Therefore, we selected trees of *L. alpina* and *L. obliqua*, measured several properties such as cellulose content and recorded which leaves were eaten. As a result, we determined that the three species of insects feed on both host plants but prefer the leaves of *L. obliqua*, with cellulose content being the main determining factor for their decisions. However, in the case of *P. arda*, there was a positive relationship between cellulose and host plant preference, whereas there was an opposite relationship for the weevils. We conclude that during feeding selection, there are some properties of the leaves that have a more important role than others and that the same property does not exert the same behavioural response in all folivorous insects.

Efficacy of transgenic maize insecticide treatment to control fall armyworm in late-season maize in Sao Paulo state, Brazil

Michelotto, MD; Neto, JC; Pirotta, MZ; Duarte, AP; de Feitas, RS; Finoto, EL. 2017

Several genetically modified maize events expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) have been commercially available in Brazil, intended to control of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The objective of this study was to evaluate the efficiency of transgenic (Bt) and conventional (non-Bt) **hybrids** in the control of fall armyworm, treated or not with insecticides throughout three late growing seasons. To this end, trials were conducted at three locations in the state of Sao Paulo, in a randomized complete block design, in factorial arrangements with 5x4, 5x4 and 8x4 factors, respectively. The first factor consisted of the number of commercial **hybrids** of different companies. The second factor was represented by the different control managements of insect (non-Bt **hybrid** with and without insecticide; Bt **hybrid** with and without insecticide). The damage caused by fall armyworm was evaluated on a visual 1-9 scale, from score 0 (no damage) to 9 (totally destroyed whorl), and grain yield (kg ha⁻¹). The data were analyzed by analysis of variance and the means compared by the Tukey test at 5% probability for each parameter evaluated at each location. For the Bt **hybrids**, the damage scores attributed to fall armyworm were lower and the proteins Cry 1F, Cry 1A105 and VIP3Aa20 were the most efficient in reducing leaf damage. Insecticide applications proved efficient in reducing leaf damage caused by the pest. Application of insecticides to Bt **hybrids** generally reduced leaf damage, especially for Cry1Ab-producing **hybrids**, which were the least effective in reducing pest damage without insecticides.

Karyotypes versus Genomes: The Nymphalid Butterflies *Melitaea cinxia*, *Danaus plexippus*, and *D-chrysippus*

Traut, W; Ahola, V; Smith, DAS; Gordon, IJ; French-Constant, RH. 2017

The number of sequenced lepidopteran genomes is increasing rapidly. However, the corresponding assemblies rarely represent whole chromosomes and generally also lack the highly repetitive W sex chromosome. Knowledge of the karyotypes can facilitate genome assembly and further our understanding of sex chromosome evolution in Lepidoptera. Here, we describe the karyotypes of the Glanville fritillary *Melitaea cinxia* (n = 31), the monarch *Danaus plexippus* (n = 30), and the African queen *D. chrysippus* (2n = 60 or 59, depending on the source population). We show by FISH that the telomeres are of the (TTAGG) n type, as found in most insects. *M. cinxia* and *D. plexippus* have "conventional" W chromosomes which are heterochromatic in meiotic and somatic cells. In *D. chrysippus*, the W is inconspicuous. Neither telomeres nor W chromosomes are represented in the published genomes of *M. cinxia* and *D. plexippus*. Representation analysis in sequenced female and male *D. chrysippus* genomes detected an evolutionarily old autosome-Z chromosome fusion in *Danaus*. Conserved synteny of whole chromosomes, so called "macro synteny", in Lepidoptera permitted us to identify the chromosomes involved in this fusion. An additional and more recent sex chromosome fusion was found in *D. chrysippus* by karyotype analysis and classical genetics. In a **hybrid** population between 2 subspecies, *D. c. chrysippus* and *D. c. dorippus*, the W chromosome was fused to an autosome that carries a wing colour locus. Thus, cytogenetics and the present state of genome data complement one another to reveal the evolutionary history of the species. (C) 2017 S. Karger AG, Basel

Genetically manipulated Brassica genotypes affect demography and performance of *Diadegma semiclausum* parasitizing *Plutella xylostella*

Nikooei, M; Fathipour, Y; Javaran, MJ; Soufbaf, M. 2017

The performance of *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) reared on canola's progenitor (*Brassica rapa* L.), two cultivated canola cultivars (Opera and RGS(003)), one **hybrid** (Hyula(401)), one gamma-ray mutant-RGS(003) and one transgenic (PF) genotype was compared using the age-stage, two-sex life table parameters. All experiments were carried out in a growth chamber at 25 +/- 1 degrees C, 65 +/- 5% RH and a photoperiod of 16:8 (L:D) h. There were significant differences in duration of different life stages of *D. semiclausum* on its host larvae reared on different plant genotypes. The shortest (12.27days) and longest (15.21days) pre-adult developmental times were observed on cultivar-RGS(003) and **hybrid**-Hyula(401), respectively. The intrinsic rate of increase (r) in *D. semiclausum* ranged between 0.189/day (cultivar-Opera) and 0.141/day (transgenic-PF). Moreover, the highest (20.078 offspring) and lowest (12.027 offspring) net reproductive rates (R₀) were observed on cultivar-Opera and **hybrid**-Hyula(401). The mean generation time (T) of *D. semiclausum* was the highest (18.34days) and lowest (15.05days) on mutant-RGS(003) and cultivar-RGS(003). The maximum and minimum parasitism values of this parasitoid were observed on canola's progenitor (44.28%) and **hybrid**-Hyula(401) (37.09%). The heaviest pupae (3.82mg) and females (3.22mg) of the parasitoid were found on canola's progenitor and cultivar-Opera, respectively. The results showed that performance of this parasitoid was better on canola's progenitor and cultivated plants known to have higher levels of glucosinolates concentration than others.

Statistical modeling of insect behavioral response to changes in weather conditions in *Brassica napus* L.

Chambo, ED; de Oliveira, NTE; Garcia, RC; Ruvolo-Takasusuki, MCC; de Toledo, VAA. 2017

Understanding specific meteorological factors that affect the foraging activity of pollinator insects can provide valuable information to ensure appropriate levels of pollination of crops. This research was carried out to verify the spectrum of pollinating insects and their foraging behavior in relation to specific meteorological variables in the *Brassica napus* (rapeseed) crop. Data collected consisted of the following observations: number of visits by each pollinator, floral resources collected by Africanized honeybee *Apis mellifera* L., number of flowers visited by Africanized honeybees in 1 min, number of flowers visited by Africanized honeybees in one specific plant during the timed observation of 1 min, and the time taken by the honeybees to visit each flower. In this study the analyses were made through different generalized linear models. The Africanized honeybees were the most abundant pollinating insects (88%) and most visitors were observed collecting nectar (90%). The Africanized honeybees visited a total of 12.9 +/- 1.40 flowers in 1 min and 2.96 +/- 1.09 flowers of a single plant in 1 min. The time the honeybees spent on the

rapeseed flowers was 4.2 +/- 1.6 s. The number of floral visitors correlated closely to the variation of abiotic factors, especially temperature, relative humidity, wind speed, and solar radiation. Africanized honeybees were more active at different times of the day as compared to the other floral visitors and they foraged more intensely on the Hyola 433 **hybrid** than on the Hyola 61 **hybrid**. The Hyola 61 **hybrid** was more attractive to *Diabrotica speciosa* and *Lepidoptera*.

Introgression between divergent corn borer species in a region of sympatry: Implications on the evolution and adaptation of pest arthropods

Wang, YZ; Kim, KS; Guo, WC; Li, QY; Zhang, YY; Wang, ZY; Coates, BS. 2017

The Asian corn borer, *Ostrinia furnacalis*, and European corn borer, *O. nubilalis* (Lepidoptera: Crambidae), cause damage to cultivated maize in spatially distinct geographies and have evolved divergent hydrocarbons as the basis of sexual communication. The Yili area of Xinjiang Uyghur Autonomous Region in China represents the only known region where *O. furnacalis* has invaded a native *O. nubilalis* range, and these two corn borer species have made secondary contact. Genetic differentiation was estimated between *Ostrinia* larvae collected from maize plants at 11 locations in Xinjiang and genotyped using high-throughput SNP and microsatellite markers. Maternal lineages were assessed by direct sequencing of mitochondrial cytochrome c oxidase subunit I and II haplotypes, and a high degree of genotypic diversity was demonstrated between lineages based on SNP genotypes. Furthermore, historical introgression was predicted among SNP genotypes only at sympatric locations in the Yili area, whereas in Xinjiang populations only *O. furnacalis* haplotypes were detected and no analogous introgressed genotypes were predicted. Our detection of putative **hybrids** and historical evidence of introgression defines Yili area as a **hybrid** zone between the species in normal ecological interactions and furthermore, might indicate that adaptive traits could spread even between seemingly divergent species through horizontal transmission. Results of this study indicate there may be a continuum in the degree of reproductive isolation between *Ostrinia* species and that the elegance of distinct and complete speciation based on modifications to the pheromone communication might need to be reconsidered.

Influence of maize kernel state and type on life history of *Plodia interpunctella* (Lepidoptera: Pyralidae)

Predojevic, DZ; Vukajlovic, FN; Tanaskovic, ST; Gvozdenac, SM; Pesic, SB. 2017

The objective of this study was to investigate the influence of different mechanical states (whole, broken and ground kernels) and types (dent, semi-flint and flint) of maize kernels on life history parameters of *Plodia interpunctella* (Hubner), the Indian meal moth. These parameters included larval development and its dynamics, adult emergence, mean development duration (MDD) and fecundity. Since the larvae are the developmental stage that causes the most damage on maize, additional data on larval development helps identify conditions that promote development. The results of our study indicate that broken maize kernels are the most suitable for *P. interpunctella* development, where the most successful larval development, the highest number of emerged adults, the shortest MDD and the highest total fecundity were recorded. On the contrary, whole kernels were the least suitable and the most resistant to infestation by *P. interpunctella*. The type of kernel also significantly affects *P. interpunctella* developmental parameters. Females reared on flint kernels (the hardest kernel type tested in our study) laid the largest total number of eggs, which implies that kernel hardness, i.e. the type of kernel should be an important parameter when choosing maize **hybrids** for cultivation and for storage. Our study warrants further investigation of maize types and their susceptibility to *P. interpunctella*. (C) 2017 Elsevier Ltd. All rights reserved.

Proboscis morphology suggests reduced feeding abilities of hybrid *Limnitis* butterflies (Lepidoptera: Nymphalidae)

Kramer, VR; Reiter, KE; Lehnert, MS. 2018

Hybridization provides the opportunity to study speciation processes. Studies of **hybridization** in butterflies provide evidence for a range of life-history traits that result in reduced fitness of the **hybrid** offspring, but no previous studies have examined the feeding ability of adult **hybrids**. Butterfly mouthpart (proboscis) architecture relates to functionality and is an important structure for fitness, because malformed proboscises would hinder feeding and nutrient acquisition. The proboscis architecture of **hybrid** *Limnitis* butterflies ('rubidus' form) was compared with parental types to determine whether **hybrids** might have a proboscis that impedes feeding. Scanning electron microscopy was used to image proboscises, and 12 measurements were recorded from each individual. Linear discriminant and hierarchical clustering analyses indicated that the proboscis architecture of **hybrid** butterflies groups separately from the parental types. The results also revealed that **hybrids** have a reduced proboscis compared with the parental types, which could be maladapted for feeding. Sexual dimorphisms for some structures in **hybrids** suggest that certain genes responsible for proboscis architecture might be sex linked. The reduced proboscis length and width of F1 **hybrid** females could be one reason why they have not been observed in the wild, providing an example of environmental selection associated with Haldane's rule.

Molecular evidence of hybridization in sympatric populations of the *Enantia jethys* complex (Lepidoptera: Pieridae)

Jasso-Martinez, JM; Machkour-M'Rabet, S; Vila, R; Rodriguez-Arnaiz, R; Castaneda-Sortibrán, AN. 2018

Hybridization events are frequently demonstrated in natural butterfly populations. One interesting butterfly complex species is the *Enantia jethys* complex that has been studied for over a century; many debates exist regarding the species composition of this complex. Currently, three species that live sympatrically in the Gulf slope of Mexico (*Enantia jethys*, *E. mazai*, and *E. albania*) are recognized in this complex (based on morphological and molecular studies). Where these species live in sympatry, some cases of interspecific mating have been observed, suggesting **hybridization** events. Considering this, we employed a multilocus approach (analyses of mitochondrial and nuclear sequences: COI, RpS5, and Wg; and nuclear dominant markers: inter-simple sequence repeat (ISSRs) to study **hybridization** in sympatric populations from Vera-cruz, Mexico. Genetic diversity parameters were determined for all molecular markers, and species identification was assessed by different methods such as analyses of molecular variance (AMOVA), clustering, principal coordinate analysis (PC(O)A), gene flow, and PhiPT parameters. ISSR molecular markers were used for a more profound study of **hybridization** process. Although species of the *Enantia jethys* complex have a low dispersal capacity, we observed high genetic diversity, probably reflecting a high density of individuals locally. ISSR markers provided evidence of a contemporary **hybridization** process, detecting a high number of **hybrids** (from 17% to 53%) with significant differences in genetic diversity. Furthermore, a directional pattern of **hybridization** was observed from *E. albania* to other species. Phylogenetic study through DNA sequencing confirmed the existence of three clades corresponding to the three species previously recognized by morphological and molecular studies. This study underlines the importance of assessing **hybridization** in evolutionary studies, by tracing the lineage separation process that leads to the origin of new species. Our research demonstrates that **hybridization** processes have a high occurrence in natural populations.

Alternative facts: a reconsideration of putatively natural interspecific hybrid specimens in the genus *Heliconius* (Lepidoptera: Nymphalidae)

Brower, AVZ. 2018

Mallet et al. (2007 BMC Evolutionary Biology, 7, 28) employed a database of putative interspecific **hybrid** specimens of the genus *Heliconius* to advance a hypothesis of "the species boundary as a continuum." Here, each of those specimens, as well as subsequently documented specimens, is individually reassessed regarding its phenotype, potential parentage and chain of custody in collections. Using a quantified scale of reliability, most of the specimens are interpreted differently than Mallet et al.'s identifications, and the actual number of interspecific **hybrids** is estimated to be much smaller than they proposed. To be specific, of 163 putative **hybrid** specimens examined, 11% suffered from ambiguous identity, 5% from confounding issues with their data labels, 50% were arguably intraspecific (depending upon alternative species concepts), and 22% were almost certainly reared, commercial specimens. Only eleven of the specimens meet the criteria established here to be legitimate and reliable interspecific **hybrids**, and all of those are between closely-related species. This result has potentially important implications for current

hypotheses of frequent genomic introgression of wing pattern alleles among *Heliconius* clades.

Climate-mediated hybrid zone movement revealed with genomics, museum collection, and simulation modeling

Ryan, SF; Deines, JM; Scriber, JM; Pfrender, ME; Jones, SE; Emrich, SJ; Hellmann, JJ. 2018

Climate-mediated changes in **hybridization** will dramatically alter the genetic diversity, adaptive capacity, and evolutionary trajectory of interbreeding species. Our ability to predict the consequences of such changes will be key to future conservation and management decisions. Here we tested through simulations how recent warming (over the course of a 32-y period) is affecting the geographic extent of a climate-mediated developmental threshold implicated in maintaining a butterfly **hybrid** zone (*Papilio glaucus* and *Papilio canadensis*; Lepidoptera: Papilionidae). These simulations predict a 68-km shift of this **hybrid** zone. To empirically test this prediction, we assessed genetic and phenotypic changes using contemporary and museum collections and document a 40-km northward shift of this **hybrid** zone. Interactions between the two species appear relatively unchanged during **hybrid** zone movement. We found no change in the frequency of **hybridization**, and regions of the genome that experience little to no introgression moved largely in concert with the shifting **hybrid** zone. Model predictions based on climate scenarios predict this **hybrid** zone will continue to move northward, but with substantial spatial heterogeneity in the velocity (55-144 km/1 degrees C), shape, and contiguity of movement. Our findings suggest that the presence of nonclimatic barriers (e.g., genetic incompatibilities) and/or nonlinear responses to climatic gradients may preserve species boundaries as the species shift. Further, we show that variation in the geography of **hybrid** zone movement could result in evolutionary responses that differ for geographically distinct populations spanning **hybrid** zones, and thus have implications for the conservation and management of genetic diversity.

Fusarium graminearum Mycotoxins in Maize Associated With *Striacosta albicosta* (Lepidoptera: Noctuidae) Injury

Smith, JL; Limay-Rios, V; Hooker, DC; Schaafsma, AW. 2018

Western bean cutworm, *Striacosta albicosta* (Smith; Lepidoptera: Noctuidae) has become a key pest of maize, *Zea mays* (L.), in Ontario, Canada which is challenging to control due to its lack of susceptibility to most Bt-maize events. Injury by *S. albicosta* may exacerbate *Fusarium graminearum* (Schwabe; Hypocreales: Nectriaceae) infection through provision of entry points on the ear. The objectives of this study were to: investigate the relationship between injury by *S. albicosta* and deoxynivalenol (DON) accumulation; evaluate non-Bt and Bt-maize **hybrids**, with and without insecticide and fungicide application; and determine optimal insecticide-fungicide application timing for reducing *S. albicosta* injury and DON accumulation. The incidence of injury by *S. albicosta* and ear rot severity were found to increase DON concentrations under favorable environmental conditions for *E. graminearum* infection. Incidence of *S. albicosta* injury was more important than severity of injury for DON accumulation which may be due to larval consumption of infected kernels. The Vip3A x Cry1Ab event provided superior protection from the incidence and severity of *S. albicosta* injury compared to non-Bt or Cry1F **hybrids**. Insecticide application to a Vip3A x Cry1Ab **hybrid** did not reduce injury further; however, lower severity of injury was observed for non-Bt and Cry1F **hybrids** when pyrethroids or diamides were applied at early VT or R1 stages. DON concentrations were reduced with application of prothioconazole fungicide tank-mixed with insecticide at late VT (before silk browning) or when insecticide was applied at early VT followed by prothioconazole at R1. The application of an insecticide/fungicide tank-mix is the most efficient approach for maize **hybrids** lacking high-dose insecticidal proteins against *S. albicosta* and *F. graminearum* tolerance. Results demonstrate that reducing the risk of DON accumulation requires a strategic approach to manage complex associations among *S. albicosta*, *E. graminearum* and the environment.

Resolving Relationships among the Megadiverse Butterflies and Moths with a Novel Pipeline for Anchored Phylogenomics

Breinholt, JW; Earl, C; Lemmon, AR; Lemmon, EM; Xiao, L; Kawahara, AY. 2018

The advent of next-generation sequencing technology has allowed for the collection of large portions of the genome for phylogenetic analysis. Hybrid enrichment and transcriptomics are two techniques that leverage next-generation sequencing and have shown much promise. However, methods for processing **hybrid** enrichment data are still limited. We developed a pipeline for anchored **hybrid** enrichment (AHE) read assembly, orthology determination, contamination screening, and data processing for sequences flanking the target "probe" region. We apply this approach to study the phylogeny of butterflies and moths (Lepidoptera), a megadiverse group of more than 157,000 described species with poorly understood deep-level phylogenetic relationships. We introduce a new, 855 locus AHE kit for Lepidoptera phylogenetics and compare resulting trees to those from transcriptomes. The enrichment kit was designed from existing genomes, transcriptomes, and expressed sequence tags and was used to capture sequence data from 54 species from 23 lepidopteran families. Phylogenies estimated from AHE data were largely congruent with trees generated from transcriptomes, with strong support for relationships at all but the deepest taxonomic levels. We combine AHE and transcriptomic data to generate a new Lepidoptera phylogeny, representing 76 exemplar species in 42 families. The tree provides robust support for many relationships, including those among the seven butterfly families. The addition of AHE data to an existing transcriptomic dataset lowers node support along the Lepidoptera backbone, but firmly places taxa with AHE data on the phylogeny. Combining taxa sequenced for AHE with existing transcriptomes and genomes resulted in a tree with strong support for (Calliduloidea + Gelechioidea + Thyridoidea) + (Papilionoidea + Pyraloidea + Macroheterocera). To examine the efficacy of AHE at a shallow taxonomic level, phylogenetic analyses were also conducted on a sister group representing more recent divergence, the Saturniidae and Sphingidae. These analyses utilized sequences from the probe region and data flanking it, nearly doubled the size of the dataset; resulting trees supported new phylogenetic relationships, especially within the Saturniidae and Sphingidae (e.g., *Hemarina* derived in the latter). We hope that our data processing pipeline, **hybrid** enrichment gene set, and approach of combining AHE data with transcriptomes will be useful for the broader systematics community.

Occurrence of Interspecific Mating between Two Species of *Danaus* & *IT Kluk*, 1780 (Lepidoptera: Nymphalidae) in Nature

Baidya, S; Basu, DN; Roy, S; Roy, AB. 2018

Mating across species occurs rarely in nature, which contends prevalent idea of biological species concept. Throughout species range, mating pattern varies and reproductive barriers are also not fixed among different species. In this study, two instances of interspecific mating between two widely common Nymphalid Tiger butterflies (*Danaus Kluk*, 1780) in Indian region are reported. Observations imply lack of absolute reproductive barriers, where possible interplay exists among prezygotic and postzygotic isolating factors compensating each other in order to restrain interspecific **hybridization**.

Efficacy of bio-rational insecticides against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomatoes

Abd El-Ghany, NM; Abdel-Razek, AS; Djelouah, K; Moussa, A. 2018

Recently, the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) becomes the most important pest affecting tomato crop worldwide. This Insect pest caused reduction of plant growth and yields in both protected and open-field. Bio-rational insecticides provide efficient, economic, and promising strategy for controlling different insect pests. The use of bio-rational insecticides as alternatives for chemicals pesticides have many advantages as decreasing the environmental risks of chemical pesticides, the specificity of most of these entomopathogens, and being harmless to vertebrates and other non-target organisms. The aim of this work was to evaluate different bio-rational insecticides including microbial pesticides, bio-chemicals derived from micro-organisms and other natural sources for controlling *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which is a major insect pest of the tomato crop in Egypt. Two **hybrid** tomato varieties; Shifa and Savera; were planted in two different plantation periods in the horticulture nursery. The effect of five different bio-rational insecticides namely; spinosad, Bacillus

thuringiensis subsp. kurstaki (Btk), azadirachtin, Metarhizium anisopliae (Metschn.) and Beauveria bassiana (Balsamo) was evaluated in trials under the greenhouse conditions. The bio-insecticides were applied at LC50 for semi-field application. Populations of *T. absoluta* were assessed by larval counts and the tomato yields were assessed for the two varieties at full plant maturity. Use of spinosad caused 78-97% reduction in *T. absoluta* population which was the most effective bio-rational insecticide among those used in this study. Btk was the second most effective bio-rational insecticide against *T. absoluta* with 78-91% reduction, followed by the botanical extract azadirachtin which showed 70-83% reduction on the target insect. Use of entomopathogenic fungi, *M. anisoplia* and *B. bassiana* caused 46 -75% reduction in comparison to the untreated plants. In conclusion, utilization of spinosad, Btk and azadirachtin were the most effective bio-rational insecticides which provide promising and safe alternative insecticides for controlling *T. absoluta*.

