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28/03/2022

HYBRIDIZATION STUDIES ON SIBLING SPECIES OF THE ANOPHELES-GAMBIAE GILES COMPLEX (DIPTERA, CULICIDAE) IN THE LABORATORY

OKEREKE, TA. 1980

A CYTOLOGICAL STUDY OF NATURAL HYBRIDS BETWEEN PROSIMULIUM-MULTIDENTATUM AND P-MAGNUM - WITH NOTES ON SEX DETERMINATION IN THE SIMULIIDAE (DIPTERA)

ROTHFELS, K; NAMBIAR, R. 1981

HYBRIDIZATION OF ANOPHELES-MINIMUS AND ANOPHELES-ACONITUS (DIPTERA, CULICIDAE) IN THAILAND

SUCHARIT, S; CHOOCHOTE, W. 1982

IMMUNOFLUORESCENT CHARACTERIZATION OF DNA.RNA HYBRIDS ON POLYTENE CHROMOSOMES OF TRICHOSIA-PUBESCENS (DIPTERA, SCIARIDAE)

BUSEN, W; AMABIS, JM; LEONCINI, O; STOLLAR, BD; LARA, FJS. 1982

ISOLATION AND CHARACTERIZATION OF ENDOGENOUS HYBRIDS IN DIPTERA

ABDELHAY, E; ALONSO, CEV; ROCHA, H; MIRANDA, M. 1983

NATURAL INTERSPECIFIC HYBRIDIZATION OF SIMULIUM-SANCTIPauli SL WITH SIMULIUM-SQUAMOSUM AND SIMULIUM-YAHENSE (DIPTERA, SIMULIIDAE)

POST, RJ. 1984

EFFECTS OF RESISTANT SORGHUM HYBRIDS ON SORGHUM MIDGE (DIPTERA, CECIDOMYIIDAE) BIOLOGY

MELTON, KD; TEETES, GL. 1984

RELATIONSHIP OF SORGHUM MIDGE (DIPTERA, CECIDOMYIIDAE) DENSITY TO DAMAGE TO RESISTANT AND SUSCEPTIBLE SORGHUM HYBRIDS

HALLMAN, GJ; TEETES, GL; JOHNSON, JW. 1984

ELECTROPHORETIC AND HYBRIDIZATION COMPARISON OF GLOSSINA-MORSITANS-MORSITANS, GLOSSINA-MORSITANS-CENTRALIS, AND GLOSSINA-MORSITANS-SUBMORSITAN (DIPTERA, GLOSSINIDAE)

GOODING, RH. 1985

THE GENETICS OF HYBRID STERILITY BETWEEN SUBSPECIES OF THE COMPLEX OF GLOSSINA-MORSITANS WESTWOOD (DIPTERA, GLOSSINIDAE)

RAWLINGS, P. 1985

COMPARATIVE FITNESS OF TEMEPHOS-RESISTANT, TEMEPHOS-SUSCEPTIBLE, AND HYBRID PHENOTYPES OF THE SOUTHERN HOUSE MOSQUITO (DIPTERA, CULICIDAE)

ELKHATIB, ZI; GEORGHIOU, GP. 1985

ADULT SORGHUM MIDGE (DIPTERA, CECIDOMYIIDAE) NONPREFERENCE FOR A RESISTANT HYBRID SORGHUM

WAQUIL, JM; TEETES, GL; PETERSON, GC. 1986

SORGHUM MIDGE (DIPTERA, CECIDOMYIIDAE) ADULT OVIPOSITIONAL BEHAVIOR ON RESISTANT AND SUSCEPTIBLE SORGHUM HYBRIDS

WAQUIL, JM; TEETES, GL; PETERSON, GC. 1986

CHROMOSOMAL LOCALIZATION OF THE WHITE GENE OF LUCILIA-CUPRINA (DIPTERA, CALLIPHORIDAE) BY INSITU HYBRIDIZATION

BEDO, DG; HOWELLS, AJ. 1987

A HYBRID POPULATION OF NEPHROTOMA (DIPTERA, TIPULIDAE) FROM A CENTRAL TEXAS REFUGIUM

OOSTERBROEK, P; TANGELDER, IRM. 1987

C-BANDING IN THE POLYTENE CHROMOSOMES OF SPECIES OF THE PLUMOSUS GROUP (DIPTERA, CHIRONOMIDAE) AND THEIR EXPERIMENTAL HYBRIDS