Deep intraspecific DNA barcode splits and hybridisation in the *Udea alpinalis* group (Insecta, Lepidoptera, Crambidae) - an integrative revision

Mally, R; Huemer, P; Nuss, M. 2018

The analysis of mitochondrial COI data for the European-Centrosian montane *Udea alpinalis* species group finds deep intraspecific splits. Specimens of *U. austriacalis* and *U. rhododendronalis* separate into several biogeographical groups. These allopatric groups are not recovered in the analyses of the two nuclear markers wingless and Elongation factor 1-alpha, except for *U. austriacalis* from the Pyrenees and the French Massif Central. The latter populations are also morphologically distinct and conspecific with *Scopula donzelalis* Guenee, 1854, which is removed from synonymy and reinstated as *Udea donzelalis* (Guenee, 1854) stat. rev. Furthermore, *Udea altaica* (Zerny, 1914), stat. n. from the Mongolian central Altai mountains, *U. juldusalis* (Zerny, 1914), stat. n. from the Tian Shan mountains of Kazakhstan, Kyrgyzstan and NW China, and *U. plumbalis* (Zerny, 1914), stat. n. from the Sayan Mountains of Northern Mongolia are raised to species level, and lectotypes are designated. Evidence of introgression of *U. alpinalis* into *U. uliginosalis* at three localities in the Central Alps is presented. A screening for *Wolbachia* using the markers *wsp*, *gatB* and *ftsZ* was negative for the *U. alpinalis* species group, but *Wolbachia* was found in single specimens of *U. fulvalis* and *U. olivalis* (both in the *U. numeralis* species group). We do not find evidence for the conjecture of several authors of additional subspecies in *U. rhododendronalis*, and synonymise *U. rhododendronalis* *luquetalis* Leraut, 1996, syn. n. and *U. r. ventosalis* Leraut, 1996, syn. n. with the nominal *U. rhododendronalis* (Duponchel, 1834).

Origin and macroevolution of micro-moths on sunken Hawaiian Islands

Johns, CA; Toussaint, EFA; Breinholt, JW; Kawahara, AY. 2018

The origins and evolution of Hawaiian biodiversity are a matter of controversy, and the mechanisms of lineage diversification for many organisms on this remote archipelago remain unclear. Here we focus on the poorly known endemic leaf-mining moth genus *Philodoria* (Lepidoptera, Gracillariidae), whose species feed on a diversity of Hawaiian plant lineages, many of which are critically endangered. We use anchored **hybrid** enrichment to assemble the first phylogenomic dataset (507 loci) for any Hawaiian animal taxon. To uncover the timing and pattern of diversification of these moths, we apply two frequently used dating calibration strategies, biogeographic calibrations and secondary calibrations. Island calibrations on their own resulted in much younger and unrealistic dates compared to strategies that relied on secondary calibrations. *Philodoria* probably originated on the now partially sunken islands of Laysan or Lisianski, approximately 21 Ma, and were associated with host plants in the families Ebenaceae, Malvaceae or Primulaceae. Major feeding groups associated with specific host-plant families originated soon after the plants colonized the islands. Allopatric isolation and host shifts, in concert and independently, probably play major roles in the diversification of *Philodoria*. Our dating results indicate that *Philodoria* is among the oldest known Hawaiian arthropod lineages, and that island calibrations alone can lead to unrealistically young dates.

Phylogenetics of moth-like butterflies (Papilionoidea: Hedyliidae) based on a new 13-locus target capture probe set

Kawahara, AY; Breinholt, JW; Espeland, M; Storer, C; Plotkin, D; Dexter, KM; Toussaint, EFA; St Laurent, RA; Brehm, G; Vargas, S; Forero, D; Pierce, NE; Lohman, DJ. 2018

The Neotropical moth-like butterflies (Hedyliidae) are perhaps the most unusual butterfly family. In addition to being species-poor, this family is predominantly nocturnal and has anti-bat ultrasound hearing organs. Evolutionary relationships among the 36 described species are largely unexplored. A new, target capture, anchored **hybrid** enrichment probe set ('BUTTERFLY2.0') was developed to infer relationships of hedyliids and some of their butterfly relatives. The probe set includes 13 genes that have historically been used in butterfly phylogenetics. Our dataset comprised of up to 10,898 aligned base pairs from 22 hedylid species and 19 out-groups. Eleven of the thirteen loci were successfully captured from all samples, and the remaining loci were captured from $\geq 94\%$ of samples. The inferred phylogeny was consistent with recent molecular studies by placing Hedyliidae sister to Hesperidae, and the tree had robust support for 80% of nodes. Our results are also consistent with morphological studies, with *Macrosoma tipulata* as the sister species to all remaining hedyliids, followed by *M. semiemis* sister to the remaining species in the genus. We tested the hypothesis that nocturnally evolved once from diurnally in Hedyliidae, and demonstrate that the ancestral condition was likely diurnal, with a shift to nocturnally early in the diversification of this family. The BUTTERFLY2.0 probe set includes standard butterfly phylogenetics markers, captures sequences from decades-old museum specimens, and is a cost-effective technique to infer phylogenetic relationships of the butterfly tree of life.

Reproductive incompatibility and fitness components in *Neoleucinodes elegantalis* races (Lepidoptera, Crambidae) from three Solanaceae hosts

Diaz-Montilla, AE; Baena-Bejarano, N; Montoya-Lerma, J; Saldamando-Benjumea, CI. 2018

Neoleucinodes elegantalis (Lepidoptera, Crambidae) is a Neotropical Solanaceae pest that has evolved into four host races due to host plant association. In this study, prezygotic and postzygotic isolation were evaluated in parental and F1 generations collected from *Solanum lycopersicum* (S. 1), *S. quitoense* (S. q) and *S. betaceum* (S. b), representing medium size (S. 1, S. q) and large size genitalia races (S. b). 617 adults were obtained in the laboratory and 106 copulated. One spermatophore per female was found in the bursa copulatrix, suggesting monoandry. Crosses between adults mostly occurred assortatively. A third of the female's eggs laid occurred, but females from female S. b x male S. 1, female S. 1 x male S. b and S. 1 x S. 1 did not. In the cross, female S. 1 x male S. q and its reciprocal F-1 progeny developed into larvae. Reproductive success only occurred in three crosses: S. q x S. q, female S. q x male S. b and its reciprocal progeny. All the progeny from these crosses reached adulthood. The parental population showed differences in pupae and adult measurements according to host. The results on reproductive isolation obtained here suggest speciation in *N. elegantalis* due to host race differentiation which is relevant for the improvement of the management of this species, especially when the species has evolved reproductive isolation among races. We suggest that the use of their host plants in sympatry might reduce *N. elegantalis* densities in the field since **hybrids** between its races have low survival rates and to evaluate the possibility of pheromone composition differentiation among races given the results on assortative mating obtained here.

Yield Losses in Transgenic Cry1Ab and Non-Bt Corn as Assessed Using a Crop-Life-Table Approach

Silva, GA; Picanco, MC; Ferreira, LR; Ferreira, DO; Farias, ES; Souza, TC; Rodrigues-Silva, N; Pereira, EJG. 2018

In this study, we constructed crop life tables for *Bacillus thuringiensis* Berliner (Bt) Cry1Ab and non-Bt corn **hybrids**, in which yield-loss factors and abundance of predaceous arthropods were recorded during 2 yr at two locations. Corn kernel/grain was the yield component that had the heaviest losses and that determined the overall yield loss in the corn **hybrids** across years and locations. Yield losses in both corn **hybrids** were primarily caused by kernel-destroying insects. *Helicoverpa zea* (Boddie) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) were the key loss factors at one location, while at the other, the key loss factor was the silk fly

larvae, *Euxesta* spp. (Diptera: Ulidiidae). Although the realized yield of corn grains was not different ($P > 0.05$) between Cry1Ab and non-Bt corn **hybrids**, the Bt corn **hybrid** reduced ($P < 0.05$) the damage by *H. zea* and *S. frugiperda* in three of the four field trials, particularly at the location where Lepidoptera were the key loss factors. As expected, no reduction in the abundance of predaceous arthropods was observed in Cry1Ab corn fields. Various species of natural enemies were recorded, particularly the earwig *Doru luteipes* (Scudder) (Dermaptera: Forficulidae), which was the most abundant and frequent predaceous insect. These results indicate that integration of pest management practices should be pursued to effectively minimize losses by kernel-destroying insects during corn reproductive stages when growing non-Bt or certain low-dose Bt corn cultivars for fall armyworm and corn earworm, such as those producing Cry1Ab or other Cry toxins.

Morphometry as a tool in species identification: a study with special reference to species of the genus *Mycalesis* (Lepidoptera: Nymphalidae)

Gooneseckera, K; van der Poorten, G; Ranawaka, GR. 2018

Morphological variability among four species of *Mycalesis* in Sri Lanka that are difficult to discriminate due to their morphological similarity was investigated to identify characters that distinguish species more accurately. Using traditional morphometrics, 90 variables from the wing, forelegs and genitalia of *M. perseus* typhlus, *M. milieus* polydecta, *M. subdita* and *M. rama* were measured and analysed. A set of 19 characters of the wing, male genitalia and forelegs were identified to discriminate species. Results of the analysis showed that male specimens were discriminated with nine wing characters and five characters of genitalia. Females could be discriminated with three wing characters and two foreleg characters. Male specimens of *M. p. typhlus* and *M. m. polydecta* showed the greatest morphological differentiation, while females of *M. subdita* and *M. rama* were the most similar species. These results were used to improve the currently available identification key. Two instances of possible **hybridisation** were discovered: one between *M.p. typhlus* and *M. m. polydecta* and the other between *M. p. typhlus* and *M. subdita*. Hence, the species of *Mycalesis*, particularly *M. p. typhlus*, *M. m. polydecta* and *M. subdita* may not be strictly reproductively isolated in Sri Lanka. Preliminary comparisons of *M. p. typhlus* in Sri Lanka with *M. p. tabitha* of India indicated that the Sri Lankan subspecies is unlikely to be a synonym.

Anchored phylogenomics illuminates the skipper butterfly tree of life

Toussaint, EFA; Breinholt, EW; Earl, C; Warren, AD; Brower, AVZ; Yago, M; Dexter, KM; Espeland, M; Pierce, NE; Lohman, DJ; Kawahara, AY. 2018

Background: Butterflies (Papilionoidea) are perhaps the most charismatic insect lineage, yet phylogenetic relationships among them remain incompletely studied and controversial. This is especially true for skippers (Hesperiidae), one of the most species-rich and poorly studied butterfly families. Methods: To infer a robust phylogenomic hypothesis for Hesperidae, we sequenced nearly 400 loci using Anchored Hybrid Enrichment and sampled all tribes and more than 120 genera of skippers. Molecular datasets were analyzed using maximum-likelihood, parsimony and coalescent multi-species phylogenetic methods. Results: All analyses converged on a novel, robust phylogenetic hypothesis for skippers. Different optimality criteria and methodologies recovered almost identical phylogenetic trees with strong nodal support at nearly all nodes and all taxonomic levels. Our results support Coeliadinae as the sister group to the remaining skippers, the monotypic *Euschemoninae* as the sister group to all other subfamilies but Coeliadinae, and the monophyly of Eudaminae plus Pyrginae. Within Pyrginae, Celaenorrhinini and Tagiadini are sister groups, the Neotropical firetips, Pyrrhopygini, are sister to all other tribes but Celaenorrhinini and Tagiadini. Achlyodini is recovered as the sister group to Carcharodini, and Erynnini as sister group to Pyrgini. Within the grass skippers (Hesperiinae), there is strong support for the monophyly of Aeromachini plus remaining Hesperinae. The giant skippers (*Agathymus* and *Megathymus*) once classified as a subfamily, are recovered as monophyletic with strong support, but are deeply nested within Hesperinae. Conclusions: Anchored Hybrid Enrichment sequencing resulted in a large amount of data that built the foundation for a new, robust evolutionary tree of skippers. The newly inferred phylogenetic tree resolves long-standing systematic issues and changes our understanding of the skipper tree of life. These results enhance understanding of the evolution of one of the most species-rich butterfly families.

Subspecies-level systematics and affinities of *Cheimas* Thieme-an endemic genus of the subparamo of the Venezuelan Cordillera de Merida (Lepidoptera: Nymphalidae, Satyrinae)

Pyrz, TW; Lorenc-Brudecka, J; Boyer, P; Zubek, A. 2018

The validity of the monobasic neotropical butterfly genus *Cheimas* Thieme (Nymphalidae, Satyrinae, Satyrini, Pronophilina) is discussed, and confirmed based on morphological and molecular data. *Cheimas opalinus* (Staudinger), endemic to the Venezuelan Cordillera de Merida, and considered prior to this study to be monotypic and restricted to the central part of the range, is demonstrated to be polytypic and more widely distributed. Five subspecies are recognised, differing mostly in their dorsal patterns, in particular the shape and colour of hindwing greenish-blue patch. Mitochondrial DNA sequences (COI) were obtained for three of them. The nominate subspecies is found in the central part of the range, in the Sierra Nevada and La Culata. The other subspecies are found as follows: *C. opalinus dominici* n. ssp.; in the Santo Do-mingo valley in the centre-north; *C. opalinus cristalinus* n. ssp. in the north; *C. opalinus iosephi* n. ssp. on the eastern slopes, and *C. opalinus rosalinus* n. ssp. in the southern Paramo El Batallon massif. A **hybrid** zone between the latter two subspecies was detected in the northern part of the Batallon massif based on unusual individual variation and intermediate phenotypes. All the populations of *Cheimas opalinus* occur in the forest-paramo ecotone at 2800-3400 m a.s.l., with the notable exception of *C. opalinus cristalinus* n. ssp. found also in mid-elevation forests down to 2300 m a.s.l.

A Survey of the Insects of the Southern High Plains (Llano Estacado) of Texas, with Particular Reference to Pollinators and Other Anthophiles

Begosh, A; Overall, LM; Smith, LM; McMurry, ST. 2018

The economic value of insect pollination to the world economy is tremendous, and pollination service is critical for our food supply and persistence of native plants. Little data are currently available on pollinator occurrence in the Southern High Plains (SHP) of Texas. Past studies on **hybrid** cottonseed production in the region documented high abundance of six species of *Agapostemon* and lower abundance of *Diadasia* spp., *Melissodes* spp., and *Perdita* spp. Historical records indicated the presence of *Bombus fraternus* and *B. pensylvanicus* which now have Endangered and Vulnerable IUCN status respectively. In 2013 and 2014, we used blue vane traps and targeted netting to collect insect pollinators and anthophiles on the three dominant land uses in the region; cropland, Conservation Reserve Program (CRP), and native grasslands. With capture methods combined, we collected 74,215 insect specimens; 63% of the specimens were Hymenoptera (289 taxa), 28.61% were Coleoptera (150 taxa), 4.48% were Diptera (115 taxa), 2.34% were Lepidoptera (60 taxa), and 1.61% were of other orders (15 taxa). Our capture included all previously documented bee genera, but we did not detect *B. fraternus*. Studies support that blue vane traps are an effective method of trapping bees and our results demonstrated that they are also effective in attracting some families of anthophilous Coleoptera and Diptera. However, we captured several species of Hymenoptera, Diptera, and Lepidoptera with aerial nets that we did not capture in blue vane traps. This study documents pollinator and anthophile species present in a highly altered landscape and is the most comprehensive list of pollinators and anthophiles in this region to date.

Control of caterpillar *Spodoptera frugiperda* (Smith, 1797) in different technologies Bts (*Bacillus thuringiensis*) in maize crop

Barcelos, PHS; Angelini, MR. 2018

The genetically modified maize containing the Bt gene is arising from the bacterium, *Bacillus thuringiensis* and is used to control lepidopteran. Thus, the aim of this study was to evaluate the efficacy of different Bt technology for the control of the *Spodoptera frugiperda*. The experimental design was a randomized block with six treatments and four replications. 7, 14, 21 and 28 days after emergence (DAE) evaluated visual damage on the leaves caused by *S. frugiperda*, where 25 plants per

plot, were evaluated according to visual scale of damage. The assessment of the number of larvae on the cartridge was performed weekly 7 to 28 sampling AED 10 plants per plot, counting the number of small larvae (< 1.5 cm) and large (> 1.5 cm) present in the cartridge. The averages of the visual damage scores of the Bt technologies were lower when compared to the conventional **hybrid** means at 7 and 14 DAE. At 21 DAE, the Optimum Intrasect technology did not differ from conventional maize, and at 28 DAE the same result was observed as in Herculex technology. At 7 DAE, plants with no damage (note 0) or slight damage (note 1) predominated in the experiment area. From 14 DAE scores above or equal to 3 were observed in the treatments Herculex, Optimum Intrasect, Powercore, VT Pro and conventional, and the percentage of plants with incidence of notes above 3 were higher than 20% only in Herculex treatments, Optimum Intrasect and conventional. The Powercore technology (2B587PW) and Viptera3 (Impact) presented effective in the culture of protection in relation to the *S. frugiperda*.

Museum specimens provide phylogenomic data to resolve relationships of sack-bearer moths (Lepidoptera, Mimallonoidea, Mimallonidae)

St Laurent, RA; Hamilton, CA; Kawahara, AY. 2018

Mimallonidae, the sack-bearer moths, are a family of predominantly Neotropical moths containing nearly 300 described species. Mimallonidae feed on over 40 host plant families and are found in a variety of environments, but phylogenetic relationships of species within the family have never been investigated. We sequenced 515 loci using anchored **hybrid** enrichment target capture on ethanol-preserved and dried museum specimens, with dates of collection ranging from 1985 to 2017. We sampled 47 species, representing 32 of the 36 described mimallonid genera. By incorporating 19 dry museum specimens, and recovering an average of over 400 loci for each, we illustrate the utility of natural history collections in anchored **hybrid** enrichment-based phylogenomics. Maximum likelihood and multi-species coalescent analyses provide robust support for the recognition of six higher-level groups within Mimallonidae, which we designate as subfamilies: Zaphantinae St Laurent & Kawahara subfam.n., Aurorianinae St Laurent & Kawahara subfam.n., Mimalloninae Burmeister, Lacosominae Dyar, Druenticinae St Laurent & Kawahara subfam.n. and Cicinninae Schaus stat.n. Our phylogenetic results also robustly support eight new tribes: Lacosomini St Laurent & Kawahara tribe n., Lacosomini Dyar stat.n., Alheitini St Laurent & Kawahara tribe n.; Druenticinae: Luramini St Laurent & Kawahara tribe n., Druenticini St Laurent & Kawahara tribe n.; Cicinninae: Bedosiini St Laurent & Kawahara tribe n., Psychocampini St Laurent & Kawahara tribe n., Cicinnini Schaus stat.n. Three new genera are also described based on our phylogenetic results: Herbinalla St Laurent & Kawahara, gen.n., Ulaluma St Laurent & Kawahara, gen.n., Bedosiallo St Laurent & Kawahara, gen.n. Naniteta Franclemont, syn.n. is a synonym of Lacosoma Grote. Six genera are paraphyletic, and in total 19 new combinations are proposed: Macessoga laxa comb.n., Lacosoma elassa comb.n., Thaelia anysia comb.n., Thaelia subrubiginosa comb.n., Herbinalla caudina comb.n., Druentica broscica comb.n., Ulaluma valva comb.n., Cicinnus eminens comb.n., Roelmana pluridiscata comb.n., Roelmana laguerrei comb.n., Psychocampa joanna comb.n., Psychocampa unalca comb.n., Psychocampa hamata comb.n., Psychocampa marona comb.n., Bedosiallo eugeniaca comb.n., Bedosiallo forbesi comb.n., Bedosiallo moengus comb.n., Bedosiallo styx comb.n. and Bedosiallo sylvia comb.n. This study is the first to implement the LEP1 probe set on a comprehensive taxonomic dataset that includes many museum specimens, and our results demonstrate that museum specimens can be used in anchored **hybrid** enrichment studies. Importantly, these data produce a robust phylogeny that will serve as a foundation for future studies on mimallonid evolution, such as host plant relationships and biogeography.

Association of citrus leafminer Phyllocnistis citrella (Lepidoptera: Gracillariidae) damage with physiological parameters and larval weight in Citrus reticulata

Arshad, M; Ullah, MI; Afzal, M; Aatif, HM; Iftikhar, Y; Molina-Ochoa, J; Foster, JE. 2018

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton causes injury to citrus and related species in the Rutaceae family. The damage that the CLM larvae can cause is significant in citrus plantations. We tested two citrus cultivars- 'Kinnow' (*Citrus reticulata* Blanco) and 'Fairchild' (a **hybrid** of *Citrus reticulata* Clementine x Orlando Tangelo) - to quantify CLM larvae infestation and effect on the physiology of the citrus cultivars. We then compared the CLM larval weight with its associated damage. To calculate infestation level, mine area and total leaf area, we used the image analysis technique. The infestation level of CLM was higher in 'Fairchild' than in 'Kinnow' cultivar of citrus. For both cultivars, larval weight of CLM was directly proportional to the amount of mines generated. Taken together, the results of this study suggest that the mines that CLM larvae generate pose significant effect on the net photosynthetic rates and water use efficiency of citrus nursery plants. These results will help improve our understanding of the interaction between CLM and citrus nursery plants and effect of the pest on the yield potential of the crop.

Phylogeographic pattern of the plane leaf miner, *Phyllonorycter platani* (STAUDINGER, 1870) (Lepidoptera: Gracillariidae) in Europe

Toth, V; Lakatos, F. 2018

Background: The plane leaf miner, *Phyllonorycter platani* is a widely distributed insect species on plane trees and has a well-documented colonisation history in Europe over the last century. However, phylogeographic data of the species are lacking. Results: We analysed 284 individuals from 38 populations across Europe, Asia, and North America. A 1242 bp fragment of the mitochondria, COI gene and an 893 bp fragment of the 28S rDNA has been Sanger sequenced. Twenty-four haplotypes were detected on the COI gene, and two alleles were identified on the 28S rDNA. We revealed two distinct clades for both markers reflecting the geographic origins, Asia and Europe. The genetic distance between the two main clades is 2.08% on the COI gene and 0.10% on the nuclear DNA. An overlapping zone of the two clades was found across Eastern Europe and the Anatolian Peninsula. We detected heterozygote individuals of the 28S rDNA gene in Moldavia, Ukraine and in the southern part of Turkey. These suggest that the two clades can **hybridise**. Furthermore, the presence of European type homozygote individuals has been confirmed in the southern part of Turkey as well. Conclusions: We have shown that both post-glacial recolonization and recent expansion events influenced the present genetic structure of *P. platani*. The genetic patterns revealed at least two refugia during the last ice age: one in the Balkan Peninsula and the other in the Caucasus region. Recent expansion was detected in some European and Central Asian populations. The two main clades (Europe/Asia) show definite genetic differences; however, several **hybrid** individuals were found in the overlapping zone as well (stretching over Eastern Europe and the Anatolian Peninsula). Discrepancies in mitochondrial and nuclear data indicate introgressions in the southern part of the Anatolian Peninsula.

Widespread hybridization among native and invasive species of Operophtera moths (Lepidoptera: Geometridae) in Europe and North America

Andersen, JC; Havill, NP; Broadley, HJ; Boettner, GH; Caccone, A; Elkinton, JS. 2019

In North America the invasive winter moth (*Operophtera brumata*) has caused defoliation in forest and fruit crop systems in British Columbia, Nova Scotia, Oregon, and in the northeastern United States (the "Northeast"). In the Northeast, it was previously shown that **hybridization** is occurring with a native congener, Bruce spanworm (*O. bruceata*)-a species that has a broad distribution across much of North America. Whether **hybridization** among winter moth and Bruce spanworm populations has occurred in all of regions where winter moth established is unknown. One factor that might influence **hybridization** between these two species is the presence of reproductive manipulating endosymbionts, such as *Wolbachia*. To determine the geographic extent of **hybridization** among populations of these two species, we classified 1400 field-collected moths from Europe and North America as either being winter moth, Bruce spanworm, or **hybrids** using 10-12 polymorphic microsatellite loci. We then screened each individual for the presence of *Wolbachia* by PCR amplification of the *wsp* gene fragment. For all **hybrids**, we determined their maternal species-lineage by PCR amplification and sequencing of the mitochondrial locus cytochrome oxidase I. We find that winter moth x Bruce spanworm **hybrid** individuals appear to be present in all regions of North America that winter moth has invaded, and that **hybrids** are of both winter moth and Bruce spanworm maternal-origins. In addition, we find *Wolbachia* infected individuals from all species in North America, and that winter moth individuals in North America have a much lower infection rate (11.5%) than individuals in Europe (55.1%).

Sequence capture across large phylogenetic scales by using pooled PCR-generated baits: A case study of Lepidoptera

Zhang, Y; Deng, SH; Liang, D; Zhang, P. 2019

Sequence capture across large phylogenetic scales is not easy because **hybridization** capture is only effective when the genetic distance between the bait and target is small. Here, we propose a simple but effective strategy to tackle this issue: pooling DNA from a number of selected representative species of different clades to prepare PCR-generated baits to minimize the genetic distance between the bait and target. To demonstrate the utility of this strategy, we newly developed a set of universal nuclear markers (including 94 nuclear protein-coding genes) for Lepidoptera, a superdiverse insect group. We used a DNA pool from six lepidopteran species (representing six superfamilies) to prepare PCR baits for the 94 markers. These homemade PCR baits were used to capture sequence data from 43 species of 17 lepidopteran families, and 94% of the target loci were recovered. We constructed two data sets from the obtained data (one containing 90 kb target coding sequences and the other containing 120 kb target + flanking coding sequences). Both data sets yielded highly similar and well-resolved trees with 90% of nodes having >95% bootstrap support. Our capture experiment indicated that using DNA mixtures pooled from different clade-representative species of Lepidoptera to prepare PCR baits can reliably capture a large number of targeted nuclear markers across different Lepidoptera lineages. We hope that this newly developed nuclear marker set will serve as a new phylogenetic tool for Lepidoptera phylogenetics, and the PCR bait preparation strategy can facilitate the application of sequence capture techniques by researchers to accelerate data collection.

Limited intrinsic postzygotic reproductive isolation despite chromosomal rearrangements between closely related sympatric species of small ermine moths (Lepidoptera: Yponomeutidae)

Hora, KH; Marec, F; Roessingh, P; Menken, SBJ. 2019

In evolutionarily young species and sympatric host races of phytophagous insects, postzygotic incompatibility is often not yet fully developed, but reduced fitness of **hybrids** is thought to facilitate further divergence. However, empirical evidence supporting this hypothesis is limited. To assess the role of reduced **hybrid** fitness, we studied meiosis and fertility in **hybrids** of two closely related small ermine moths, *Yponomeuta padella* and *Yponomeuta cagnagella*, and determined the extent of intrinsic postzygotic reproductive isolation. We found extensive rearrangements between the karyotypes of the two species and irregularities in meiotic chromosome pairing in their **hybrids**. The fertility of reciprocal F-1 and, surprisingly, also of backcrosses with both parental species was not significantly decreased compared with intraspecific offspring. The results indicate that intrinsic postzygotic reproductive isolation between these closely related species is limited. We conclude that the observed chromosomal rearrangements are probably not the result of an accumulation of postzygotic incompatibilities preventing **hybridization**. Alternative explanations, such as adaptation to new host plants, are discussed.

Two consecutive Wolbachia-mediated mitochondrial introgressions obscure taxonomy in Palearctic swallowtail butterflies (Lepidoptera, Papilionidae)

Gaunet, A; Dinca, V; Dapporto, L; Montagud, S; Voda, R; Schar, S; Badiane, A; Font, E; Vila, R. 2019

Swallowtail butterflies (Papilionidae) are among the most spectacular and well-known Lepidoptera in the European fauna, but their systematics is not fully elucidated. A notable case is that of *Iphiclidus feisthamelii* which, after more than 180 years since description, still has a debated status, being often considered as a subspecies of *Iphiclidus podalirius*. To elucidate the relationship between the two taxa and the evolutionary processes that led to their separation, we combine mitochondrial and nuclear DNA (mtDNA and nDNA) data, Wolbachia screening, genitalia morphology and wing UV reflectance. Our results show that the two taxa clearly differ in male and female genital morphology, male wing UV reflectance and nDNA. Two Wolbachia strains were found to widely infect the studied samples, apparently explaining the phylogeographic pattern displayed by mtDNA. The available data point towards a historical Wolbachia infection that spread from *I. podalirius* to *I. feisthamelii* and produced a mitochondrial introgression. Currently, a new Wolbachia strain is spreading across mainland populations of *I. podalirius*, mediating once more a mitochondrial genetic sweep, which has already infected and introgressed *I. feisthamelii* populations in south-eastern France. We conclude that, given the marked differences in morphology and nDNA between the two taxa, and the apparent restriction of **hybridization** to a narrow contact area where non-**hybrid** specimens are common, the taxon *feisthamelii* should be considered as a separate species. Within this species, two well-differentiated nDNA lineages that represent European and Maghrebian populations are documented, here proposed as subspecies. The case of, presumably, two consecutive Wolbachia-mediated mitochondrial introgression events, further supports the view that infection by this endosymbiont may be frequently related to mito-nuclear discordance in insects.

Karyotype reinvestigation does not confirm the presence of two cryptic species and interspecific hybridization in the *Polyommatus* (*Agrodiaetus*) *damocles* complex in the Crimea (Lepidoptera, Lycaenidae)

Lukhtanov, VA; Efetov, KA; Dantchenko, AV. 2019

The karyotype of the blue butterflies from the Angarskiy Pass (Crimea), previously attributed to *Polyommatus* (*Agrodiaetus*) *poseidon* (Herrich-Schaffer, 1851), was re-examined. In all 19 studied individuals, we found the haploid chromosome number $n = 26$, including 7 pairs of relatively large and 19 pairs of relatively small chromosomes. According to the chromosome number and karyotype structure, the studied population does not differ from *P. (A.) damocles krymaeus* (Sheljuzhko, 1928) from the eastern part of the Crimean Mountains. This result does not confirm the previously formulated hypotheses, according to which (1) two morphologically similar but karyologically different species, *P. (A.) poseidon* and *P. (A.) damocles krymaeus*, occur sympatrically in the Crimea and (2) there is **hybridization** between these taxa on the Angarskiy Pass. Thus, only three species of the subgenus *Agrodiaetus* Hubner, 1822 have been reliably established for the Crimea: *P. (A.) damone* pljushtchi Lukhtanov & Budashkin, 1993, *P. (A.) damocles krymaeus* (Sheljuzhko, 1928) and *P. (A.) ripartii* budashkini Kolev & de Prins, 1995.

DESCRIPTION OF LARVAL AND PUPAL STAGES OF A BILATERAL GYNANDROMORPH REARED FROM A CROSS BETWEEN CITHERONIA REGALIS (FABR.) AND C. SPLENDENS (DRUCE) (LEPIDOPTERA: SATURNIIDAE)

Adamski, D; Marques, D; Mayo, KG; Mayo, R. 2019

A detailed study of the larval and pupal stages of a bilateral gynandromorph obtained from a **hybrid** cross between *Citheronia regalis* (Fabr.) and *C. splendens* (Druce) (Lepidoptera: Saturniidae) is presented. Color illustrations and metric data document male *splendens* characteristics on one side and female *regalis* characteristics on the other side of the last instar larva. Differences in color, shape, and setal positions on the head capsule and the anal plate are documented. A video study of the larval gynandromorph is presented from a hyperlink within this publication. The pupa shows evidence of male and female genitalia, however, no asymmetry was observed among the sclerites of the antennae or of the wings. Images of the adult gynandromorph are included.

Phylogenomics resolves major relationships and reveals significant diversification rate shifts in the evolution of silk moths and relatives

Hamilton, CA; St Laurent, RA; Dexter, K; Kitching, IJ; Breinholt, JW; Zwick, A; Timmermans, MJTN; Barber, JR; Kawahara, AY. 2019

Background: Silkmooths and their relatives constitute the ecologically and taxonomically diverse superfamily Bombycoidea, which includes some of the most charismatic species of Lepidoptera. Despite displaying spectacular forms and diverse ecological traits, relatively little attention has been given to understanding their

evolution and drivers of their diversity. To begin to address this problem, we created a new Bombycoidea-specific Anchored Hybrid Enrichment (AHE) probe set and sampled up to 571 loci for 117 taxa across all major lineages of the Bombycoidea, with a newly developed DNA extraction protocol that allows Lepidoptera specimens to be readily sequenced from pinned natural history collections. Results: The well-supported tree was overall consistent with prior morphological and molecular studies, although some taxa were misplaced. The bombycid *Arotros Schaus* was formally transferred to *Apatelodidae*. We identified important evolutionary patterns (e.g., morphology, biogeography, and differences in speciation and extinction), and our analysis of diversification rates highlights the stark increases that exist within the Sphingidae (hawkmoths) and Saturniidae (wild silkmoths). Conclusions: Our study establishes a backbone for future evolutionary, comparative, and taxonomic studies of Bombycoidea. We postulate that the rate shifts identified are due to the well-documented bat-moth "arms race". Our research highlights the flexibility of AHE to generate genomic data from a wide range of museum specimens, both age and preservation method, and will allow researchers to tap into the wealth of biological data residing in natural history collections around the globe.

The Parasitic Wasp, *Cotesia congregata* (Say), Consists of Two Incipient Species Isolated by Asymmetric Reproductive Incompatibility and Hybrid Inability to Overcome Host Defenses

Bredlau, JP; Kuhar, D; Gundersen-Rindal, DE; Kester, KM. 2019

Parasitic wasps are highly diverse and play a major role in suppression of herbivorous insect pest populations. Several previously identified species of parasitic wasps have been found to be complexes of cryptic species resulting from adaptations to specific hosts or host foodplants. *Cotesia congregata* (Say) (Hymenoptera: Braconidae), which has long served as a model system for host-parasitoid interactions, can be used for investigating the process of diversification among sympatric populations that differ in host and host foodplant usage. Two incipient species of *C. congregata* have been identified in the USA mid-Atlantic region, "MsT wasps" originate from *Manduca sexta* (L.) (Lepidoptera: Sphingidae) on tobacco and "CcC wasps" originate from *Ceratomia catalpae* (Boisduval) (Lepidoptera: Sphingidae) on catalpa. Both wasp sources can develop in either host species. Hybrids resulting from MsT male xCcC female crosses are fertile, whereas **hybrids** from CcC male xMsT female crosses are typically sterile. In this study, we compared relative expression in vivo of seven *C. congregata* bracovirus (CcBV) genes among MsT and CcC parental and **hybrid** crosses. Also, we established **hybrid** crosses between MsT and CcC wasps and four additional host foodplant sources of *C. congregata*. Patterns of relative expression in vivo of MsT and CcC CcBV genes differed; a few were not expressed in hosts parasitized by CcC wasps. Overall, relative expression of CcBV genes from MsT and CcC wasps did not differ with respect to the host species parasitized. Low or absent expression of CcBV genes was found in hosts parasitized by sterile **hybrids**. For the most part, the other four host-foodplant wasp sources were reproductively compatible with either MsT or CcC wasps and **hybrid** crosses with the alternative wasp source were asymmetrally sterile. Crosses involving CcC males or MsT females produced sterile **hybrids** that lacked mature ovaries. Cumulatively, results indicate that *C. congregata* is composed of two sympatric incipient species that can utilize multiple host species rather than several host-associated races or cryptic species.

Reproductive biology of two synchronopatric neotropical species of *Epidendrum* (Orchidaceae)

Sao Leao, LC; de Sa-Haiad, B; Rodarte, ATD; Pimentel, RR; Benevides, CR; de Santiago-Fernandes, LDR; de Lima, HA. 2019

The reproductive system of Orchidaceae is predominantly xenogamous and highly dependent on animal pollen vectors. Nectar is the main floral resource, offered to pollinators in perigonal nectaries, cuniculi or spurs; these structures are often difficult to locate and to evaluate their functionality. The Neotropical *Epidendrum denticulatum* Barb. Rodr. and *Epidendrum orchidiflorum* Salzm. ex. Lindl. bloom synchronously throughout the year in the municipality of Marica (Rio de Janeiro State, SE Brazil). The flowers of *E. denticulatum* open in the morning, have a conspicuous pink colour, a nectar guide, and nuptial and extranuptial nectaries. During the day, they are visited by species of Hesperidae (Lepidoptera), which remove the pollinia. The flowers of *E. orchidiflorum* open at night, are green with nuptial and extranuptial nectaries, and have a strong odour at night, suggesting nocturnal pollination agents, possibly moths. To date, no information is available on the reproductive biology and pollination mechanisms for *E. orchidiflorum*, nor is micromorphological, anatomical or histochemical data related to the presence and functionality of secretory structures associated with pollination in either species. Also, no studies have examined the occurrence of mechanisms that hinder or prevent interspecific pollination. Here, through reproductive, morphological, structural and histochemical analyses, we evaluated the floral biology and reproductive systems of these synchronopatric *Epidendrum* species. Both species have extranuptial nectaries, a functional cuniculus, and osmophores. Hand-pollination experiments revealed that the species are self and intercompatible, although individuals with intermediate morphology were not identified. Pre-pollination barriers related to the floral biology of the species seem to be sufficient to prevent **hybridization**, but other biological aspects certainly contribute to the genetic integrity of the populations, such as the low rates of visits to flowers, low percentage of fruit and seed set, low seedling recruitment, and high investment in vegetative propagation.

Anchored hybrid enrichment phylogenomics resolves the backbone of erebine moths

Homziak, NT; Breinholt, JW; Branham, MA; Storer, CG; Kawahara, AY. 2019

The subfamily Erebinidae (Lepidoptera, Erebiidae) includes approximately 10,000 species with many still undescribed. It is one of the most diverse clades within the moth superfamily Noctuoidea and encompasses a diversity of ecological habits. Erebine caterpillars feed on a broad range of host plants including several economically important crops. Adults possess a unique array of adaptations for predator defense, including some of the most sensitive hearing organs (tympana) across the Lepidoptera and striking wing coloration to startle visual predators. Despite the relevance of these moths to agriculture and ecological research, a robust phylogenetic framework is lacking. Here we used anchored **hybrid** enrichment, a relatively new approach in phylogenomics, to resolve relationships among the subfamily. Using the recently developed Lep1 anchored **hybrid** enrichment probe set, 658 gene fragments with an average length of 320 bp were captured from an exemplar set of 75 erebine species, representing 73 genera and 23 tribes. While the total number of erebine tribes is not firmly established, this represents at least 75% of known tribal level diversity. Anchored **hybrid** enrichment data were partitioned by locus and by codon position for maximum likelihood phylogenetic analysis and coalescent-based species-tree approaches. Results from our study provided strong nodal support (BP >= 95) for nearly all nodes in the partitioned ML tree, solidifying many relationships that were previously uncertain or moderately supported based on morphology or a smaller number of gene fragments. Likelihood analyses confidently resolved the placement of *Acantholipini* as a sister tribe to *Synpini* and all other Erebinidae. The remaining tribes were placed in a single, strongly supported clade split into two major subclades. Additionally, 25 tropical species that did not have previous tribal assignments are confidently placed on the phylogeny. Statistical comparisons with Shimodaira-Hasegawa (SH) tests found that our maximum likelihood trees were significantly more likely than alternative hypotheses. This study demonstrates the utility of anchored phylogenomics for resolving relationships within subfamilies of Lepidoptera.

First record of *Azalea* rough bollworm, *Earias roseifera* (Lepidoptera: Nolidae) in Europe

Taddei, A; Tantardini, A; Eda, K. 2019

The first record of the *Azalea* rough bollworm, *Earias roseifera* Butler, 1881 in Europe is reported. Larvae were collected on twigs, sprouts and buds of several *azalea* **hybrids** growing in a botanical garden in the province of Como (Northern Italy). The larvae fed mainly on the flower and vegetative buds, which resulted in a significant reduction in the amount of blossom. Specimens were identified using both morphological characters and a molecular analysis of the DNA barcode (COX1 sequence).

Orientation of some *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) strains to *Lolium perenne* L. (Poales: Poaceae) and *Galleria*

mellonella (L., 1758) (Lepidoptera: Pyralidae)

Yildirim, S; Sahin, YS; Susurluk, IA. 2019

Entomopathogenic nematodes (EPNs) have different host-finding (foraging) behavior that varies from species to species. Besides their foraging behavior, the orientation of some EPNs can vary depending on plant roots. In the present study, the orientation of some *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) strains to perennial ryegrass *Lolium perenne* L. (Poales: Poaceae) roots and greater wax moth *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae were investigated. For this purpose, three different strains of *H. bacteriophora* were used. One of them was a **hybrid** strain (HBH) and the two were parents (HB1138 and HB4) of the **hybrid** strain. Three experimental combinations were conducted in the study conducted in 2019 under laboratory conditions in Bursa, Turkey: (A) plant roots, larvae and control; (B) plant roots, control I and control II; and (C) mechanically damaged plant roots, control-I and control-II in Y-tube olfactometers filled with moist sand. The results indicated that for combination A, most orientation to plants and larvae was seen in HB1138; for combination B, most orientation to plant roots was seen in HBH; and for combination C, strain HB4 showed the most orientation to mechanically damaged plant roots. According to the results, each strain of the same EPN species may have a different response to plant roots and host insects.

Colonization of Northern Europe by *Zygaena filipendulae* (Lepidoptera)

Zagrobelyny, M; Dalsten, L; Hille, A. 2019

Northern and mountainous ice sheets have expanded and contracted many times due to ice ages. Consequently, temperate species have been confined to refugia during the glacial periods wherefrom they have recolonized warming northern habitats between ice ages. In this study, we compare the gene CYP405A2 between different populations of the common burnet moth *Zygaena filipendulae* from across the Western Palearctic region to illuminate the colonization history of this species. These data show two major clusters of *Z. filipendulae* populations possibly reflecting two different refugial populations during the last ice age. The two types of *Z. filipendulae* only co-occur in Denmark, Sweden, and Scotland indicating that Northern Europe comprise the **hybridization** zone where individuals from two different refugia met after the last ice age. Bayesian phylogeographic and ecological clustering analyses show that one cluster probably derives from an Alpine Maritime refugium in Southern France with ancestral expansive tendencies to the British Isles in the west, touching Northern Europe up to Denmark and Sweden, and extending throughout Central Europe into the Balkans, the Peloponnese, and South East Europe. The second cluster encompasses East Anatolia as the source area, from where multiple independent dispersal events to Armenia, to the Alborz mountains in north-western Iran, and to the Zagros mountains in western Iran are suggested. Consequently, the classical theory of refugia for European temperate species in the Iberian, Italian, and Balkan peninsulas does not fit with the data from *Z. filipendulae* populations, which instead support more Northerly, mountainous refugia.

Impact of Simulated Corn Earworm (Lepidoptera: Noctuidae) Kernel Feeding on Field Corn Yield

Olivi, BM; Gore, J; Musser, FM; Catchot, AL; Cook, DR. 2019

Corn earworm, *Helioverpa zea* (Boddie) (Lepidoptera: Noctuidae), has not been considered an economic pest of field corn. Historical losses estimates ranged from 1.5 to 2.5%, and a large number of foliar insecticide applications would be needed to minimize infestations. In recent years, Bt, *Bacillus thuringiensis* (Berliner) field corn, *Zea mays* (L.) Poales: Poaceae, technologies that exhibit activity against corn earworm have been introduced. However, it is unclear how much damage to corn ears (number of damaged kernels) is required to reduce yield. In this study manual damage methods were utilized to inflict defined levels of kernel damage and to impose damage at levels greater than observed with natural corn earworm infestations. Bt corn **hybrids** expressing the Agrisure Viptera (Vip 3A) trait were used to minimize injury from natural infestations of corn earworm. Manual kernel damage was imposed at R3 stage to mimic corn earworm feeding while avoiding interference with pollination. These methods were used in experiments where treatments were applied to individual ears and hand-harvested and in experiments where treatments were applied to all primary ears in the plot and machine-harvested. Damage of ≥ 60 kernels per ear was required to significantly reduce yield regardless of harvest method. Kernel damage from natural corn earworm infestations reported in other studies was much lower than 60 kernels per ear. Timely planting is a key component of all integrated pest management programs. Field corn planted during the recommended planting window for optimum yield is unlikely to experience corn earworm damage great enough to reduce yield.

Molecular species delimitation in the genus *Rhamma* Johnson, 1992 (Lepidoptera: Lycaenidae, Theclinae)

Prieto, C; Nunez, R; Hausmann, A. 2019

In this work, we used mtDNA data as a tool to delimit species and we compared the resulting molecular operational taxonomic units (barcode index number, BIN) with morphology-based identifications in the Colombian species of *Rhamma* Johnson, 1992 exploring the usefulness of DNA barcodes for taxonomy, species identification and delimitation. We obtained cytochrome oxidase I (COI) sequences for 134 morphologically identified specimens, representing 12 species of *Rhamma* from Colombia. Ten of these species have not been previously barcoded. DNA barcodes suggested the potential for eight additional cryptic species in Colombia but we were readily able to morphologically diagnose just one of these lineages as a new species which recently was described in a separate paper as *Rhamma dawkinsi* Prieto & Lorenc-Brudecka, 2017. The morphological species were separated into three categories: species showing a perfect match between morphological species and BINs (33%, four species); species sharing a BIN completely or partly (single specimens) with another morphological species (42%, five species placed in three BINs); and morphological species splitting up into more than one BIN (25%, three species placed in 10 BINs). The high percentages of incongruence between morphology-based identification and species delineation through BINs, could be explained as a consequence of high rates of introgressive **hybridization**. However, DNA barcodes can be considered diagnostic even in cases where specimens of a species were assigned to two or more distinct BINs and in species showing a low but constant divergence causing their assignment to a single BIN, which is often the case in young, allopatric species. We retain 10 of the 12 species (83%) to be diagnostic in molecular identification.

The phylogenetic history of the old world butterfly subtribe *Mycalesina* extended: the *Mycalesis* (Lepidoptera: Nymphalidae) of Sri Lanka

Goonasekera, K; Lee, PLM; van der Poorten, G; Ranawaka, GR. 2019

Sri Lanka, together with the Western Ghats, is one of the world's biodiversity hotspots, yet little is known about the genetic diversity of the butterflies here. Within the framework of the recently reconstructed molecular phylogeny for the subtribe *Mycalesina*, we determined the phylogenetic relationships and biogeographic history of the five species of *Mycalesis* butterflies that are found in Sri Lanka. Sequences of Elongation Factor 1- α (EF1- α), Wingless (Wg1) and the barcode region of the Cytochrome c Oxidase sub unit 1 (COI) were used to confirm the identity of species, to resolve taxonomic queries and to infer the phylogenetic history of the group. Time-calibrated analysis of genetic data suggests that the *Mycalesis* species that occur in Sri Lanka diverged in the Miocene (5-23 million years ago). The results of phylogenetic analyses confirmed the following; the endemism of *Mycalesis rama* to Sri Lanka; the subspecies classification of *M. perseus typhlus* and, the close sister relationship of the Sri Lankan *M. pamia pamia* to *M. p. junonia* of India. However, whether or not *M. subdita* of Sri Lanka and India are genetically similar remains unknown. Lastly, genetic evidence emerged suggesting that *M. mineus* forms a cryptic species complex in the Oriental region, and that in Sri Lanka, there may be occasional **hybridization** between *M. mineus* and *M. perseus*. As a case study of island colonization and diversification by the *Mycalesis* species, this study further extends our understanding of the Old World butterfly subtribe of *Mycalesina*.