MICHAILOVA, P. 1987

SURVIVAL OF PERMETHRIN-SUSCEPTIBLE, RESISTANT, AND F1-HYBRID STRAINS OF HEMATOBIA-IRRITANS (DIPTERA, MUSCIDAE) ON EAR-TAGGED STEERS

MCDONALD, PT; SCHMIDT, CD; FISHER, WF; KUNZ, SE. 1987

GENETIC-BASIS OF STERILITY IN HYBRID MALES FROM CROSSES OF GLOSSINA-MORSITANS-MORSITANS AND GLOSSINA-MORSITANS-CENTRALIS (DIPTERA, GLOSSINIDAE)

GOODING, RH. 1987

HYBRIDIZATION IN PRINCIPAL PARASITOIDS OF SYNANTHROPIC DIPTERA - THE GENUS MUSCIDIFURAX (HYMENOPTERA, PTEROMALIDAE)

LEGNER, EF. 1988

HYBRID MALE-STERILITY IN CROSSES BETWEEN FIELD AND LABORATORY STRAINS OF ANOPHELES-QUADRIMACULATUS (SAY) (DIPTERA, CULICIDAE)

LANZARO, GC; NARANG, SK; MITCHELL, SE; KAISER, PE; SEAWRIGHT, JA. 1988

MEIOSIS IN AN EXPERIMENTAL HYBRID OF GLYPTOTENDIPES-SALINUS MICHAILOVA X GLYPTOTENDIPES-BARBIPES (STAEGER) (DIPTERA, CHIRONOMIDAE)

MICHAILOVA, P; BELCHEVA, R. 1989

GENETIC-BASIS OF STERILITY IN HYBRIDS FROM CROSSES OF GLOSSINA-MORSITANS-SUBMORSITANS AND GLOSSINA-MORSITANS-MORSITANS (DIPTERA, GLOSSINIDAE)

GOODING, RH. 1989

FIELD-EVALUATION OF THE SUSCEPTIBILITY OF 8TH SORGHUM HYBRIDS TO THE SORGHUM MIDGE, CONTARINIA-SORGHICOLA (COQUILLET) (DIPTERA, CECIDOMYIIDAE) (1,2)

SEGARRACARMONA, AE; SOTOMAYORRIOS, A; TORRESCARDONA, S; QUILESBELEN, A. 1989

INVOLVEMENT OF THE X-CHROMOSOME IN FERTILITY OF MALE AND FEMALE HYBRIDS OF GLOSSINA-MORSITANS-MORSITANS AND GLOSSINA-MORSITANS-CENTRALIS (DIPTERA, GLOSSINIDAE)

GOODING, RH. 1989

GENETICS OF INTERSPECIFIC HYBRIDIZATION IN THE TRISERIATUS AND ZOOSOPHUS GROUPS OF AEDES (PROTOMACLEAYA) (DIPTERA, CULICIDAE)

TAYLOR, DB. 1990

THE CHIRONOMIDAE (DIPTERA) OF BONE PILE POND, DELTA-MARSH, MANITOBA, CANADA

WRUBLESKI, DA; ROSENBERG, DM. 1990

Adult midge flies, Chironomidae, were collected by emergence traps from a shallow, semi-isolated bay of the Delta Marsh, Manitoba, over a two-year period. Three habitats were sampled - sago pondweed (*Potamogeton pectinatus*), hybrid cattail (*Typha x glauca*), and hardstem bulrush (*Scirpus acutus*). The pondweed habitat was the most productive of the three habitats sampled, both in terms of numbers and biomass of emerging chironomids. Eighty-four species were collected, the most

abundant being *Tanypus punctipennis*, *Psectrotanypus alaskensis*, *Cricotopus sylvestris*, *Corynoneura* cf. *scutellata*, *Limnophyes prolongatus*, *L. immucronatus*, *Glyptotendipes barbipes*, *G. lobiferus*, *Chironomus tentans*, *Dicrotendipes nervosus*, *Paratanytarsus* sp. 1, and *Cladotanytarsus* sp. Most of the dominant species were bi- or multivoltine, but considerable inter-annual variation was noted in emergence rates and patterns.