A revisit to the *Melitaea taxon nigrogygia* Verity, 1939 (Lepidoptera: Nymphalidae) and designation of a Lectotype

Russell, P; Bartolozzi, L. 2019

The two syntypes of *Melitaea phoebe nigrogygia* Verity, 1939 present in the Florence University Natural History Museum are examined from high quality photographs of both sides. It is concluded that there is a high probability that they represent two different species: *M. phoebe* ([Denis & Schiffermüller], 1775) and *M. ornata* Christoph, 1898. Alternatively one or both could be **hybrids** between the two species; **hybrids** have been encountered nearby in Slovenia. In order to solve the nomenclatural problem, a Lectotype has been chosen, which fixes the name *nigrogygia* Verity, 1939 to be associated with *Melitaea ornata*.

Diversity of Insect Pests Damaging Quality of "Huitlacoche" (Corn Smut) at Saltillo, Coahuila, Mexico

Sanchez-Vega, M; Mendez-Lopez, A; Salazar-Torres, JC; Leal-Robles, AI; Martinez-Amador, SY; Perez-Perez, JE. 2019

Huitlacoche or corn smut, *Ustilago maydis* (DC.) Corda, is a biotrophic fungal pathogenic. The scales that grow in ears of maize, *Zea mays* L., are used as fresh or raw vegetables. In some regions of Mexico, maize is planted to harvest "huitlacoche", a crop also susceptible to attack by insect pests that affect its quality. The purpose of this study was to recognize entomofauna in maize ears infected with huitlacoche (corn smut) and identify pest species that attack huitlacoche in five maize **hybrids**. We collected a total of 515 specimens in four orders, seven families, and 11 species. The orders Coleoptera and Lepidoptera comprised 71.4% of the total number of families, 81.8% of the species, and 99.6% of individuals. The percentage of damaged ears ranged from 47.5 to 65.0%. Species best represented were *Cotinis mutabilis* (Gory and Percheron), *Spodoptera frugiperda* (J.E. Smith), *Heliopsis virescens* (Fabricius), and *Carpophilus lugubris* Murray considered primary pest of huitlacoche because they were present in all maize studied. Shannon-Wiener diversity (0.592 bits per individual) and Simpson (0.302 bits per individual) indices showed less diversity of individuals and moderate species dominance. Greatest richness of species was in **hybrids** 'AN388' and 'H-318', each with seven.

The complete mitochondrial genome of *Antheraea proylei* strain In981 (Lepidoptera: Saturniidae)

Yang, J; Zhang, RS; Chen, DB; Chen, MM; Li, YP; Liu, YQ. 2019

In the present study, we report the complete mitochondrial genome of *Antheraea proylei* strain In981, a **hybrid** of Chinese oak silkworm (*A. pernyi*) and Indian oak silkworm (*A. roylei*). The circular molecule is 15,573 bp in length, with 37 typical coding genes (13 protein-coding genes, 2 ribosomal RNA genes, and 22 transfer RNA genes) and one non-coding A + T-rich region of 552 bp long. Its gene components and gene order are identical to the common type found in Bombycoidea species. Phylogenetic analyses revealed that In981 is closely related to *A. pernyi* rather than *A. roylei*. This is the first report on the complete mitochondrial genome of *A. proylei*.

Alternative splicing regulation of doublesex gene by RNA-binding proteins in the silkworm *Bombyx mori*

Zheng, ZZ; Sun, X; Zhang, B; Pu, J; Jiang, ZY; Li, MW; Fan, YJ; Xu, YZ. 2019

Doublesex is highly conserved and sex-specifically spliced in insect sex-determination pathways, and its alternative splicing (AS) is regulated by Transformer, an exonic splicing activator, in the model system of *Drosophila melanogaster*. However, due to the lack of a transformer gene, AS regulation of doublesex remains unclear in Lepidoptera, which contain the economically important silkworm *Bombyx mori* and thousands of agricultural pests. Here, we use yeast three-**hybrid** system to screen for RNA-binding proteins that recognize sex-specific exons 3 and 4 of silkworm doublesex (*Bm-dsx*); this approach identified BxRBP1/Lark binding to the exon 3, and BxRBP2/TBPH and BxRBP3/Aret binding to the exon 4. Investigation of tissues shows that BxRBP1 and BxRBP2 have no sex specificity, but BxRBP3 has - three of its four isoforms are expressed with a sex-bias. Using novel sex-specific silkworm cell lines, we find that BxRBP1 and BxRBP3 directly interact with each other, and cooperatively function as splicing repressors. Over-expression of BxRBP1 and BxRBP3 isoforms efficiently inhibits splicing of the exons 3 and 4 in the female-specific cells and generates the male-specific isoform of *Bm-dsx*. We also demonstrate that the sex-determination upstream gene *Masc* regulates alternatively transcribed BxRBP3 isoforms. Thus, we identify a new regulatory mechanism of doublesex AS in the silkworm, revealing an evolutionary divergence in insect sex-determination.

South and Central America Cut Flower Production and Postharvest Survey

Loyola, CE; Dole, JM; Dunning, R. 2019

Imports of cut flowers into the United States have doubled in the last 20 years and come mainly from Colombia and Ecuador. We surveyed the cut flower industry in South and Central America, focusing on Colombia and Ecuador, to determine their production and postharvest problems. We received a total of 51 responses, of which 62% of the respondents had 100 or more employees. The most commonly grown or handled crops were rose (*Rosa* **hybrids**), carnation (*Dianthus caryophyllus*), chrysanthemum (*Chrysanthemum xgrandiflorum*), alstroemeria (*Alstroemeria* cultivars), gerbera (*Gerbera jamesonii*), and hydrangea (*Hydrangea* species), in order of ranking. The most significant production problem was insect management, with disease management and crop timing the next most important issues. The most important species-specific issues in production were phytosanitary problems, disease (causal organism not specified), leaf miner (Lepidoptera, Symphyta, or Diptera), and thrips (Thysanoptera). The main overall postharvest problem was temperature management, followed by hydration and flower food management and botrytis (*Botrytis cinerea*). In regard to on-farm postharvest handling, damage to the flowers was the most mentioned issue. For the postharvest during storage and transport phase, temperature management, air transport, damage, and botrytis were the most important problems. The most mentioned customer complaints were damage, botrytis, and phytosanitary problems. The results of this survey can be used by researchers to focus their work on topics of most need. Improved production and postharvest handling will support the continued growth of the cut flower industry.

First record of *Isia alcumena*, *Spodoptera cosmioides* and *S. eridania* (LEPIDOPTERA: NOCTUOIDEA) attacking Passion Fruit (*Passiflora edulis* Sims) in Brazil

Ferreira, TE; Faleiro, FG; Junqueira, NTV; de Camargo, AJA; Teston, JA; Specht, A. 2019

Brazil is considered the center of origin and diversity of passifloras. These species have a great importance to in natura fruits consumption, industrialization, medicinal use and also as ornamental plants. The different *Passiflora* species are host of a great diversity of arthropods that can cause injuries, economic damages and, in some situations, the plant death. Among the arthropods, leafhopper caterpillars are considered frequent and severe pests in the main passion fruit producing regions. The present work is an occurrence report of *Isia alcumena* and *Spodoptera cosmioides* attacking passion fruit plants (*Passiflora edulis* Sims) in the Federal District, Brazil. The *S. cosmioides* and *S. eridania* caterpillars were collected while consuming leaves of *Passiflora edulis* intra-specific **hybrid** located in the Germplasm Active Bank 'Flor da Paixão' at Embrapa Cerrados. The caterpillars of *I. alcumena* were collected on leaves of the *Passiflora edulis* cv. BRS Rubi do Cerrado at the Agricultural Support Unit, Embrapa Cerrados. After collected, the caterpillars were individualized in a breeding system with leaves of the host plants. The development of the caterpillar was accompanied until the emergence of the adults, which were identified and fixed in entomological pins for permanent dry preservation.

Antioxidant Systems as a Response to Midgut Cellular of *Bombyx mori* Lineu, 1758 (Lepidoptera: Bombycidae) Infection for Baculoviruses

AVessaro-Silva, S; Neto, MHM; Brancalhão, RMC; Ribeiro, LFC; Guimaraes, ATB; de Oliveira, CMT. 2019

Bombyx mori nucleopolyhedrovirus (BmNPV) is a DNA virus that infects different tissues in *Bombyx mori* at immature stage. Caterpillars become infected after ingesting polyhedral occlusion bodies (POB) present in contaminated mulberry leaves and spread through the body after passing the epithelium of the midgut. As this organ is responsible for digestion, most absorption of nutrients requires an intact epithelium to maintain gastrointestinal physiology. Considering the importance of this organ in the feeding of caterpillars and in the production of quality silk threads, and because it is also the first barrier faced by the BmNPV, the study analyzed details of cytopathological events in the intestinal cells as well as evaluated the action of the antioxidant systems as a response to cellular infection. For this purpose, *B. mori* **hybrid** caterpillars of fifth instar were inoculated with a suspension of 7.8×10^7 POB ml⁻¹ and, from the first to the eighth day post-inoculation (dpi), segments of the midgut were collected and processed for light and electronic microscopy. The nuclei of columnar cells showed polyhedral occlusion bodies in the seventh dpi and fragmentation of those cells, with peritrophic matrix disorganization. Analysis of antioxidant systems shows some moments of changes of the catalase enzymes and superoxide dismutase. Analysis of the cholinergic system revealed changes only at the beginning of the infection. Thus, the article acknowledges the antioxidant system as a barrier to stop viral infection, albeit it cannot stop infection from occurring, once a coevolutionary bond is maintained between virus and host.

Absorption spectra and electric field distributions of butterfly wing scale models coated with a metal film studied by finite-difference time-domain method

Rakstrithong, P; Locharoenrat, K. 2019

Inspired by alternative **hybrid**-biophotonic structures and modern computational electromagnetics in plasmonics, herein, we attempted to understand the plasmonic properties of a metal film (gold or palladium) on the surface features of butterfly wing scales, as they might represent the dominant features of structure-enhanced and/or structure-attenuated optical properties. Light-harvesting plasmonic antenna was loaded on these natural substrates. We examined the plasmonic properties of three models representing the scales of three lepidoptera species. Each scale model was assumed to have a 100 nm metal coating. In addition to the electron micrograph of the lepidopterans' wings, the optical properties of the investigated structures were numerically studied using the finite-difference time-domain technique. We first constructed the biophotonic models of butterfly structures coated with a metal film, and then they were verified by scanning electron microscopy images using Lumerical Software, which provided an accurate solution of Maxwell's equation for the micro/nanostructures. The metal samples were palladium or gold, while the investigated scales of butterfly species were *Catopsilia pomona*, *Danaus genutia*, and *Cetbosia penthesilea*. Electric field and absorption spectra were observed under broadband light irradiations at perpendicular- and parallel-polarized light illuminations. As a result of the formation of variations of metals on the different features of wing scales, we observed changes in the absorption intensities and a redshift in the main peak absorbance. The spectra further showed a close relationship with the electric field distribution. A metal film coated on the butterfly wing scales acted as an optical plasmonic sensitivity to amplify and attenuate the visible light, whereas the existence of wave propagating modes from the well-defined structural variations resulted in a reduction and enhancement of the bandwidth of absorbance. Among the three simulation models, the *Cetbosia penthesilea* scale model coated with a gold film demonstrated the best plasmonic properties to the electric field, in terms of its potential application for further biophotonic structure fabrication.

Hybridization and transgressive exploration of colour pattern and wing morphology in *Heliconius* butterflies

Merot, C; Debat, V; Le Poul, Y; Merrill, RM; Naisbit, RE; Tholance, A; Jiggins, CD; Joron, M. 2020

Hybridization can generate novel phenotypes distinct from those of parental lineages, a phenomenon known as transgressive trait variation. Transgressive phenotypes might negatively or positively affect **hybrid** fitness, and increase available variation. Closely related species of *Heliconius* butterflies regularly produce **hybrids** in nature, and **hybridization** is thought to play a role in the diversification of novel wing colour patterns despite strong stabilizing selection due to interspecific mimicry. Here, we studied wing phenotypes in first- and second-generation **hybrids** produced by controlled crosses between either two co-mimetic species of *Heliconius* or between two nonmimetic species. We quantified wing size, shape and colour pattern variation and asked whether **hybrids** displayed transgressive wing phenotypes. Discrete traits underlain by major-effect loci, such as the presence or absence of colour patches, generate novel phenotypes. For quantitative traits, such as wing shape or subtle colour pattern characters, **hybrids** only exceed the parental range in specific dimensions of the morphological space. Overall, our study addresses some of the challenges in defining and measuring phenotypic transgression for multivariate traits and our data suggest that the extent to which transgressive trait variation in **hybrids** contributes to phenotypic diversity depends on the complexity and the genetic architecture of the traits.

From refugia to contact: Pine processionary moth hybrid zone in a complex biogeographic setting

Ipekdağ, K; Burban, C; Saune, L; Battisti, A; Kerdelhue, C. 2020

Contact zones occur at the crossroad between specific dispersal routes and are facilitated by biogeographic discontinuities. Here, we focused on two *Lepidoptera* sister species that come in contact near the Turkish Straits System (TSS). We aimed to infer their phylogeographic histories in the Eastern Mediterranean and finely analyze their co-occurrence and **hybridization** patterns in this biogeographic context. We used molecular mitochondrial and nuclear markers to study 224 individuals from 42 localities. We used discordances between markers and complementary assignment methods to identify and map **hybrids** and parental individuals. We confirmed the parapatric distribution of *Thaumetopoea pityocampa* (*Lepidoptera*: Notodontidae) in the west and *Thaumetopoea wilkinsoni* in the east and identified a narrow contact zone. We identified several glacial refugia of *T. wilkinsoni* in southern Turkey with a strong east-west differentiation in this species. Unexpectedly, *T. pityocampa* crossed the TSS and occur in northern Aegean Turkey and some eastern Greek islands. We found robust evidence of introgression between the two species in a restricted zone in northwestern Turkey, but we did not identify any F-1 individuals. The identified **hybrid** zone was mostly bimodal. The distributions and genetic patterns of the studied species were strongly influenced both by the Quaternary climatic oscillations and the complex geological history of the Aegean region. *T. pityocampa* and *T. wilkinsoni* survived the last glacial maximum in disjoint refugia and met in western Turkey at the edge of the recolonization routes. Expanding population of *T. wilkinsoni* constrained *T. pityocampa* to the western Turkish shore. Additionally, we found evidence of recurrent introgression by *T. wilkinsoni* males in several *T. pityocampa* populations. Our results suggest that some prezygotic isolation mechanisms, such as differences in timing of the adult emergences, might be a driver of the isolation between the sister species.

An application of the synthetic sex attractants from the series "EFETOV-2" for studying Procridinae in Italy (Lepidoptera: Zygaenidae)

Efetov, KA; Kucherenko, EE; Tarmann, GM. 2020

The enantiomers of 2-butyl 2-dodecenoate synthesised in the Crimean Federal University are known as the sex attractants for Procridinae species (*Lepidoptera*: Zygaenidae). These compounds, alone and in combination, were applied for studying the Zygaenidae fauna in Italy for four years from 2015 to 2018. We evaluated the effectiveness of the three following variants: EFETOV-2 (the racemic mixture of (2R)-butyl 2-dodecenoate and (2S)-butyl 2-dodecenoate), EFETOV-S-2 (the R-enantiomer alone) and EFETOV-S-S-2 (the S-enantiomer alone). The males of three genera of Procridinae were attracted to the lures from the series "EFETOV-2": *Rhagades Wallengren*, 1863 (one species), *Adscita Retzius*, 1783 (six species), and *Jordanita Verity*, 1946 (four species). The sex attractants for species *Adscita* (*Adscita*) *italica* (Alberti, 1937), *A. (A.) alpina* (Alberti, 1937), *A. (A.) dujardini* Efetov & Tarmann, 2014, and one endemic species *Jordanita* (*Jordanita*) *tenuicornis*

(Zeller, 1847) were found for the first time. Moreover, a **hybridisation** zone between *A. (A.) italica* and *A. (A.) alpina* was discovered by the application of the sex attractants. *Rhagades (Rhagades) pruni* ([Denis & Schiffermuller], 1775) was recorded for the first time for more than 100 years in Piemonte and for the first time ever in the Ligurian Alps.

Incomplete Sterility of Chromosomal Hybrids: Implications for Karyotype Evolution and Homoploid Hybrid Speciation

Lukhtanov, VA; Dinca, V; Friberg, M; Vila, R; Wiklund, C. 2020

Heterozygotes for major chromosomal rearrangements such as fusions and fissions are expected to display a high level of sterility due to problems during meiosis. However, some species, especially plants and animals with holocentric chromosomes, are known to tolerate chromosomal heterozygosity even for multiple rearrangements. Here, we studied male meiotic chromosome behavior in four **hybrid** generations (F1-F4) between two chromosomal races of the Wood White butterfly *Leptidea sinapis* differentiated by at least 24 chromosomal fusions/fissions. Previous work showed that these **hybrids** were fertile, although their fertility was reduced as compared to crosses within chromosomal races. We demonstrate that (i) F1 **hybrids** are highly heterozygous with nearly all chromosomes participating in the formation of trivalents at the first meiotic division, and (ii) that from F1 to F4 the number of trivalents decreases and the number of bivalents increases. We argue that the observed process of chromosome sorting would, if continued, result in a new homozygous chromosomal race, i.e., in a new karyotype with intermediate chromosome number and, possibly, in a new incipient homoploid **hybrid** species. We also discuss the segregational model of karyotype evolution and the chromosomal model of homoploid **hybrid** speciation.

Genome Assembly of the Dogface Butterfly *Zerene cesonia*

Rodriguez-Caro, L; Fenner, J; Benson, C; Van Belleghem, SM; Counterman, BA. 2020

Comparisons of high-quality, reference butterfly, and moth genomes have been instrumental to advancing our understanding of how **hybridization**, and natural selection drive genomic change during the origin of new species and novel traits. Here, we present a genome assembly of the Southern Dogface butterfly, *Zerene cesonia* (Pieridae) whose brilliant wing colorations have been implicated in developmental plasticity, **hybridization**, sexual selection, and speciation. We assembled 266,407,278 bp of the *Z. cesonia* genome, which accounts for 98.3% of the estimated 271 Mb genome size. Using a **hybrid** approach involving Chicago libraries with Hi-Rise assembly and a diploid Meraculous assembly, the final haploid genome was assembled. In the final assembly, nearly all autosomes and the Z chromosome were assembled into single scaffolds. The largest 29 scaffolds accounted for 91.4% of the genome assembly, with the remaining similar to 8% distributed among another 247 scaffolds and overall N50 of 9.2 Mb. Tissue-specific RNA-seq informed annotations identified 16,442 protein-coding genes, which included 93.2% of the arthropod Benchmarking Universal Single-Copy Orthologs (BUSCO). The *Z. cesonia* genome assembly had similar to 9% identified as repetitive elements, with a transposable element landscape rich in helitrons. Similar to other Lepidoptera genomes, *Z. cesonia* showed a high conservation of chromosomal synteny. The *Z. cesonia* assembly provides a high-quality reference for studies of chromosomal arrangements in the Pierid family, as well as for population, phylo, and functional genomic studies of adaptation and speciation.

Molecular taxonomy of *Tomares* hairstreaks (Lepidoptera, Lycaenidae, Theclinae)

Nazari, V; ten Hagen, W. 2020

Tomares hairstreaks comprise about 10 species distributed from Europe and North Africa to Central Asia. The taxonomy of the genus is hampered by the absence of diagnostic characters by which specimens can be unambiguously assigned to species. Our investigation of morphology and DNA barcode variations within and between *Tomares* species shows that while well-defined species (*T. ballus*, *T. mauritanicus*, *T. callimachus*, *T. desinens* and *T. fedtschenkoi*) diverge, poorly characterized taxa (*T. nogelii*, *T. nesimachus*, *T. dobrogensis*, *T. romanovi* and *T. telemachus*) show very little to no differentiation in mtDNA. We reinstate *Tomares callimachus* spp. *hafis* (Kollar, 1849) as a valid subspecies (stet. rev.) and propose taxa *telemachus* Zhdanko, 2000 and *uighurica* Kocak, Seven & Kemal, 2000 as synonyms of *T. romanovi* and *T. nogelii* *nogelii* respectively (syn. nov.). We relegate *Polyommatus epiphania* Boisduval, 1848, recently revived as a valid subspecies of *T. callimachus*, back to synonymy under the latter, and reconsider the status of *T. nogelii* *dobrogensis* (Caradja, 1895) in the light of new molecular data. We use a nuclear gene (EF-1 alpha) in addition to COI barcodes to reconstruct the phylogeny of the group.

A phylogenomics approach to characterizing sensory neuron membrane proteins (SNMPs) in Lepidoptera

Zhang, HJ; Xu, W; Chen, QM; Sun, LN; Anderson, A; Xia, QY; Papanicolaou, A. 2020

Sensory neuron membrane proteins (SNMPs) play a critical role in the insect olfactory system but there is a deficit of functional studies beyond *Drosophila*. Here, we use a combination of available genome sequences, manual curation, genome and transcriptome data, phylogenetics, expression profiling and gene knockdown to investigate SNMP superfamily in various insect species with a focus on Lepidoptera. We curated 81 genes from 36 insect species and identified a novel lepidopteran SNMP gene family, SNMP3. Phylogenetic analysis shows that lepidopteran SNMP3, but not the previously annotated lepidopteran SNMP2, is the true homologue of the dipteran SNMP2. Digital expression, microarray and qPCR analyses show that the lepidopteran SNMP1 is specifically expressed in adult antennae. SNMP2 is widely expressed in multiple tissues while SNMP3 is specifically expressed in the larval midgut. Microarray analysis suggest SNMP3 may be involved in the silkworm immunity response to virus and bacterial infections. We functionally characterized SNMP1 in the silkworm using RNA interference (RNAi) and behavioral assays. Our results suggested that *Bombyx mori* SNMP1 is a functional orthologue of the *Drosophila melanogaster* SNMP1 and plays a critical role in pheromone detection. Split-ubiquitin yeast **hybridization** study shows that BmorSNMP1 has a protein-protein interaction with the pheromone receptor (BmorOR1), and the co-receptor (BmorOrco). Concluding, we propose a novel molecular model in which BmorOrco, BmorSNMP1 and BmorOR1 form a heteromer in the detection of the silkworm sex pheromone bombykol.

A new species of *Cossidophaga* Baranov from Indonesia (Diptera: Tachinidae), a parasitoid of the carpenter moth, *Polyphagozerra coffeae* (Nietner) (Lepidoptera: Cossidae)

Tachi, T; Shima, H; Tavares, WD; Tarigan, M. 2020

The carpenter moth *Polyphagozerra coffeae* (Nietner) (Lepidoptera: Cossidae) is an important pest of commercial plantations of *Eucalyptus pellita* F. Muell. and the **hybrid** *E. pellita* x *Eucalyptus grandis* W. Hill. ex Maiden (Myrtaceae) in Riau, Indonesia. This study describes a new species of tachinid fly, *Cossidophaga coffeae* sp. nov., record its host, *P. coffeae*, and redescribe the type species of *C. atkinsoni* (Aubertin).

A deeper meaning for shallow-level phylogenomic studies: nested anchored hybrid enrichment offers great promise for resolving the tiger moth tree of life (Lepidoptera: Erebidae: Arctiinae)

Dowdy, NJ; Keating, S; Lemmon, AR; Lemmon, EM; Conner, WE; Chialvo, CHS; Weller, SJ; Simmons, RB; Sisson, MS; Zaspel, JM. 2020

Anchored **hybrid** enrichment (AHE) has emerged as a powerful tool for uncovering the evolutionary relationships within many taxonomic groups. AHE probe sets have been developed for a variety of insect groups, though none have yet been shown to be capable of simultaneously resolving deep and very shallow (e.g., intraspecific) divergences. In this study, we present NOC1, a new AHE probe set (730 loci) for Lepidoptera specialized for noctuoids and assess its ability to deliver phylogenetic utility at all taxonomic levels. We test the NOC1 probe set with 142 individuals from 116 species sampled from all the major lineages of Arctiinae (Erebidae), one of the most diverse groups of noctuoids (>11 000 species) for which no well-resolved, strongly supported phylogenetic hypothesis exists. Compared to previous methods, we generally recover much higher branch support (BS), resulting in the most well-supported, well-resolved phylogeny of Arctiinae to date. At the most shallow-levels, NOC1 confidently resolves species-level and intraspecific relationships and potentially uncovers cryptic species diversity within the genus *Hypoprepia*. We also implement a 'sensitivity analysis' to explore different loci combinations and site sampling strategies to determine whether a reduced probe set can yield results similar to those of the full probe set. At both deep and shallow levels, only 50-175 of the 730 loci included in the complete NOC1 probe set were necessary to resolve most relationships with high confidence, though only when the more rapidly evolving sites within each locus are included. This demonstrates that AHE probe sets can be tailored to target fewer loci without a significant reduction in BS, allowing future studies to incorporate more taxa at a lower per-sample sequencing cost. NOC1 shows great promise for resolving long-standing taxonomic issues and evolutionary questions within noctuid lineages, one of the most speciose superfamilies within Lepidoptera.

BACK TO THE FUTURE: UPDATES ON THE INVASION HISTORY OF JUNONIA BUTTERFLIES IN FLORIDA AND THE MYSTERY OF CHOKOLOOSKEE

Lalonde, MML; Marcus, JM. 2020

The tropical buckeye, *Junonia zonalis* (Lepidoptera: Nymphalidae) is a recent addition to the butterfly fauna of mainland Florida. It appears that this species began to invade the mainland from the Florida Keys or Cuba by the 1930s, **hybridizing** with *J. coenia* and bringing with it mitochondrial haplotype group A, which is common in the Caribbean but is essentially absent from North American *Junonia*. By the 1940s, *J. zonalis* appears to have established populations in Miami, but eventually may have been extirpated. Later, new populations of *J. zonalis* may have become established on mainland Florida by subsequent waves of *J. zonalis* migrants. Substantial fluctuations in both population size and mitochondrial haplotype group A frequency seem to be characteristic of Florida mainland populations of *J. zonalis*. Populations of *J. zonalis* in the Florida Keys and Cuba have maintained nearly constant mitochondrial haplotype group A frequencies over many decades and may be more stable than those on the Florida mainland. *Junonia zonalis* specimens attributed to Chokoloskee, Florida, from the early 1900s have questionable provenance. Based on their haplotype frequency and other evidence these Chokoloskee specimens may have been collected in Cuba. Similarly, one specimen of *J. zonalis* likely collected during the 1880s and labeled "Indian River, Fla." probably also originated from outside of Florida.

Genomic evidence suggests *Mesapamea remmi* is an imaginary species (Lepidoptera: Noctuidae)

Sihvonen, P; Lee, KM; Lundsten, KE; Mutanen, M. 2020

Taxonomic status of the noctuid moth *Mesapamea remmi* Rezbanyai-Reser (Lepidoptera) has remained controversial since its description in 1985. Based on morphology, it has been considered a valid species or a **hybrid** between *Mesapamea secalis* (Linnaeus) and *Mesapamea didyma* (Esper). We studied this case of uncertain identity by using traditional Sanger sequencing techniques (COI and seven nuclear genes) as well as double digest restriction-site associated DNA (ddRAD) sequencing, which resulted in analyses including 9402 loci and 1 512 976 bp across the genome. Our analyses showed that genomic data do not support the **hybrid** hypothesis and that *M. remmi* is not separated genetically from *M. secalis*; both are clearly distinct from *M. didyma*. Reproductive organs of *M. remmi* males are unique and diagnosable, whereas females are teratological in the sense that ductus seminalis is missing and corpus bursae is malformed, the latter being connected directly to the ovipore. These data support the view that *M. remmi* is not a valid species and we consider *M. remmi* Rezbanyai-Reser, 1985 to be a junior synonym of *M. secalis* (Linnaeus, 1758). We demonstrate that genomic approaches provide an efficient way to address various difficult and long-standing taxonomic issues, in this case oversplitting of species.

N-glycosylation in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) midgut membrane-bound glycoproteins

Fuzita, FJ; Chandler, KB; Haserick, JR; Terra, WR; Ferreira, C; Costello, CE. 2020

Spodoptera frugiperda is a widely distributed agricultural pest. It has previously been established that glycoproteins in the midgut microvillar membrane of insects are targets for toxins produced by different organisms as well as plant lectins. However, there is still little information about the N-glycome of membrane-bound midgut glycoproteins in Lepidoptera and other insect groups. The present study used mass spectrometry-based approaches to characterize the N-glycoproteins present in the midgut cell microvilli of *Spodoptera frugiperda*. We subjected midgut cell microvilli proteins to proteolytic digestion and enriched the resulting glycopeptides prior to analysis. We also performed endoglycosidase release of N-glycans in the presence of (H₂O)-O-18 determining the compositions of released N-glycans by MALDI-TOF MS analysis and established the occupancy of the potential N-glycosylation sites. We report here a total of 160 glycopeptides, representing 25 N-glycan compositions associated with 70 sites on 35 glycoproteins. Glycan compositions consistent with oligomannose, paucimannose and complex/**hybrid** N-glycans represent 35, 30 and 35% of the observed glycans, respectively. The two most common N-glycan compositions were the complex/**hybrid** Hex(3)HexNAc(4)dHex(4) and the paucimannose structure that contains only the doubly-fucosylated trimannosylchitobiose core Hex(3)HexNAc(2)dHex(2), each appearing in 22 occupied sites (13.8%). These findings enlighten aspects of the glycobiology of lepidopteran midgut microvilli.

Museum identifies genetic erosion in two butterfly species across the 20th century in Finland

Gauthier, J; Pajkovic, M; Neuenschwander, S; Kaila, L; Schmid, S; Orlando, L; Alvarez, N. 2020

Erosion of biodiversity generated by anthropogenic activities has been studied for decades and in many areas at the species level, using taxa monitoring. In contrast, genetic erosion within species has rarely been tracked, and is often studied by inferring past population dynamics from contemporaneous estimators. An alternative to such inferences is the direct examination of past genes, by analysing museum collection specimens. While providing direct access to genetic variation over time, historical DNA is usually not optimally preserved, and it is necessary to apply genotyping methods based on **hybridization**-capture to unravel past genetic variation. In this study, we apply such a method (i.e., HyRAD), to large time series of two butterfly species in Finland, and present a new bioinformatic pipeline, namely PopHyRAD, that standardizes and optimizes the analysis of HyRAD data at the within-species level. In the localities for which the data retrieved have sufficient power to accurately examine genetic dynamics through time, we show that genetic erosion has increased across the last 100 years, as revealed by signatures of allele extinctions and heterozygosity decreases, despite local variations. In one of the two butterflies (*Erebia embla*), isolation by distance also increased through time, revealing the effect of greater habitat fragmentation over time.

Moth versus fly: a preliminary study of the pollination mode of two species of endemic Asteraceae from St Helena (*Commidendrum robustum* and *C. rugosum*) and its conservation implications

Paajanen, MPT; Cronk, Q. 2020

Commidendrum robustum (Roxb.) DC. (St Helena gumwood) and *C. rugosum* (Dryand.) DC. (St Helena scrubwood) are ecologically important, endemic woody Asteraceae from the isolated South Atlantic island of St Helena. Once very abundant, they now exist in sparse fragmented populations due to 500 years of environmental destruction. They are sister taxa that evolved on the island and are reported to **hybridise**. *Commidendrum rugosum* has a saucer-like erect capitulum, whereas *C. robustum* has a somewhat globular hanging capitulum. Using daytime timelapse photography to follow capitula through their life cycle, we found that *C. rugosum* appears to be myophilous, visited largely by flies (including the endemic syrphid, *Sphaerophoria beattiei* Doesburg & Doesburg) and occasionally by Lepidoptera. *Commidendrum robustum*, on the other hand, although visited by flies, strongly attracts moths (especially noted at the Millennium Forest site). Our data suggest that moth visits may reduce visits from flies due to the sensitivity of flies to interference by other insects. We conclude that *C. robustum* may have a mixed syndrome of myophily/phalaenophily and that there is apparently some divergence of the pollination niche between the two species. Its potential in attracting moths, coupled with its former abundance, suggests that it may have been a major food source for adults of the numerous endemic moths. Pollinator activity was measured by insect visitation rates (mean visits per capitulum per day, V) and insect residence time (mean pollinator kiloseconds per capitulum per day, R). Both are higher for *C. robustum* (*C. rugosum*, V = 16.4, R = 3.101; *C. robustum*, V = 34.0, R = 8.274), reflecting the abundance of moths on the capitula at the Millennium Forest site. The conservation implications of the pollination mode are that: (1) there is considerable pollinator activity on the capitula and pollination is not currently a limiting factor for plant reproduction; (2) gene exchange between geographically-isolated populations of *C. rugosum* is likely to be minimal due to the apparent reliance of the species for pollination on small flies (especially *Sphaerophoria beattiei*), which are believed to be not effective as pollinators over long distances (> 1 km). A possible exception is the strong-flying drone-fly, *Eristalis tenax* Linn. which, although not as abundant as *Sphaerophoria*, does visit the flowers; (3) there is considerable overlap between the two species in flower visitors and interspecific pollen transfer is possible where the two species grow intermixed (which has potential positive and negative implications for species survival).

Consequences of leaf biochemical characters for Citrus Leafminer, *Phyllocnistis citrella stainton* (Lepidoptera: Gracillariidae) along the microclimatic gradient of Citrus plants

Abbas, MW; Raza, AM; Dageri, A; Arshad, M; Khan, MA; Ullah, MI; Afzal, M. 2020

The leaf traits and microclimate in different positions of a host plant often influence the host choice and performance of herbivore insects. In the present study, the relation of leaf biochemical characters with the infestation of citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on six citrus cultivars; Meyer Lime (lemon x mandarin **hybrid**), Kinnow, and Feutrell's Early (Citrus mandarins), Fairchild (C. tangerines), Succari and Musambi (C. sinensis) was determined. It was also hypothesized that the CLM damage increases from lower to upper canopy of the plant. The highest level of infestation was observed on the upper canopy of citrus plants, followed by a middle and lower canopy in the case of all citrus cultivars. The CLM damage also varied between six citrus cultivars with the highest level of 24.24% for Meyer Lime and 21.42% for Kinnow cultivars and the least affected cultivar by CLM feeding was Musambi with 9.31% infestation. Among the leaf traits, nitrogen and moisture contents showed significant and positive relation with CLM infestation in all citrus cultivars. Total minerals in leaves didn't show the significant ($P > 0.05$) relation with CLM damage in all citrus cultivars. Crude proteins showed significant relation with CLM only in the case of the Meyer Lime cultivar. Our findings suggest that the moisture level and nitrogen contents in the leaves are important factors for enhancing the activity of CLM and this interaction can impact the growth and productivity of citrus plants.

The Reliability of Genitalia Morphology to Monitor the Spread of the Invasive Winter Moth (Lepidoptera: Geometridae) in Eastern North America

Griffin, BP; Chandler, JL; Andersen, JC; Havill, NP; Elkinton, JS. 2020

Winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae), causes widespread defoliation in both its native and introduced distributions. Invasive populations of winter moth are currently established in the United States and Canada, and pheromone-baited traps have been widely used to track its spread. Unfortunately, a native species, the Bruce spanworm, *O. bruceata* (Hulst), and *O. bruceata* x *brumata* **hybrids** respond to the same pheromone, complicating efforts to detect novel winter moth populations. Previously, differences in measurements of a part of the male genitalia called the uncus have been utilized to differentiate the species; however, the accuracy of these measurements has not been quantified using independent data. To establish morphological cutoffs and estimate the accuracy of uncus-based identifications, we compared morphological measurements and molecular identifications based on microsatellite genotyping. We find that there are significant differences in some uncus measurements, and that in general, uncus measurements have low type I error rates (i.e., the probability of having false positives for the presence of winter moth). However, uncus measurements had high type II error rates (i.e., the probability of having false negatives for the presence of winter moth). Our results show that uncus measurements can be useful for performing preliminary identifications to monitor the spread of winter moth, though for accurate monitoring, molecular methods are still required. As such, efforts to study the spread of winter moth into interior portions of North America should utilize a combination of pheromone trapping and uncus measurements, while maintaining vouchers for molecular identification.

A highly contiguous genome assembly of the bat hawkmoth *Hyles vespertilio* (Lepidoptera: Sphingidae)

Pippel, M; Jebb, D; Patzold, F; Winkler, S; Vogel, H; Myers, G; Hiller, M; Hundsdoerfer, AK. 2020

Background: Adapted to different ecological niches, moth species belonging to the *Hyles* genus exhibit a spectacular diversity of larval color patterns. These species diverged similar to 7.5 million years ago, making this rather young genus an interesting system to study a wide range of questions including the process of speciation, ecological adaptation, and adaptive radiation. Results: Here we present a high-quality genome assembly of the bat hawkmoth *Hyles vespertilio*, the first reference genome of a member of the *Hyles* genus. We generated 51x Pacific Biosciences long reads with an average read length of 8.9 kb. Pacific Biosciences reads longer than 4 kb were assembled into contigs, resulting in a 651.4-Mb assembly consisting of 530 contigs with an N50 value of 7.5 Mb. The circular mitochondrial contig has a length of 15,303 bp. The *H. vespertilio* genome is very repeat-rich and exhibits a higher repeat content (50.3%) than other Bombycoidea species such as *Bombyx mori* (45.7%) and *Manduca sexta* (27.5%). We developed a comprehensive gene annotation workflow to obtain consensus gene models from different evidence including gene projections, protein homology, transcriptome data, and ab initio predictions. The resulting gene annotation is highly complete with 94.5% of BUSCO genes being completely present, which is higher than the BUSCO completeness of the *B. mori* (92.2%) and *M. sexta* (90%) annotations. Conclusions: Our gene annotation strategy has general applicability to other genomes, and the *H. vespertilio* genome provides a valuable molecular resource to study a range of questions in this genus, including phylogeny, incomplete lineage sorting, speciation, and **hybridization**. A genome browser displaying the genome, alignments, and annotations is available at <https://genome-public.pks.mpg.de/cgi-bin/hgTracks?db=HLhylVes1>.

Responses of stemborer *Chilo partellus* to volatiles emitted by maize landraces exposed to signal grass (*Brachiaria brizantha*)

Magara, HJO; Mutyambai, DM; Charles, MAO; Otieno, SA; Nyaga, TM; Niassy, S; Khan, ZR. 2020

This study sought to evaluate the oviposition responses of *Chilo partellus* (Lepidoptera: Crambidae) on maize plants exposed to *Brachiaria brizantha* Stapf following oviposition by *C. partellus* and nonexposed maize. Two Kenyan maize landraces (Jowi and Nyamula), Latin America landrace (Cuba 91) and WH505 (**hybrid** variety) (control) were studied. The result demonstrated that *C. partellus* deposited a significantly lower number of eggs and egg batches on Nyamula, Jowi and Cuba 91 maize plants exposed to *B. brizantha* than *C. partellus* eggs compared to nonexposed ones and the exposed **hybrid** variety. This was because they emitted electrophysiologically active compounds such as (E)-4, 8-dimethyl-1, 3, 7-nonatriene, decanal, (E)-caryophyllene, linalool, linalool (plus nananal), E-beta-fernesene, methyl salicylate and (3E,7E)-4, 8, 12-trimethyl-1, 3, 7, 11-tri-decatetraene that deterred *C. partellus* from ovipositing more eggs on these plants. Therefore, herbivore-induced plant volatiles (HIPVs) of *B. brizantha* can be employed to protect the maize crop against *C. partellus*.

A new target capture phylogeny elucidates the systematics and evolution of wing coupling in sack-bearer moths

St Laurent, RA; Mielke, CGC; Herbin, D; Dexter, KM; Kawahara, AY. 2020

The frenulum is a wing coupling structure that is found on the wings of most families of Lepidoptera. It is a single bristle or set of bristles that originate from the base of the hindwing that often interlocks with the forewing during flight. This wing coupling mechanism is thought to have been a major evolutionary innovation that allowed for enhanced flight in Lepidoptera. The sack-bearer moths (Mimallonidae) are unusual among Lepidoptera in that not all species within the family have a frenulum. We test the hypothesis that the frenulum is not necessary and is therefore lost in mimallonids that have longer male forewings, because such wings are perhaps better suited to be coupled by other means. To understand the evolution of the frenulum, we inferred the most taxonomically and genetically sampled anchored **hybrid** enrichment-based phylogeny of Mimallonidae, including 604 loci from all 41 genera and from 120 species, covering about 40% of the described species in the family. The maximum likelihood tree robustly supports major relationships within the family, and ancestral state reconstruction clearly recovers the frenulum as the plesiomorphic condition in Mimallonidae. Our results show that the frenulum is more often observed in species that have shorter, rather than longer, male forewings. The frenulum has historically been used as an important character for intrafamilial classification in Mimallonidae, but our results conclusively show that this character system is more variable than previously thought. Based on our results, we erect two new subfamilies, Roelofinae St Laurent & Kawahara, subfam.n. and Meneviinae St Laurent, Herbin, & Kawahara, subfam.n., for four genera previously considered incertae sedis. In the predominantly frenulum-lacking clade Cicinninae, we describe a new genus, Cerradocinnus St Laurent, Mielke, & Kawahara, gen.n., and the genus Gonogramma stat. rev. is revalidated to include many species previously placed in Cicinnus sensu lato. With these changes, Cicinnus can now be considered monophyletic. Thirty-three species are transferred to Gonogramma from Cicinnus sensu lato. This published work has been registered on Zoobank, .

Cold adaptation across the elevation gradient in an alpine butterfly species complex

Neve, G; Despres, L. 2020

1. Temperature acts as a major factor on the timing of activity and behaviour in butterflies, and it might represent a key driver of butterfly diversification along elevation gradients. Under this hypothesis, local adaptation should be found along the elevation gradient, with butterflies from high elevation populations able to remain active at lower ambient temperature than those from low elevation. 2. The warming-up rate and the thoracic temperature at take-off of 123 individuals of the Alpine butterfly species complex *Coenonympha arcania* - *C. macromma* - *C. gardetta* were recorded in controlled conditions. 3. Warming-up rate increased with elevation in *C. arcania*: high-elevation males of *C. arcania* were able to warm up more quickly compared to low-elevation ones. 4. High-elevation *C. gardetta* had a darker underwing pattern than low-elevation ones. This high-elevation species was significantly smaller (lower weight and wing surface) than the two other species and had a faster warming-up rate. 5. This study's results suggest that the ability to warm up quickly and to take flight at a high body temperature evolved adaptively in the high-altitude *C. gardetta* and that low temperature at high altitude may explain the absence of *C. arcania*, while the **hybrid** nature of *C. macromma* is probably the explanation of its elevation overlap with both the other species and its local replacement of *C. gardetta*.
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A Toll-Spatzle Pathway in the Immune Response of *Bombyx mori*

Yu, B; Sang, Q; Pan, GQ; Li, CF; Zhou, ZY. 2020

Simple Summary The Toll-Spatzle pathway is a crucial defense mechanism in insect innate immunity through inducing the expression of antimicrobial peptides (AMPs) against pathogens. As an important economical insect, *Bombyx mori* (*B. mori*) has been used as an insect model of Lepidoptera. However, the recognition of the Toll-Spatzle pathway in silkworm is very limited. In this study, we reported that *B. mori* Spatzle2 (*BmSpz2*) could be activated by pathogens. The activated *BmSpz2* could bind with *B. mori* Toll11 (*BmToll11*) or *B. mori* Toll9-1 (*BmToll9-1*). Further investigations showed that overexpression of *BmToll11* or *BmToll9-1* could significantly upregulate AMPs expression. The findings of this study confirmed that a Toll-Spz pathway regulated immune response to microbial infection in *B. mori* and may help improve the understanding of the signaling pathways involved in the innate immune responses of Lepidoptera insect. The Toll-Spatzle pathway is a crucial defense mechanism in insect innate immunity, it plays an important role in fighting against pathogens through the regulation of antimicrobial peptide gene expression. Although Toll and Spatzle (*Spz*) genes have been identified in *Bombyx mori*, little is known regarding the specific *Spz* and Toll genes members involved in innate immunity. There is also limited direct evidence of the interaction between *Spz* and Toll. In this study, the dual-luciferase reporter assay results showed that *BmToll11* and *BmToll9-1* could activate both drosomycin and dipterin promoters in S2 cells. Furthermore, *BmToll11*, *BmToll9-1*, and five *BmSpz* genes were found to be significantly upregulated in *B. mori* infected by *Escherichia coli* and *Staphylococcus aureus*. Additionally, the yeast two-**hybrid** assay results confirmed that *BmSpz2*, but not other *BmSpz*s, could interact with both *BmToll11* and *BmToll9-1*. These findings suggest that the activated *BmSpz2* can bind with *BmToll11* and *BmToll9-1* to induce the expression of AMPs after the silkworm is infected by pathogens.

De Novo Genome Assembly of the Meadow Brown Butterfly, *Maniola jurtina*

Singh, KS; Hosken, DJ; Wedell, N; French-Constant, R; Bass, C; Baxter, S; Paszkiewicz, K; Sharma, MD. 2020

Meadow brown butterflies (*Maniola jurtina*) on the Isles of Scilly represent an ideal model in which to dissect the links between genotype, phenotype and long-term patterns of selection in the wild - a largely unfulfilled but fundamental aim of modern biology. To meet this aim, a clear description of genotype is required. Here we present the draft genome sequence of *M. jurtina* to serve as a founding genetic resource for this species. Seven libraries were constructed using pooled DNA from five wild caught spotted females and sequenced using Illumina, PacBio RSII and MinION technology. A novel **hybrid** assembly approach was employed to generate a final assembly with an N50 of 214 kb (longest scaffold 2.9 Mb). The sequence assembly described here predicts a gene count of 36,294 and includes variants and gene duplicates from five genotypes. Core BUSCO (Benchmarking Universal Single-Copy Orthologs) gene sets of Arthropoda and Insecta recovered 90.5% and 88.7% complete and single-copy genes respectively. Comparisons with 17 other Lepidopteran species placed 86.5% of the assembled genes in orthogroups. Our results provide the first high-quality draft genome and annotation of the butterfly *M. jurtina*.

Multiplex PCR for identification of two butterfly sister species: *Eurema mandarina* and *Eurema hecabe*

Miyata, MN; Kageyama, D; Nomura, M. 2020

In insects, closely related species are often difficult or impossible to distinguish solely by morphological traits. Mitochondrial DNA (mtDNA) markers are often useful and reliable for distinguishing closely related species. However, useful mtDNA markers can be unavailable, particularly when such species pairs experienced **hybrid** introgression in the past. Although polymorphic nuclear DNA markers would be necessary to distinguish such species pairs, recombination, multiple copies, and slower mutation rates of the nuclear DNA compared with those of mtDNA often make it challenging. The objective of this study was to develop a multiplex polymerase chain reaction that can reliably amplify and distinguish the *Tpi* sequences of *Eurema mandarina* and *Eurema hecabe*. Results We successfully analyzed the nucleotide sequences of the Z chromosome-linked triose phosphate isomerase (*Tpi*) gene to develop a multiplex polymerase chain reaction (PCR) that amplified ca. 120-bp products for *E. mandarina* and ca. 375-bp products for *E. hecabe*. We suggest that multiplex PCR using *Tpi* with appropriately designed primers can be used to accurately and reliably distinguish between other closely related Lepidoptera species.

Comparative mitochondrial genome analysis of *Dendrolimus houi* (Lepidoptera: Lasiocampidae) and phylogenetic relationship among Lasiocampidae species

Han, XH; He, H; Shen, HY; Tang, JH; Dong, WY; Shi, YF; Wu, SQ; Zhang, FP; Liang, GH. 2020

Dendrolimus houi is one of the most common caterpillars infesting Gymnosperm trees, and widely distributed in several countries in Southeast Asia, and exists solely or coexists with several congeners and some Lasiocampidae species in various forest habitats. However, natural **hybrids** occasionally occur among some closely related species in the same habitat, and host preference, extreme climate stress, and geographic isolation probably lead to their uncertain taxonomic consensus. The mitochondrial DNA (mtDNA) of *D. houi* was extracted and sequenced by using high-throughput technology, and the mitogenome composition and characteristics were compared and analyzed of these species, then the phylogenetic relationship was constructed using the maximum likelihood method (ML) and the Bayesian method (BI) based on their 13 protein-coding genes (PCGs) dataset, which were combined and made available to download which were combined and made available to download among global Lasiocampidae species data. Mitogenome of *D. houi* was 15,373 bp in length, with 37 genes, including 13 PCGs, 22 tRNA genes (tRNAs) and 2 rRNA genes (rRNAs). The positions and sequences of genes were consistent with those of most known Lasiocampidae species. The nucleotide composition was highly A+T biased, accounting for similar to 80% of the whole mitogenome. All start codons of PCGs belonged to typical start codons ATN except for COI which used CGA, and most stop codons ended with standard TAA or TAG, while COI, COII, ND4 ended with incomplete T. Only tRNA(Ser (AGN)) lacked DHU arm, while the remainder formed a typical "clover-shaped" secondary structure. For Lasiocampidae species, their complete mitochondrial genomes ranged from 15,281 to 15,570 bp in length, and all first genes started from trnM in the same direction. And base composition was biased toward A and T. Finally, both two methods (ML and BI) separately revealed that the same phylogenetic relationship of *D. spp.* as ((((*D. punctatus* + *D. tabulaeformis*) + *D. spectabilis*) + *D. superans*) + (*D. kikuchii* of Hunan population + *D. houi*) as in previous research, but results were different in that *D. kikuchii* from a Yunnan population was included, indicating that different geographical populations of insects have differentiated. And the phylogenetic relationship among Lasiocampidae species was ((((*Dendrolimus*) + *Kunugia*) + *Euthrix*) + *Trabala*). This provides a better theoretical basis for Lasiocampidae evolution and classification for future research directions.

Comparative efficacy of gypsy moth (Lepidoptera: Erebidiae) entomopathogens on transgenic blight-tolerant and wild-type American, Chinese, and hybrid chestnuts (Fagales: Fagaceae)

Brown, AJ; Newhouse, AE; Powell, WA; Parry, D. 2020

American chestnut (*Castanea dentata* [Marsh.] Borkh.) was once the dominant hardwood species in Eastern North America before an exotic fungal pathogen, *Cryphonectria parasitica* (Murrill) Barr, functionally eliminated it across its range. One promising approach toward restoring American chestnut to natural forests is development of blight-tolerant trees using genetic transformation. However, transformation and related processes can result in unexpected and unintended phenotypic changes, potentially altering ecological interactions. To assess unintended tritrophic impacts of transgenic American chestnut on plant-herbivore interactions, gypsy moth (*Lymantria dispar* L.) caterpillars were fed leaf disks excised from two transgenic events, Darling 54 and Darling 58, and four control American chestnut lines. Leaf disks were previously treated with an LD50 dose of either the species-specific *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) or the generalist pathogen *Bacillus thuringiensis* subsp. *kurstaki* (Btk). Mortality was quantified and compared to water blank controls. Tree genotype had a strong effect on the efficacies of both pathogens. Larval mortality from Btk-treated foliage from only one transgenic event, Darling 54, differed from its isogenic progenitor, Ellis 1, but was similar to an unrelated wild-type American chestnut control. LdMNPV efficacy was unaffected by genetic transformation. Results suggest that although genetic modification of trees may affect interactions with other nontarget organisms, this may be due to insertion effects, and variation among different genotypes (whether transgenic or wild-type) imparts a greater change in response than transgene presence.

Invasions of insect pests and fungal pathogens of woody plants into the northwestern part of European Russia

Selikhovkin, AV; Drenkhan, R; Mandelshtam, MY; Musolin, DL. 2020

Invasions of insects and fungi is a serious problem for the existence of woody plants in the northwest of the European part of Russia. The following species of moths (Lepidoptera: Gracillariidae) that produce mines in the leaves of woody plants recently arrived in the region: *Phyllonorycter issikii* (feeding on lime), *Cameraria ohridella* (feeding on chestnut), and, likely, *Acrocercops brongniardella* (feeding on oak). Increasing average monthly temperatures during the growing season is a favorable factor which can promote the spread of pests and pathogens and increase their population density. The particularly warm season of 2018 likely contributed to the noticeable increase in the population density of the invasive mining moths as well as the adventive poplar mining moth *Phyllonorycter populifoliella*. Spreading species of stem-boring and bark beetles, as well as diseases associated with them, might be particularly dangerous; in particular, *Scolytus* spp. (Coleoptera: Curculionidae: Scolytinae) are involved in spreading Dutch elm disease (caused by *Ophiostoma novo-ulmi*). It has been demonstrated that **hybrids** of *Oph. novo-ulmi* are spread in the region and can be highly pathogenic. Emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae) is another serious potential aggressive invader. The northwestern border of its invasive range is currently in the environs of the city of Tver, but its arrival by highways, with transport or planting materials to the northwest of the European part of Russia is likely. The recent invasion of the ascomycete *Hymenoscyphus fraxineus* has already led to a noticeable deterioration of the condition of ash trees in Saint Petersburg and the Leningrad Region. The combined effect of the buprestid beetle *A. planipennis* and fungus *El. fraxineus* can have fatal consequences for ash. It is necessary to continue monitoring invasive species range dynamics and studying their adaptation to local conditions and the interaction of invasive insects with local and invasive woody plant pathogenic organisms.

Abundance of the Sugarcane Borer (Lepidoptera: Crambidae) and Foraging Ants (Hymenoptera: Formicidae) in Sugarcane Grown on Organic and Mineral Soils in Florida

Roldan, EL; Beuzelin, JM; VanWeelden, MT; Cherry, RH. 2020

A study was conducted in Florida to determine sugarcane borer, *Diatraea saccharalis* (F.), injury and infestation levels in sugarcane (*Saccharum* spp. **hybrids**), *D. saccharalis* parasitism rates, and ant foraging activity in 32 commercial fields as affected by soil type (shallow organic vs deep organic vs mineral). In 2017 and 2018, each field was sampled four times during the summer for *D. saccharalis* by inspecting 100 sugarcane stalks and for foraging ants using plastic tubes baited with hot dog at 12 locations. One non-parasitized *D. saccharalis* larva was collected in 2017 and in 2018 out of 12,100 and 12,600 stalks sampled, respectively. Additional sampling of 50 stalks per field in October showed that 0.6% (2017) and 0.1% (2018) of the sugarcane stalks had bored internodes, and one *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) cocoon mass was observed. Seven ant species foraged in sugarcane fields, including the red imported fire ant, *Solenopsis invicta* Buren, which was the most abundant ant. *Solenopsis invicta* was not affected by soil type; however, sugarcane fields on shallow organic soils might represent a more suitable environment. The third most abundant foraging ant, *Nylanderia bourbonica* (Forel), was more abundant in mineral soil fields than in shallow and deep organic soil fields. Results suggest that *D. saccharalis* population levels in Florida sugarcane are extremely low under current production conditions regardless of soil type. In addition, the observation of *C. flavipes*, *S. invicta*, and six other ant species suggest that biological control contributes to these low *D. saccharalis* population levels.

Identification of Natural Hybrids between *Ahlbergia frivaldszkyi* (Lederer, 1853) and *Callophrys rubi* (Linnaeus, 1758) (Lepidoptera, Lycaenidae) Using Mitochondrial and Nuclear Markers

Shapoval, NA; Yakovlev, RV; Kuftina, GN; Lukhtanov, VA; Knyazev, SA; Romanovich, AE; Krupitsky, AV. 2021

Simple Summary Butterfly specimens with unusual morphological characters (e.g., unusual wing coloration) have contradictory interpretations in the literature and have been considered by different authors either as previously undescribed taxa, putative **hybrids**, or aberrations of well-known species. Such individuals clearly represent a taxonomic problem that needs to be addressed by scientists. The application of molecular techniques could shed light on the origin of morphological uncertainty. Here we use a combination of mitochondrial and nuclear DNA markers to analyze three lycaenid butterflies with unusual wing pattern, which are thought to represent naturally occurring **hybrids** due to their intermediate phenotype. We confirm their **hybrid** origin and indicate that the specimens are wild-caught **hybrids** between females of *Callophrys rubi* and males of *Ahlbergia frivaldszkii*. Our data indicate that gene flow across species boundaries in these butterflies can occur long after speciation. Natural **hybridization** is rather widespread and common in animals and can have important evolutionary consequences. In terms of taxonomy, exploring **hybridization** and introgression is crucial in defining species boundaries and testing taxonomic hypotheses. In the present paper, we report on natural **hybrid** specimens between *Ahlbergia frivaldszkii* (Lederer, 1853) and *Callophrys rubi* (Linnaeus, 1758). To test the hypothesis of their **hybrid** origin, we employed the molecular mitochondrial (COI gene) and nuclear (wingless, RPS5, and Ca-ATPase genes) markers commonly used in phylogenetic studies and explored the morphology of the specimens. Our analysis revealed that **hybrids** bear mitochondrial haplotypes of *C. rubi*, while nuclear fragments are heterozygous, sharing a combination of *A. frivaldszkii* and *C. rubi* lineages. The **hybrid** specimens combine morphological characters of both genera. Our results for the first time empirically demonstrate the possibility of genetic introgression between these species and between the genera *Callophrys* and *Ahlbergia* on the whole.

Genomic introgression from a distant congener in the Levant fritillary butterfly, *Melitaea acentria*

Pazhenkova, EA; Lukhtanov, VA. 2021

Introgressive **hybridization** is more common in nature than previously thought, and its role and creative power in evolution is hotly discussed but not completely understood. Introgression occurs more frequently in sympatry between recently diverged taxa, or when the speciation process has not yet been completed. However, there are relatively few documented cases of **hybridization** that erodes reproductive barriers between distantly related species. Here, we use whole genome and mitochondrial data to examine how introgression from a distant congener affects pattern of genetic differentiation in the Levant fritillary butterfly *Melitaea acentria*. We show that this local taxon has evolved as a peripatric geographic isolate of the widespread *Melitaea perseia*, and that there has been significant unidirectional gene flow from the sympatric, nonclosely related *Melitaea didyma* to *M. acentria*. We found direct evidence of ongoing sporadic **hybridization** between *M. didyma* and *M. acentria*, which are separated by at least 5 million years of independent evolution. Elevated differentiation and lower level of introgression on the sex Z chromosome compared to autosomes suggest that the Z chromosome has accumulated loci acting as intrinsic postzygotic barriers. Our results show that introgression from *M. didyma* has been an additional source of nucleotide diversity in the *M. acentria* population, providing material for drift and selection.

First report of *Tetracona (Agrotera) amathealis* (Walker, 1859) on a *Eucalyptus grandis* W. Hill x *Eucalyptus pellita* F. Muell. (Myrtaceae) hybrid in Indonesia (Lepidoptera: Crambidae, Spilomelinae)

Melia, T; Sinulingga, NGHB; Maretha, MV; Wijaya, R; Efendi, RR; Oliveira, LSS; Kkadan, SK; Tavares, WD; Tarigan, M; Duran, A. 2021

Tetracona amathealis (Walker, 1859) (Lepidoptera: Crambidae) is previously known from Australia and New Guinea, where it feeds on the native *Eucalyptus tereticornis* Sm. (Myrtaceae). The objective of this study was the report, for the first time, *T. amathealis* in Indonesia as well as to record new host plants for this insect. Were collected manually from a *Eucalyptus grandis* W. Hill. x *Eucalyptus pellita* F. Muell. **hybrid** commercial plantation in Riau, Sumatra, Indonesia and a sample sent for identification via molecular analysis. Our findings revealed that the molecular approach used (through sequencing of its mt-COI gene) successfully allowed the identification of the species as *T. amathealis*. This insect is recorded for the first time in Indonesia on a new host, a *E. grandis* x *E. pellita* **hybrid**.

Food Plant Shifts Drive the Diversification of Sack-Bearer Moths

St Laurent, RA; Carvalho, APS; Earl, C; Kawahara, AY. 2021

Lepidoptera are a highly diverse group of herbivorous insects; however, some superfamilies have relatively few species. Two alternative hypotheses for drivers of Lepidoptera diversity are shifts in food plant use or shifts from concealed to external feeding as larvae. Many studies address the former hypothesis but with bias toward externally feeding taxa. One of the most striking examples of species disparity between sister lineages in Lepidoptera is between the concealed-feeding sack-bearer moths (Mimallonioidea), which contain about 300 species, and externally feeding Macroheterocera, which have over 74,000 species. We provide the first dated tree of Mimallonidae to understand the diversification dynamics of these moths in order to fill a knowledge gap pertaining to drivers of diversity within an important concealed-feeding clade. We find that Mimallonidae is an ancient Lepidoptera lineage that originated in the Cretaceous similar to 105 million years ago and has had a close association with the plant order Myrtales for the past 40 million years. Diversification dynamics are tightly linked with food plant usage in this group. Reliance on Myrtales may have influenced diversification of Mimallonidae because clades that shifted away from the ancestral condition of feeding on Myrtales have the highest speciation rates in the family.

Characterization of sensory neuron membrane proteins (SNMPs) in cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae)

Xu, W; Zhang, HJ; Liao, YL; Papanicolaou, A. 2021

Sensory neuron membrane proteins (SNMPs) play a critical role in insect chemosensory system. Previously, three SNMPs were identified, characterized and functionally investigated in a lepidopteran model insect, *Bombyx mori*. However, whether these results are consistent across other lepidopteran species are unknown. Here genome and transcriptome data analysis, expression profiling, quantitative real-time PCR (qRT-PCR) and the yeast **hybridization** system were utilized to examine snmp genes of *Helicoverpa armigera*, one of the most destructive lepidopteran pests in cropping areas. In silico expression and qRT-PCR analyses showed that, just as the *B. mori* snmp genes, *H. armigera* snmp1 (*Harmsnmp1*) is specifically expressed in adult antennae. *Harmsnmp2* is broadly expressed in multiple tissues including adult antennae, tarsi, larval antennae and mouthparts. *Harmsnmp3* is specifically expressed in larval midguts. Further RNAseq analysis suggested that the expression levels of *Harmsnmp2* and *Harmsnmp3* differed significantly depending on the plant species on which the larvae fed, indicating they may be involved in plant-feeding behaviours. Yeast **hybridization** results revealed a protein-protein interaction between *HarmSNMP1* and the sex pheromone receptor, *HarmOR13*. This study demonstrated that SNMPs may share same functions and mechanisms in different lepidopteran species, which improved our understanding of insect snmp genes and their functions in lepidopterans.

Congruence between morphology-based species and Barcode Index Numbers (BINs) in Neotropical Eumaeini (Lycaenidae)

Prieto, C; Faynel, C; Robbins, R; Hausmann, A. 2021

Background: With about 1,000 species in the Neotropics, the Eumaeini (Theclinae) are one of the most diverse butterfly tribes. Correct morphology-based identifications are challenging in many genera due to relatively little interspecific differences in wing patterns. Geographic infraspecific variation is sometimes more substantial than variation between species. In this paper we present a large DNA barcode dataset of South American Lycaenidae. We analyze how well DNA barcode BINs match morphologically delimited species. Methods: We compare morphology-based species identifications with the clustering of molecular operational taxonomic units (MOTUs) delimited by the RESL algorithm in BOLD, which assigns Barcode Index Numbers (BINs). We examine intra- and interspecific divergences

for genera represented by at least four morphospecies. We discuss the existence of local barcode gaps in a genus by genus analysis. We also note differences in the percentage of species with barcode gaps in groups of lowland and high mountain genera. Results: We identified 2,213 specimens and obtained 1,839 sequences of 512 species in 90 genera. Overall, the mean intraspecific divergence value of CO1 sequences was 1.20%, while the mean interspecific divergence between nearest congeneric neighbors was 4.89%, demonstrating the presence of a barcode gap. However, the gap seemed to disappear from the entire set when comparing the maximum intraspecific distance (8.40%) with the minimum interspecific distance (0.40%). Clear barcode gaps are present in many genera but absent in others. From the set of specimens that yielded COI fragment lengths of at least 650 bp, 75% of the a priori morphology-based identifications were unambiguously assigned to a single Barcode Index Number (BIN). However, after a taxonomic a posteriori review, the percentage of matched identifications rose to 85%. BIN splitting was observed for 17% of the species and BIN sharing for 9%. We found that genera that contain primarily lowland species show higher percentages of local barcode gaps and congruence between BINs and morphology than genera that contain exclusively high montane species. The divergence values to the nearest neighbors were significantly lower in high Andean species while the intra-specific divergence values were significantly lower in the lowland species. These results raise questions regarding the causes of observed low inter and high intraspecific genetic variation. We discuss incomplete lineage sorting and **hybridization** as most likely causes of this phenomenon, as the montane species concerned are relatively young and **hybridization** is probable. The release of our data set represents an essential baseline for a reference library for biological assessment studies of butterflies in mega diverse countries using modern high-throughput technologies and highlights the necessity of taxonomic revisions for various genera combining both molecular and morphological data.

A global comparison of DNA sequences of *Pelopidas* (Lepidoptera: Hesperiidae) reveals discordance between morphological and genetic data, and an insular 'ghost' population

Gooneseckera, K; Lee, PLM; Van der Poorten, G; Ranawaka, G. 2021

The *Pelopidas* are a widely distributed genus of hesperiids ranging from the Afrotropical to Australasia. Based on traditional classification, four species are recorded from Sri Lanka: *Pelopidas agna*, *P. mathias*, *P. subochracea* and *P. conjuncta*. The aim is to test the taxonomic hypotheses of the Sri Lankan *Pelopidas* as they are currently recognised based on morphology. We achieve this by analysing the phylogenetic relationships among the *Pelopidas* as well as between the *Pelopidas* and tribe Baorini both inside and outside of Sri Lanka. We used DNA sequence data from the cytochrome oxidase c subunit I (COI) "barcode" region and two nuclear DNA regions, elongation factor 1- α (EF1- α) and wingless (Wg1) collected for Sri Lankan *Pelopidas* and combined with all available *Pelopidas* sequence data from public databases. The reconstructed phylogeny showed that *P. mathias*, *P. agna* and *P. conjuncta* were not monophyletic. Additionally, *P. agna* in Sri Lanka may now be a **hybrid** population that retains the *P. agna* morphology but possesses the mitochondrial DNA of *P. mathias* (termed a 'ghost' population). Finally, two lineages of *P. conjuncta* were identified in Sri Lanka, appearing more closely related to other species of *Pelopidas* than to each other. This study demonstrates the phylogenetic complexity within the *Pelopidas* and the uniqueness of the Sri Lankan *Pelopidas* that have not been previously appreciated. This new knowledge is important in planning conservation management and may also guide future work on the potential mechanisms of evolutionary diversification of island butterflies.

***Cotesia cassina* sp. nov. from southwestern Colombia: a new gregarious microgastrine wasp (Hymenoptera, Braconidae) reared from the pest species *Opsiphanes cassina* Felder & Felder (Lepidoptera, Nymphalidae) feeding on *Elaeis oil palm trees* (Arecaceae)**

Salgado-Neto, G; Vasquez, CAN; Max, DS; Whitfield, JB. 2021

A new species of microgastrine wasp, *Cotesia cassina* Salgado-Neto, Vasquez & Whitfield, sp. nov., is described from southwestern Colombia in Tumaco, Narino. This species is a koinobiont gregarious larval endoparasitoid, and spins a common mass of cocoons underneath the host caterpillars of *Opsiphanes cassina* (Felder & Felder) (Lepidoptera, Nymphalidae), feeding on oil palm trees (interspecific **hybrid** *Elaeis oleifera* x *E. guineensis*) (Arecaceae). While superficially similar, both morphologically and biologically, to *C. invirae* Salgado-Neto & Whitfield from southern Brazil, the two species are distinct based on DNA barcodes, host species, geographical range and morphological characters.

Clustering of loci controlling species differences in male chemical bouquets of sympatric *Heliconius* butterflies

Byers, KJRP; Darragh, K; Garza, SF; Almeida, DA; Warren, IA; Rastas, PMA; Merrill, RM; Schulz, S; McMillan, WO; Jiggins, CD. 2021

The degree to which loci promoting reproductive isolation cluster in the genome—that is, the genetic architecture of reproductive isolation—can influence the tempo and mode of speciation. Tight linkage between these loci can facilitate speciation in the face of gene flow. Pheromones play a role in reproductive isolation in many Lepidoptera species, and the role of endogenously produced compounds as secondary metabolites decreases the likelihood of pleiotropy associated with many barrier loci. *Heliconius* butterflies use male sex pheromones to both court females (aphrodisiac wing pheromones) and ward off male courtship (male-transferred antiaphrodisiac genital pheromones), and it is likely that these compounds play a role in reproductive isolation between *Heliconius* species. Using a set of backcross **hybrids** between *H. melpomene* and *H. cydno*, we investigated the genetic architecture of putative male pheromone compound production. We found a set of 40 significant quantitative trait loci (QTL) representing 33 potential pheromone compounds. QTL clustered significantly on two chromosomes, chromosome 8 for genital compounds and chromosome 20 for wing compounds, and chromosome 20 was enriched for potential pheromone biosynthesis genes. There was minimal overlap between pheromone QTL and known QTL for mate choice and color pattern. Nonetheless, we did detect linkage between a QTL for wing androconial area and *optix*, a color pattern locus known to play a role in reproductive isolation in these species. This tight clustering of putative pheromone loci might contribute to coincident reproductive isolating barriers, facilitating speciation despite ongoing gene flow.

Hybrid effects in field populations of the African monarch butterfly, *Danaus chrysippus* (L.) (Lepidoptera: Nymphalidae)

Smith, DAS; Bennie, JJ; Gordon, IJ; Martin, S; Ileri, P; Omufwoko, KS; French-Constant, RH. 2021

Heterosis, Haldane and Bateson-Dobzhansky-Muller effects have been widely documented amongst a range of plants and animals. However, typically these effects are shown by taking parents of known genotype into the laboratory and measuring components of the F₁ progeny under laboratory conditions. This leaves in doubt the real significance of such effects in the field. Here we use the well-known colour pattern genotypes of the African monarch or queen (*Danaus chrysippus*), which also control wing length, to test these effects both in the laboratory and in a contact zone in the field. By measuring the wing lengths in animals of known colour pattern genotype we show clear evidence for all three **hybrid** effects at the A and BC colour patterning loci, and importantly, that these same effects persist in the same presumptive F₁s when measured in **hybrid** populations in the field. This demonstrates the power of a system in which genotypes can be directly inferred in the field and highlights that all three **hybrid** effects can be seen in the East African contact zone of this fascinating butterfly.

Comparative transcriptome analysis at the onset of speciation in a mimetic butterfly—The Ithomiini *Melinaea marsaeus*

Piron-Prunier, F; Persyn, E; Legeai, F; McClure, M; Meslin, C; Robin, S; Alves-Carvalho, S; Mohammad, A; Blugeon, C; Jacquin-Joly, E; Montagne, N; Elias, M; Gauthier, J. 2021

Ecological speciation entails divergent selection on specific traits and ultimately on the developmental pathways responsible for these traits. Selection can act on gene sequences but also on regulatory regions responsible for gene expression. Mimetic butterflies are a relevant system for speciation studies because wing colour

pattern (WCP) often diverges between closely related taxa and is thought to drive speciation through assortative mating and increased predation on **hybrids**. Here, we generate the first transcriptomic resources for a mimetic butterfly of the tribe Ithomiini, *Melinaea marsaeus*, to examine patterns of differential expression between two subspecies and between tissues that express traits that likely drive reproductive isolation; WCP and chemosensory genes. We sequenced whole transcriptomes of three life stages to cover a large catalogue of transcripts, and we investigated differential expression between subspecies in pupal wing discs and antennae. Eighteen known WCP genes were expressed in wing discs and 115 chemosensory genes were expressed in antennae, with a remarkable diversity of chemosensory protein genes. Many transcripts were differentially expressed between subspecies, including two WCP genes and one odorant receptor. Our results suggest that in *M. marsaeus* the same genes as in other mimetic butterflies are involved in traits causing reproductive isolation, and point at possible candidates for the differences in those traits between subspecies. Differential expression analyses of other developmental stages and body organs and functional studies are needed to confirm and expand these results. Our work provides key resources for comparative genomics in mimetic butterflies, and more generally in Lepidoptera.

Combining modelling, field data and genetic variation to understand the post-reintroduction population genetics of the Marsh Fritillary butterfly (*Euphydryas aurinia*)

Davis, ML; Barker, C; Powell, I; Porter, K; Ashton, P. 2021

The Marsh Fritillary butterfly (*Euphydryas aurinia*) is a Eurasian species which has suffered significant reductions in occurrence and abundance over the past century, particularly across the western side of its range, due to agricultural intensification and habitat loss. This loss has been particularly severe in the UK with extensive localised extinctions. Following sympathetic management, reintroduction was undertaken at four Cumbria (northern UK) sites in 2007 with stock from a captive admixture population descended from Cumbrian and Scottish founders. Annual population monitoring of the reintroductions was undertaken. Nine years post-reintroduction, the level of population genetic variation was assessed using microsatellites. Variation in historical Cumbrian samples was determined using museum samples and Scottish samples from current populations were assayed to characterise natural population variation. Half of the Scottish sites also served as indicators of the alleles present in the founder populations. The genetic contribution of the founder populations allied to population size data allowed patterns of genetic variation to be modelled. Alleles from Cumbrian and Scottish founders are present in the reintroduced populations. The four sites have levels of variation akin to natural populations and exhibit differentiation as predicted by statistical modelling and comparable with natural populations. This suggests that reintroduction following captive breeding can produce self-sustaining populations with natural levels of genetic diversity. These populations appear to be undergoing the same evolutionary dynamics with bottlenecks and drift as natural populations. Implications for insect conservation Reintroduction of captive bred individuals is a viable strategy for producing populations with natural levels of genetic diversity and evolutionary dynamics. Hybridisation of populations on the brink of extinction with those thriving can preserve some of the genetic distinctiveness of the declining population.

The intraspecific variability of *Colias croceus* (Geoffroy, 1785) and *C. erate* (Esper, 1805) (Lepidoptera, Pieridae) from the perspective of comparative morphology

Dzurinka, M; Mutanen, M; Semelakova, M; Csanady, A; Mikitova, B; Panigaj, L. 2021

The sibling species *Colias croceus* and *Colias erate* show significant intraspecific variability, presumably resulting from frequent **hybridization**. For this reason, some specimens are not clearly identifiable. The yellow forms of males and the white forms of females are particularly problematic in terms of their identification. In this study, we investigated the reliability of external features for effective identification of this species pair using geometric morphometrics. The study specimens were tentatively identified to the species level based on genital characteristics and several genetic markers. Geometric morphometric analyses were focused on the variability of wings and male genital valvae of Slovak butterfly populations. The morphometric analysis of valvae enabled identification of most of the specimens, whereas based on the putatively diagnostic features of forewings and hindwings, several butterflies were incorrectly assigned to a species. Contrary to earlier observations, we observed that androconial scales are also present in some forms of *C. erate*.

Genome and transcriptome analysis of the beet armyworm *Spodoptera exigua* reveals targets for pest control

Simon, S; Breeschoten, T; Jansen, HJ; Dirks, RP; Schranz, ME; Ros, VID. 2021

The genus *Spodoptera* (Lepidoptera: Noctuidae) includes some of the most infamous insect pests of cultivated plants including *Spodoptera frugiperda*, *Spodoptera litura*, and *Spodoptera exigua*. To effectively develop targeted pest control strategies for diverse *Spodoptera* species, genomic resources are highly desired. To this aim, we provide the genome assembly and developmental transcriptome comprising all major life stages of *S. exigua*, the beet armyworm. *Spodoptera exigua* is a polyphagous herbivore that can feed on > 130 host plants, including several economically important crops. The 419 Mb beet armyworm genome was sequenced from a female *S. exigua* pupa. Using a **hybrid** genome sequencing approach (Nanopore long-read data and Illumina short read), a high-quality genome assembly was achieved (N50 = 1.1 Mb). An official gene set (18,477 transcripts) was generated by automatic annotation and by using transcriptomic RNA-seq datasets of 18 *S. exigua* samples as supporting evidence. In-depth analyses of developmental stage-specific expression combined with gene tree analyses of identified homologous genes across Lepidoptera genomes revealed four potential genes of interest (three of them *Spodoptera*-specific) upregulated during first- and third-instar larval stages for targeted pest-outbreak management. The beet armyworm genome sequence and developmental transcriptome covering all major developmental stages provide critical insights into the biology of this devastating polyphagous insect pest species worldwide. In addition, comparative genomic analyses across Lepidoptera significantly advance our knowledge to further control other invasive *Spodoptera* species and reveals potential lineage-specific target genes for pest control strategies.

Diversity of Phytophagous Insects with Potential to Become Key Pests in Genetically Modified Bt Cotton

Sanchez-Vega, M; Aguirre-Uribe, LA; Flores-Davila, M; Cerna-Chavez, E; Ochoa-Fuentes, YM; Hernandez-Juarez, A; Mendez-Lopez, A. 2021

Reduction in pests targeted by Bt cotton is widely studied; however, other phytophages not affected by Bt cotton-expressed toxins are increasing, suggesting these potential pests might occupy ecological niches of primary pests and become new key pests requiring use of broad-spectrum insecticide. During the 2017 spring-summer crop season, insects were collected on 10 genetically modified (GM) **hybrids** of cotton (*Gossypium hirsutum* L.), planted at "Rincan del Buitre" ranch, belonging to "Universidad Autonoma Agraria Antonio Narro" (UAAAN) at San Pedro, Coahuila, Mexico. The 13,422 phytophagous insects collected from GM cotton were in five orders: Coleoptera, Diptera, Hemiptera, Lepidoptera, and Thysanoptera; approximate to 12,766 of the individual insects were in Hemiptera, mainly whiteflies (Aleyrodidae), for a total of 657 other insects collected. Coleoptera were 18.5% of the families, 19.4% of species, and 6.6% of individuals; Diptera had 29.6, 27.8, and 17.2%, respectively; Hemiptera (without Aleyrodidae) had 25.9, 27.8, and 18.9%, respectively; Lepidoptera had 18.5, 19.4, and 44.6%, respectively, and Thysanoptera had 3.7, 2.8, and 2.5%, respectively. Species with more individuals than 5% of the total were *Anagasta kuehniella* Zeller (32.7%), *Spodoptera exigua* Hübner (11.0%), *Epichlorops* sp. Becker (10.7%), *Lygus oblineatus* Say (9.0%), *Diabrotica balteata* Leconte (6.9%), *Anthonomus grandis* Boheman (5.9%), and *Bemisia tabaci* Gennadius (approximate to 12,766 individuals). Species with greatest incidence among cotton **hybrids** were *Epichlorops* sp. (70%), *L. oblineatus* and *D. balteata* (each 60%), and *A. grandis* and *Aphis gossypii* Glover (each 30%). Monitoring increasing abundance of secondary pests on Bt crops can indicate if the pests threaten sustainable production of the crop.

Contribution of Larvae Developing on Corn and Dry Beans to the Adult Population of Western Bean Cutworm in Michigan

Bunn, DC; de Oliveira, ED; Springborn, F; Gonzalez-Meler, MA; Miller, N. 2021

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), is historically a pest of both corn (*Zea mays* L. (Poales: Poaceae)) and dry beans (*Phaseolus* sp. L. (Fabales: Fabaceae)) in the western Great Plains. However, it has recently undergone an eastward range expansion establishing itself across the Corn Belt in 25 states and 4 Canadian provinces. To mitigate the effects of infestation in Michigan, foliar insecticides are used in dry beans, whereas management of the pest in corn relies more heavily on the use of Bt-expressing **hybrids**. In this study stable carbon isotope analysis was used to determine what crop adult moths developed on as larvae with analysis showing that very few of the adult moths developed on dry beans. These results suggest that beans and corn are not suitable as co-refuges and that mainly adults which developed on corn are contributing to the next generation of western bean cutworm in Michigan.

Low tunnels with shading meshes: An alternative for the management of insect pests in kale cultivation

Ponce, FD; Trento, DA; Toledo, CAD; Antunes, DT; Zanuzo, MR; Dallacort, R; Oliveira, RC; Seabra, S. 2021

Kale (*Brassica oleracea* L. var. *acephala*) is one of the most cultivated leafy vegetables in Brazil. Abiotic and biotic factors can damage kale crops, reducing production and quality and making it impossible to market the product. Thus, because consumers demand sustainable production of food, the objective of this study was to evaluate the production of kale and the damage caused by pests of **hybrid** kale grown in different seasons and under different types of mesh. Two growing seasons, six growing environments and two kale **hybrids** were evaluated. The parameters evaluated were yield as measured by the total number of leaves, marketable leaves, and damaged leaves. The settlement of *Myzus persicae* and the damage caused by *Plutella xylostella*, *Hellula undalis* and *Diabrotica speciosa* were quantified. During the dry season, there was a lower yield per plant, but there was greater commercial production due to lower levels of insect infestation on plants. The use of nonwoven shade mesh reduced the damage from *P. xylostella* and *D. speciosa* on plants grown during the rainy season and reduced *M. persicae* during the dry season. However, cultivation under the protection of the organza fabric provided more effective protection against *P. xylostella* and *D. speciosa*, resulting in greater production of kale leaves.

Septin homologs cooperating in the Proliferative Stage of Microsporidia *Nosema bombycis*

Huang, J; Chen, J; Liu, FY; He, Q; We, Y; Sun, Q; Long, MX; Li, T; Pan, GQ; Zhou, ZY. 2021

The single-celled pathogen *Nosema bombycis*, that can infect silkworm *Bombyx mori* and other lepidoptera including Spodoptera, is the first identified Microsporidia which has diplokaryotic nuclei throughout the life cycle. Septin proteins can form highly ordered filaments, bundles or ring structures related to the cytokinesis in fungi. Here, three septin proteins (NbSeptin1, NbSeptin2 and NbSeptin3) from *Nosema bombycis* CQ I are described. These proteins, appear to be conserved within the phylum Microsporidia. NbSeptins transcripts were detected throughout the pathogen developmental cycle and were significantly enhanced from second days of infection, which lead to our hypothesis that NbSeptins play a role in merogony. Immunofluorescence assay (IFA) revealed a broad distribution of NbSeptins in meronts and partly co-localization of NbSeptins. Interestingly, in some of meronts, NbSeptin2 and NbSeptin3 showed localization between the nuclei of the diplokaryon. Yeast two-**hybrid** and co-immunoprecipitation analysis verified that NbSeptins can interact with each other. Our findings suggest that NbSeptins can cooperate in the proliferation stage of *Nosema bombycis* and contribute towards the understanding of the roles of septins in microsporidia development.

Wolbachia affects mitochondrial population structure in two systems of closely related Palaearctic blue butterflies

Bartonova, AS; Konvicka, M; Maresova, J; Wiemers, M; Ignatev, N; Wahlberg, N; Schmitt, T; Fric, ZF. 2021

The bacterium *Wolbachia* infects many insect species and spreads by diverse vertical and horizontal means. As co-inherited organisms, these bacteria often cause problems in mitochondrial phylogeny inference. The phylogenetic relationships of many closely related Palaearctic blue butterflies (Lepidoptera: Lycaenidae: Polyommatainae) are ambiguous. We considered the patterns of *Wolbachia* infection and mitochondrial diversity in two systems: *Aricia agestis*/*Aricia artaxerxes* and the *Pseudophilotes baton* species complex. We sampled butterflies across their distribution ranges and sequenced one butterfly mitochondrial gene and two *Wolbachia* genes. Both butterfly systems had uninfected and infected populations, and harboured several *Wolbachia* strains. *Wolbachia* was highly prevalent in *A. artaxerxes* and the host's mitochondrial structure was shallow, in contrast to *A. agestis*. Similar bacterial alleles infected both *Aricia* species from nearby sites, pointing to a possible horizontal transfer. Mitochondrial history of the *P. baton* species complex mirrored its *Wolbachia* infection and not the taxonomical division. *Pseudophilotes baton* and *P. vicrama* formed a **hybrid** zone in Europe. *Wolbachia* could obscure mitochondrial history, but knowledge on the infection helps us to understand the observed patterns. Testing for *Wolbachia* should be routine in mitochondrial DNA studies.

Historical biogeography of Heteropterinae skippers via Beringian and post-Tethyan corridors

Toussaint, EFA; Ellis, EA; Gott, RJ; Warren, AD; Dexter, KM; Storer, C; Lohman, DJ; Kawahara, AY. 2021

Skippers are a species rich and widespread group of butterflies with evolutionary patterns and processes largely unstudied despite some recent efforts. Among Hesperidae, the subfamily Heteropterinae is a moderately diverse clade comprising ca. 200 species distributed from North to South America and from Africa to the Palearctic region. While some regions are species rich, others are far less diverse. Using anchored phylogenomics, we infer a robust timetree and estimate ancestral ranges to understand the biogeographic history of these skippers. Inferences based on up to 383 exons recover a robust backbone for the subfamily along with the monophyly of all genera. Bayesian divergence time estimates suggest an origin of Heteropterinae in the late Eocene, ca. 40 million years ago. Maximum likelihood ancestral range estimates indicate an origin of the group in the New World. The eastern Palearctic was likely colonized via a Beringian route and a reverse colonization event resulted in two independent and extant American clades. We estimate a vicariant event between Central and South America that significantly predates estimates of the proto-Caribbean seaway closure, indicating active overwater dispersal in the Oligocene. The colonization of Africa from the east Palearctic is synchronous with the closure of the Tethys Ocean, while the colonization of Madagascar appears to be comparatively recent. Our results shed light on the systematics and biogeography of Heteropterinae skippers and unveil the evolutionary history of a new leaf in the skipper tree-of-life.

First report of Spodoptera frugiperda (Lepidoptera: Noctuidae) on Onion (Allium cepa L.) in South Kivu, Eastern DR Congo

Cokola, MC; Ndjaji, SS; Bisimwa, EB; Ahoton, LE; Francis, F. 2021

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797). has become one of the most devastating pests in the world with diverse host range. To develop effective integrated management strategies against *S. frugiperda* it is crucial to know its alternative host plants. In this study, we provide the first information on the occurrence of *S. frugiperda* in onion crops in Africa. Monitoring was carried out during the 2019 and 2020 growing seasons in onion monoculture and in intercropping with groundnut to detect the presence and assess the incidence of *S. frugiperda*. The highest average incidence (2.88%) and larval density (0.79 per 9 m²) were observed in onion monoculture. The classification of the onion leaf damage rating scale indicated low *S. frugiperda* infestation. This suggests that future studies will need to assess this aspect which we consider to be a survival strategy to maintain its population throughout the year. which contributes to sustain the insect in the study area during times when its preferred hosts are not present. Furthermore, genetic studies are needed to provide a better explanation of the ecology of this pest and to find out which of the two existing strain infests onions or whether it is an unknown **hybrid** population of *S. frugiperda*.

The Phylogenetics and Biogeography of the Central Asian Hawkmoths, *Hyles hippophaes* and *H. chamyla*: Can Mitogenomics and Machine Learning Bring Clarity?

Patzold, F; Marabuto, E; Daneck, H; O'Neill, MA; Kitching, IJ; Hundsdoerfer, AK. 2021

The western Palaearctic species of the hawkmoth genus *Hyles* (Lepidoptera: Sphingidae) have long been the subject of molecular phylogenetic research. However, much less attention has been paid to the taxa inhabiting the central and eastern Palaearctic, particularly Central Asia, where almost 50% of the species diversity of the genus occurs. Yet, many taxonomic conundrums hinder a proper assessment of the true diversity in these moths. One still unresolved group of species includes *Hyles hippophaes* and *Hyles chamyla*. Despite a largely overlapping morphology and ecology, a plethora of infraspecific taxa display some unique divergent characters over a wide geographical area. In this study, we undertook a taxonomic assessment of each population and resolved this species complex using an integrative approach. A combination of new computational techniques (DAISY-II) in comparative morphology and recent advances in DNA extraction methods and sequencing of museum specimens (WISC) alongside more traditional genetic approaches allowed testing of the three main phenotypes—*bienerti*, *chamyla* and *apocyni*—in terms of their morphological, mitochondrial and biogeographical integrity, and to elucidate their evolutionary relationships. Our results support the existence of two closely related species, *Hyles chamyla* and *H. hippophaes*, but the former species *H. apocyni* (here discussed as the ecological form *apocyni* of *H. chamyla*) is best regarded as a **hybrid** between *H. chamyla* and *H. h. bienerti*. The results indicate that the evolutionary relationship between *H. chamyla* and *H. hippophaes* is one of admixture in the context of ongoing ecological differentiation, which has led to shared morphological characters and a blurring of the species boundaries. These results clarify the evolutionary relationships of this species complex and open future research lines, including the analysis of nuclear markers and denser sampling, particularly of *H. hippophaes* and *H. vespertilio* in western Europe.

Complex basis of hybrid female sterility and Haldane's rule in *Heliconius* butterflies: Z-linkage and epistasis

Rosser, N; Edelman, NB; Queste, LM; Nelson, M; Seixas, F; Dasmahapatra, KK; Mallet, J. 2022

Hybrids between species are often sterile or inviable. Hybrid unfitness usually evolves first in the heterogametic sex—a pattern known as Haldane's rule. The genetics of Haldane's rule have been extensively studied in species where the male is the heterogametic (XX/XY) sex, but its basis in taxa where the female is heterogametic (ZW/ZZ), such as Lepidoptera and birds, is largely unknown. Here, we analyse a new case of female **hybrid** sterility between geographic subspecies of *Heliconius pardalinus*. The two subspecies mate freely in captivity, but female F1 **hybrids** in both directions of cross are sterile. Sterility is due to arrested development of oocytes after they become differentiated from nurse cells, but before yolk deposition. We backcrossed fertile male F1 **hybrids** to parental females and mapped quantitative trait loci (QTLs) for female sterility. We also identified genes differentially expressed in the ovary as a function of oocyte development. The Z chromosome has a major effect, similar to the 'large X effect' in *Drosophila*, with strong epistatic interactions between loci at either end of the Z chromosome, and between the Z chromosome and autosomal loci on chromosomes 8 and 20. By intersecting the list of genes within these QTLs with those differentially expressed in sterile and fertile **hybrids**, we identified three candidate genes with relevant phenotypes. This study is the first to characterize **hybrid** sterility using genome mapping in the Lepidoptera and shows that it is produced by multiple complex epistatic interactions often involving the sex chromosome, as predicted by the dominance theory of Haldane's rule.

Biology, host specificity and DNA barcoding of cryptic *Eueupithecia* species (Lepidoptera: Geometridae), and implications for biological control of *Parkinsonia aculeata* (Fabaceae) in Australia

Rafter, MA; McKay, F; Parisi, M; Sosa, A; Heard, TA; White, A; Fichera, G; Brookes, D; Nagalingam, K; Kaye, L; Raghu, S. 2022

Parkinsonia aculeata L. (Fabaceae: Caesalpinioideae), native to the Americas, is a designated Weed of National Significance in Australia. The leaf-feeding geometrid moth species, *Eueupithecia cisplatensis* Prout (Lepidoptera: Geometridae), was identified as a potential biological control agent of *P. aculeata* following native range surveys in Argentina. After importation into quarantine for host specificity assessment, this species was identified as a cryptic species complex of two morphologically similar species. The new species, *Eueupithecia vollonoides* Hausmann (Lepidoptera: Geometridae), was subsequently described. The biology and host range of both moth species were examined to determine their suitability as biological control agents. Host specificity was evaluated in the native range, by surveying insects found on closely related plant species, and in the laboratory, by no-choice larval development tests. Native range surveys and laboratory assays revealed a high level of specificity to *P. aculeata* by both *Eueupithecia* species. Of the 71 plant species from the Fabaceae family that were tested, *E. cisplatensis* completed development on only one non-target species, *Parkinsonia praecox*. This plant species is not present in Australia, and no *Eueupithecia* species were found to use *P. praecox* in the field in its native range. Hence, the risk of non-target effects if these species were to be released in Australia was considered to be low. One mitochondrial (COI) gene region and two nuclear (28S and CAD) gene regions were tested for their ability to differentiate between the two cryptic species. COI haplotypes from *E. cisplatensis* and *E. vollonoides* belong to two divergent haplotype groups and can reliably distinguish either species from the other. Some 28S and CAD gene haplotypes are shared at low frequencies, perhaps because of rare or historical **hybridisation** or incomplete lineage sorting, thus cannot reliably discriminate between the species. This pattern of mito-nuclear discordance requires further elucidation using population genetics studies to determine its biological significance. Based on the results of host specificity tests, *E. cisplatensis* and *E. vollonoides* were approved for release in Australia in May 2013 and May 2014, respectively.

Integrative Taxonomy Reveals a New *Melitaea* (Lepidoptera: Nymphalidae) Species Widely Distributed in the Iberian Peninsula

Hinojosa, JC; Toth, JP; Monasterio, Y; Mesa, LS; Sariat, MGM; Escobes, R; Vila, R. 2022

The *Melitaea phoebe* group is constituted by six species distributed throughout the Palearctic. One of the most widespread species is *Melitaea ornata* Christoph, 1893, present from France (Provence) to Central Asia. Recently, populations of *M. ornata* were discovered in a mountainous region of south-eastern Iberia, although doubts about their taxonomy existed. To clarify the taxonomic status of these populations and to revise the distribution of this taxon in Iberia, we have sequenced mitochondrial (COI barcode region) and nuclear (wg, RPS5, MDH, and EF-1 α) markers, and analyzed the male genitalia for 72 Iberian individuals and for all the species of the *M. phoebe* group. This information was complemented with phenological and ecological data. Our results unveiled that the Iberian *M. ornata*-like taxon is in fact distributed through most of the Iberian Peninsula, except for the south-west and north-east. In contrast to the univoltine *M. ornata*, the Iberian taxon can be bivoltine in the wild. The Iberian taxon was retrieved to be related to *M. ornata*, but the differences in the genetic markers and genitalia were comparable to those found between species in the group. Based on the evidence here presented and according to species delimitation results, we propose to consider the Iberian taxon as a novel species, tentatively named *Melitaea pseudornata* Munoz Sariat & Sanchez Mesa, 2019, stat. nov.

Effects of organic-inorganic hybrid nanoflowers' framework on hemocytes and enzymatic responses of the model organism, *Galleria mellonella* (Lepidoptera: Pyralidae)

Eskin, A; Ekremoglu, M; Altinkaynak, C; Ozdemir, N. 2022

Organic-inorganic **hybrid** nanoflowers have gained considerable attention over the last years due to their morphologies, enhanced enzymatic activities, and relatively high stability. The framework of these **hybrid** nanostructures has been associated with the formation of $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ (pure copper phosphate nanoflowers). In this study, we investigated the formation of pure copper phosphate nanoflowers (p-CPnfs), and their effects on hemocytes and enzymatic responses in *Galleria mellonella* (Lepidoptera: Pyralidae) as a model organism. Therefore, the effects of p-CPnfs on *G. mellonella* hemocytes and on the activities of hemolymph acetylcholine esterase (AChE), superoxide dismutase (SOD), and catalase (CAT) enzymes were evaluated after a 24-h p-CPnfs treatment in the *G. mellonella* larvae.

Different concentrations of p-CPnfs (100, 500, 1000, 2000, 6000 ppm) were cautiously force-fed to the fourth instar larvae through the mouth of the insect by means of a micro-fine insulin syringe without causing any physical damage. 1000, 2000, and 6000 ppm of p-CPnfs significantly increased the total hemocyte counts (THCs) of the *G. mellonella* larvae. 500, 1000, 2000, and 6000 ppm of p-CPnfs increased the SOD activity of the larvae. 1000, 2000, and 6000 ppm of p-CPnfs increased the CAT activity in the hemolymph of the larvae. Unlike the results seen in SOD and CAT enzyme activities, all concentrations of p-CPnfs decreased AChE activity in the larval hemolymph. In addition to these results, LC50 value was found to be 8410 ppm for the force-fed larvae at the end of the 24-h period.

Virulence potential of two entomopathogenic nematodes, their associated bacteria, and its metabolites to larvae of *Pieris brassicae* L. (Lepidoptera, Pieridae) in cabbage under greenhouse and field bioassays

Abbas, W; Javed, N; Haq, IU; Ahmed, S. 2022

The cabbage butterfly (*Pieris brassicae* L.) is a major insect pest of cabbage in Pakistan. *Heterorhabditis bacteriophora* (at 1500 infective juvenile/ ml concentration), *Steinernema glaseri* (at 1500 infective juvenile/ ml concentration), *H. bacteriophora* + *S. glaseri* (at 1500 infective juvenile/ ml concentration), *Xenorhabdus* spp., (at 4 x 10(12) concentration) *Photorhabdus* spp., (at 4 x 10(12) concentration) metabolites of *Xenorhabdus* spp. (at 50% concentration) and metabolites of *Photorhabdus* spp (at 50% concentration) were sprayed on cabbage **hybrid** CB-60 grown in pots placed in the greenhouse. After 7 days, the highest *P. brassicae* larval mortality (90.43%) was observed where *H. bacteriophora* + *S. glaseri* were sprayed. In field experiment, best-performing treatments from greenhouse experiment were selected i.e. *H. bacteriophora* + *S. glaseri* (at 1500 infective juvenile/ ml concentration), *Xenorhabdus* spp., (at 4 x 10(12) concentration) and metabolites of *Xenorhabdus* spp. (at 50% concentration) were sprayed on a highly susceptible cabbage **hybrid** (CB-60). *Heterorhabditis bacteriophora* + *Steinernema glaseri* @ 1500 infective juvenile/ ml was found to be more superior with maximum mortality (80.43) of cabbage butterfly larvae in comparison with other treatments. From the present study, it may be concluded that EPNs (*H. bacteriophora* and *S. glaseri*) application is more effective as compared to their symbiotic bacteria and metabolite for cabbage butterfly management.

New Insights into the Evolution of the W Chromosome in Lepidoptera

Dalikova, M; Zrzava, M; Hladova, I; Nguyen, P; Sonsky, I; Flegrova, M; Kubickova, S; Volenikova, A; Kawahara, AY; Peters, RS; Marec, F. NA

Moths and butterflies (Lepidoptera) represent the most diverse group of animals with heterogametic females. Although the vast majority of species has a WZ/ZZ (female/male) sex chromosome system, it is generally accepted that the ancestral system was Z/ZZ and the W chromosome has evolved in a common ancestor of Tischeriidae and Ditrysia. However, the lack of data on sex chromosomes in lower Lepidoptera has prevented a formal test of this hypothesis. Here, we performed a detailed analysis of sex chromosomes in *Tischeria ekebladella* (Tischeriidae) and 3 species representing lower Ditrysia, *Cameraria ohridella* (Gracillariidae), *Plutella xylostella* (Plutellidae), and *Tineola bisselliella* (Tineidae). Using comparative genomic **hybridization** we show that the first 3 species have well-differentiated W chromosomes, which vary considerably in their molecular composition, whereas *T. bisselliella* has no W chromosome. Furthermore, our results suggest the presence of neo-sex chromosomes in *C. ohridella*. For Z chromosomes, we selected 5 genes evenly distributed along the Z chromosome in ditrysian model species and tested their Z-linkage using qPCR. The tested genes (Henna, laminin A, Paramyosin, Tyrosine hydroxylase, and 6-Phosphogluconate dehydrogenase) proved to be Z-linked in all species examined. The conserved synteny of the Z chromosome across Tischeriidae and Ditrysia, along with the W chromosome absence in the lower ditrysian families Psychidae and Tineidae, suggests a possible independent origin of the W chromosomes in these 2 lineages.

The role of latitudinal, genetic and temperature variation in the induction of diapause of *Papilio glaucus* (Lepidoptera: Papilionidae)

Ryan, SF; Valella, P; Thivierge, G; Aardema, ML; Scriber, JM. NA

A key adaptation in insects for dealing with variable environmental conditions is the ability to diapause. The tiger swallowtail butterflies, *Papilio glaucus* and *P. canadensis* are ideal species to explore the genetic causes and population genetic consequences of diapause because divergence in this trait is believed to be a salient factor in maintaining a **hybrid** zone between these species. Yet little is known about the factors that influence diapause induction in this system. Here we explored how spatial (latitudinal), environmental (temperature) and genetic (**hybridization**) factors affect diapause induction in this system. Specifically, a series of growth chamber experiments using wild caught individuals from across the eastern United States were performed to: (1) evaluate how critical photoperiod varies with latitude, (2) isolate the stage in which induction occurs, (3) test whether changes in temperature affected rates of diapause induction, and (4) explore how the incidence of diapause is affected in **hybrid** offspring. We find that induction occurs in the larval stage, is not sensitive to a relatively broad range of temperatures, appears to have a complex genetic basis (i.e., is not simply a dominant trait following a Mendelian inheritance pattern) and that the critical photoperiod increases by 0.4 h with each increasing degree in latitude. This work deepens our understanding of how spatial, environmental and genetic variation influences a key seasonal adaptation (diapause induction) in a well-developed ecological model system and will make possible future studies that explore how climatic variation affects the population dynamics and genetics of this system.

Olfactory neuron responsiveness and pheromone blend preference in hybrids between *Ostrinia furnacalis* and *Ostrinia nubilalis* (Lepidoptera : Crambidae)

Domingue, MJ; Musto, CJ; Linn, CE; Roelofs, WL; Baker, TC. NA

The olfactory receptor neuron (ORN) and behavioral responses of **hybrids** between the Asian corn borer (ACB), *Ostrinia furnacalis*, and the E-strain European corn borer (ECB(E)), *Ostrinia nubilalis* were examined and compared to the parental populations. In **hybrids** and both parents, the large-spike-size ORN was capable of responding to all four pheromone components of ACB and ECB, despite differences in which compounds elicited the greatest spike frequency in each population. There was a small-spiking ORN more narrowly tuned to the minor pheromone components in both ACB and ECB(E). In **hybrids** the homologous small-spiking ORN was tuned primarily to the ECB(E) minor pheromone component, with some responsiveness to the ACB minor component. Both species and all the **hybrids** had an intermediate spike-size ORN tuned primarily to their common behavioral antagonist. Dominance of responsiveness to the ECB(E) versus the ACB minor pheromone component on the small-spiking ORN may explain the greater tendency of **hybrids** to fly upwind to the ECB(E) pheromone blend than the ACB blend. This finding points toward a distinct evolutionary role for this ORN in allowing a pheromone shift. Published by Elsevier Ltd.

Cross-species amplification and polymorphism of microsatellite loci in *Helicoverpa armigera* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in Brazilian cropping systems

Leite, NA; Correa, AS; Alves-Pereira, A; Campos, JB; Zucchi, MI; Omoto, C. NA

The Old World bollworm *Helicoverpa armigera* (Hubner) was recently discovered in Brazil. This species is closely related to the New World bollworm *H. zea* (Boddie), and mating between these species has already been reported under laboratory conditions. Here, we tested the cross-species amplification of 20 microsatellite (SSR) loci in field populations of *H. armigera* and *H. zea* collected from Brazilian cropping systems. Seven SSR loci were successfully amplified and polymorphic in both species except for the locus HaC14, which was monomorphic for *H. zea*. All SSR loci were in linkage equilibrium, and deviations from Hardy-Weinberg equilibrium were only observed for the locus HaRSR1 in the HaRS-2 population, where null alleles were present. A moderate level of polymorphism was detected in *H. armigera* and *H. zea* populations with a mean allele number of 4.14, and 2.24, respectively. Interestingly, most of the populations of the recent invader *H. armigera* showed

higher genetic diversity and inbreeding coefficients than *H. zea* populations. The genetic identity of each species was recovered using a STRUCTURE analysis, where the populations formed two clusters ($K = 2$) according to their species. STRUCTURE also suggested the occurrence of potential **hybrid** offspring between *H. armigera* and *H. zea* individuals in natural conditions. These SSR loci will be valuable in characterizing population differentiation, invasion routes, adaptation, reproductive behavior, and intra- and interspecific gene flow in *H. armigera* and *H. zea* populations in Brazil, the USA, and other areas where these two pests occur.

Association of Insect-Derived Ear Injury With Yield and Aflatoxin of Maize Hybrids Varying in Bt Transgenes

Pruter, LS; Brewer, MJ; Weaver, MA; Murray, SC; Isakeit, TS; Bernal, JS. NA

Environmental factors have been associated with the production of aflatoxin in maize, *Zea mays* L., and it is inconclusive whether transgenic, *Bacillus thuringiensis* (Bt), maize has an impact on aflatoxin accumulation. Maize **hybrids** differing in transgenes were planted in two locations from 2014 to 2017. Yield, aflatoxin, and ear injury caused by corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), were measured across three groups of **hybrids** differing in transgenes including near-isogenic **hybrids**, and water-stressed conditions. The **hybrid** groups consisted of non-Bt **hybrids** with no Bt transgenes, a second group with one or more Cry-Bt transgenes, and the third group with vegetative insecticidal Bt protein and Cry-Bt transgenes (CryNip-Bt). Across the six data sets derived from 11 experiments, the Cry-Bt and CryNip-Bt **hybrids** had less ear injury and aflatoxin on average than non-Bt **hybrids**. The effects of ear injury on yield and aflatoxin were more prominent and consistent in Corpus Christi, TX, where **hybrids** experienced more water-limited conditions than in College Station, TX. The trend of increased aflatoxin among **hybrids** with increased ear injury was further resolved when looking at Cry-Bt and CryNip-Bt isogenic **hybrids** in Corpus Christi. The results supported that the maize **hybrids** with the inclusion of Cry-Bt and CryNip-Bt transgenes warrant further investigation in an integrated approach to insect and aflatoxin management in sub-tropical rain-fed maize production regions. Research outcomes may be improved by focusing on areas prone to water-stress and by using **hybrids** with similar genetic backgrounds.

Towards unravelling Wolbachia global exchange: a contribution from the *Bicyclus* and *Mylothris* butterflies in the Afrotropics

Duploux, A; Pranter, R; Warren-Gash, H; Tropek, R; Wahlberg, N. NA

Background: Phylogenetically closely related strains of maternally inherited endosymbiotic bacteria are often found in phylogenetically divergent, and geographically distant insect host species. The interspecies transfer of the symbiont *Wolbachia* has been thought to have occurred repeatedly, facilitating its observed global pandemic. Few ecological interactions have been proposed as potential routes for the horizontal transfer of *Wolbachia* within natural insect communities. These routes are however likely to act only at the local scale, but how they may support the global distribution of some *Wolbachia* strains remains unclear. Results: Here, we characterize the *Wolbachia* diversity in butterflies from the tropical forest regions of central Africa to discuss transfer at both local and global scales. We show that numerous species from both the *Mylothris* (family Pieridae) and *Bicyclus* (family Nymphalidae) butterfly genera are infected with similar *Wolbachia* strains, despite only minor interclade contacts across the life cycles of the species within their partially overlapping ecological niches. The phylogenetic distance and differences in resource use between these genera rule out the role of ancestry, **hybridization**, and shared host-plants in the interspecies transfer of the symbiont. Furthermore, we could not identify any shared ecological factors to explain the presence of the strains in other arthropod species from other habitats, or even ecoregions. Conclusion: Only the systematic surveys of the *Wolbachia* strains from entire species communities may offer the material currently lacking for understanding how *Wolbachia* may transfer between highly different and unrelated hosts, as well as across environmental scales.

Homology of olfactory receptor neuron response characteristics inferred from hybrids between Asian and European corn borer moths (Lepidoptera: Crambidae)

Domingue, MJ; Musto, CJ; Linn, CE; Roelofs, WL; Baker, TC. NA

First generation **hybrid** males from crosses between the Asian corn borer (ACB), *Ostrinia furnacalis*, and the "univoltine Z-strain" European corn borer (ECB), *Ostrinia nubilalis*, were examined with respect to behavioral and physiological responses to ACB and ECB pheromones. The **hybrid** males often flew to the pheromone of ECB Z-strain, but very rarely to the ACB pheromone. We mapped the tuning profiles of each ORN of the F(1) **hybrids** with respect to the relevant pheromone components and a common behavioral antagonist by employing differential cross-adaptation and varying doses of the ligands. In the trichoid sensilla of F(1) **hybrid** males, the three co-compartmentalized ORNs produced spikes that were very difficult to distinguish by size, unlike the parental populations. Comparing the responses to ACB and ECB components at different doses reveals overlapping profiles similar to males of both parental types, but more responsiveness to the ECB pheromone components. We were unable to detect any differences in the ORN tuning profiles when comparing males with different behavioral phenotypes. While the two ECB pheromone races have similar ORN tuning properties that are different from those in ACB, the spike-amplitude patterns of ECB E-strain and ACB have greater homology when compared to ECB Z-strain. (C) 2009 Elsevier Ltd. All rights reserved.

Four hundred shades of brown: Higher level phylogeny of the problematic Euptychiina (Lepidoptera, Nymphalidae, Satyrinae) based on hybrid enrichment data

Espeland, M; Breinholt, JW; Barbosa, EP; Casagrande, MM; Huertas, B; Lamas, G; Marin, MA; Mielke, OHH; Miller, JY; Nakahara, S; Tan, D; Warren, AD; Zacca, T; Kawahara, AY; Freitas, AVL; Willmott, KR. NA

Relationships within satyrine butterflies have been notoriously difficult to resolve using both morphology and Sanger sequencing methods, and this is particularly true for the mainly Neotropical subtribe Euptychiina, which contains about 400 described species. Known larvae of Euptychiina feed on grasses and sedges, with the exception of the genus Euptychia, which feed on mosses and lycopsids, and the butterflies occur widely in rainforest, cloudforest and grassland habitats, where they are often abundant. Several previous molecular and morphological studies have made significant progress in tackling the systematics of the group, but many relationships remain unresolved, with long-branch-attraction artifacts being a major problem. Additionally, the monophyly of the clade remains uncertain, with Euptychia possibly not being closely related to the remainder of the clade. Here we present a backbone phylogeny of the subtribe based on 106 taxa, 368 nuclear loci, and over 180,000 bps obtained through **hybrid** enrichment. Using both concatenation and species tree approaches (IQ-TREE, EXABAYES, ASTRAL), we can for the first time strongly confirm the monophyly of Euptychiina with Euptychia being the sister group to the remainder of the clade. The Euptychiina is divided into nine well supported clades, but the placement of a few genera such as Hermeuptychia, Pindis and the Chloreuptychia catharina group still remain uncertain. As partially indicated in previous studies, the genera Cissia, Chloreuptychia, Magneuptychia, Megisto, Splendeuptychia and Euptychoides, among others, were found to be highly polyphyletic and revisions are in preparation. The phylogeny will provide a strong backbone for the analysis of datasets in development that are much more taxonomically comprehensive but have orders of magnitude fewer loci. This study therefore represents a critical step towards resolving the higher classification and studying the evolution of this highly diverse lineage.

Assessing ecological and physiological costs of melanism in North American *Papilio glaucus* females: two decades of dark morph frequency declines

Scriber, JM. NA

Polymorphisms for melanistic form of insects may provide various selective advantages. However, melanistic alleles may have significant/subtle pleiotrophic "costs." Several potential pleiotrophic effects of the W (=Y)-linked melanism gene in *Papilio glaucus* L. (Lepidoptera) showed no costs for melanistic versus yellow in adult size, oviposition preferences, fecundity, egg viability, larval survival/growth rates, cold stress tolerance, or postdiapause emergence times. Sexual selection (males

choosing yellow rather than mimetic dark females) had been suggested to provide a balanced polymorphism in *P. glaucus*, but spermatophore counts in wild females and direct field tethering studies of size-matched pairs of virgin females (dark and yellow), show that male preferences are random or frequency-dependent from Florida to Michigan, providing no yellow counter-advantages. Recent frequency declines of dark (melanic/mimetic) females in *P. glaucus* populations are shown in several major populations from Florida (27.3 degrees N latitude) to Ohio (38.5 degrees N). Summer temperatures have increased significantly at all these locations during this time (1999-2018), but whether dark morphs may be more vulnerable (in any stage) to such climate warming remains to be determined. Additional potential reasons for the frequency declines in mimetic females are discussed: (i) genetic introgression of Z-linked melanism suppressor genes from *P. canadensis* (R & J) and the **hybrid** species, *P. appalachiensis* (Pavulaan & Wright), (ii) differential developmental incompatibilities, or Haldane effects, known to occur in **hybrids**, (iii) selection against intermediately melanic ("dusty") females (with the W-linked melanic gene, b+) which higher temperatures can cause.

Divergence in *Heliconius* flight behaviour is associated with local adaptation to different forest structures

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Microhabitat choice plays a major role in shaping local patterns of biodiversity. In butterflies, stratification in flight height has an important role in maintaining community diversity. Despite its presumed importance, the role of behavioural shifts in early stages of speciation in response to differences in habitat structure is yet to be established. Here, we investigated variation in flight height behaviour in two closely related *Heliconius* species, *H. erato cyrbia* and *H. himera*, which produce viable **hybrids** but are isolated across an environmental gradient, spanning lowland wet forest to high-altitude scrub forest. Speciation in this pair is associated with strong assortative mating, but ecological isolation and local adaptation are also considered essential for complete reproductive isolation. We quantified differences in flight height and forest structure across the environmental gradient and tested the importance of resource distribution in explaining flight behaviour. We then used common garden experiments to test whether differences in flight height reflect individual responses to resource distribution or genetically determined shifts in foraging behaviour. We found that the two species fly at different heights in the wild, and demonstrated that this can be explained by differences in the vertical distribution of plant resources. In both the wild and captivity, *H. himera* choose to fly lower and feed at lower positions, closely mirroring differences in resource availability in the wild. Given expectations that foraging efficiency contributes to survival and reproductive success, we suggest that foraging behaviour may reflect local adaptation to divergent forest structures. Our results highlight the potential role of habitat-dependent divergence in behaviour during the early stages of speciation.

ABERRANT COLOR PATTERNS IN THE PAPILIO AND AN UPDATE ON THE SEMI-MELANIC "FLETCHER" VARIANTS, INCLUDING FEMALES (LEPIDOPTERA: PAPILIONIDAE)

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Specimens resembling the male melanic aberration called "fletcher" have been reported since the 1800's, primarily from *Papilio canadensis* populations across northern USA and Canada. These records have generally exhibited similar but very distinctive wing color patterns on the upper and undersides, especially the orange and blue on the hindwings. This paper reviews the 120-year history of "fletcher"-type records and presents a number of new records, including some from *P. glaucus* and one from *P. rutulus*. Other female variants are also figured and compared since "fletcher" phenotypes have historically almost always been males. We report on the first known female specimen of "fletcher" from Vermont and discuss another female fletcher phenotype captured in eastern Washington (also in 2008). It remains unclear whether interspecific introgression between *P. canadensis*, *P. glaucus*, and *P. rutulus* plays any role in generating these rare phenotypes, but fletcher specimens are all from areas near the **hybrid** zone. Natural environmental conditions may play some role in causing this phenotype, especially since none have been generated under lab conditions.

Hidden Phylogenomic Signal Helps Elucidate Arsenurine Silkmoth Phylogeny and the Evolution of Body Size and Wing Shape Trade-Offs

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One of the key objectives in biological research is understanding how evolutionary processes have produced Earth's diversity. A critical step toward revealing these processes is an investigation of evolutionary tradeoffs—that is, the opposing pressures of multiple selective forces. For millennia, nocturnal moths have had to balance successful flight, as they search for mates or host plants, with evading bat predators. However, the potential for evolutionary trade-offs between wing shape and body size are poorly understood. In this study, we used phylogenomics and geometric morphometrics to examine the evolution of wing shape in the wild silkmoth subfamily Arsenurinae (Saturniidae) and evaluate potential evolutionary relationships between body size and wing shape. The phylogeny was inferred based on 782 loci from target capture data of 42 arsenurine species representing all 10 recognized genera. After detecting in our data one of the most vexing problems in phylogenetic inference—a region of a tree that possesses short branches and no "support" for relationships (i.e., a polytomy), we looked for hidden phylogenomic signal (i.e., inspecting differing phylogenetic inferences, alternative support values, quartets, and phylogenetic networks) to better illuminate the most probable generic relationships within the subfamily. We found there are putative evolutionary trade-offs between wing shape, body size, and the interaction of fore- and hindwing (HW) shape. Namely, body size tends to decrease with increasing HW length but increases as forewing (FW) shape becomes more complex. Additionally, the type of HW (i.e., tail or no tail) a lineage possesses has a significant effect on the complexity of FW shape. We outline possible selective forces driving the complex HW shapes that make Arsenurinae, and silkmoths as a whole, so charismatic. [Anchored **hybrid** enrichment; Arsenurinae; geometric morphometrics; Lepidoptera; phylogenomics; Saturniidae.]