EVIDENCE FOR THE MULTIPLE EXOTIC ORIGIN OF AUSTRALIAN POPULATIONS OF THE SHEEP BLOWFLY, *LUCILIA-CUPRINA* (WIEDEMANN) (DIPTERA, CALLIPHORIDAE)

NORRIS, KR. 1990

Some *Lucilia cuprina* populations in the eastern coastal areas of Australia are shown to be a blend of the two subspecies, *Lucilia cuprina cuprina* and *L. cuprina dorsalis*. The possible history of the introduction to Australia of these two subspecies, respectively from the Pacific and South Africa, is discussed. The hybrid nature of the Australian population makes it vital to take into consideration the genetic constitution of cultures before launching comparative studies.

DETECTION OF EPIZOOTIC HEMORRHAGIC-DISEASE VIRUS IN *CULICOIDES-VARIIPENNIS* (DIPTERA, CERATOPOGONIDAE)

WILSON, WC. 1991

Nucleic acid hybridization was used to detect epizootic hemorrhagic disease (EHD) virus serotype 1 and serotype 2 in *Culicoides variipennis* (Coquillett). Adult females were inoculated intrathoracically with virus, then were assayed daily for the presence of viral RNA for 2 wk, at which time maximum virus replication is likely to occur. Viral RNA of EHD serotypes 1 and 2 was first detected by hybridization on days 9 and 7 after infection, respectively, and then for up to 14 days after infection. EHD serotype 1 viral RNA was detected by hybridization in infected flies fixed in ethanol at room temperature for 7 d and in unfixed (frozen) infected flies. However, weak false positives diminished prospects for application of this method.

DIFFERENTIAL REGULATION OF INSECT GLOBIN AND ACTIN MESSENGER-RNAS DURING LARVAL DEVELOPMENT IN *CHIRONOMUS-THUMMI*

SAFFARINI, DA; TREWITT, PM; LUHM, RA; BERGTROM, G. 1991

S1 nuclease protection assays were used to measure changes in the steady-state levels of six different globin (Gb) mRNAs in the midge, *Chironomus thummi thummi* (*C. thummi*, Diptera) during larval development. Two distinct patterns of change were observed. GbI, IV, VIIIB-4 and VIIIB-5 transcripts were present in 3rd instar larvae, rose from low levels immediately post-moult to peak levels by day 2-3 of the 4th instar, and then declined, reaching near-basal levels by day 7-8. In contrast, transcripts of GbIII (known from previous studies to be 4th instar-specific) and VI, which were undetectable in the 3rd instar, rose to high levels by day 2 of the 4th instar, but remained elevated thereafter. Our data further showed that closely linked Gb genes were not necessarily expressed in a coordinate manner. Unlike the Gb mRNAs, actin (Act) mRNA levels (measured by slot-blot hybridization to a heterologous probe) increased progressively as a proportion of total RNA during 4th instar development. Therefore, the regulation of *C. thummi* Gb transcript levels is specific, differing from that of Act and among the Gb mRNAs themselves. Elevated 20-hydroxyecdysone (HE) titer at the 3rd-4th instar moult correlates with the low steady-state levels of Gb mRNAs immediately post-moult. However, other aspects of Gb mRNA profiles cannot be explained on the basis of a direct repressive effect by HE on Gb gene transcription.

MECHANISMS OF HYBRID SUNFLOWER RESISTANCE TO THE SUNFLOWER MIDGE (DIPTERA, CECIDOMYIIDAE)

ANDERSON, MD; BREWER, GJ. 1991

A study was conducted to identify mechanisms of resistance in sunflower to the sunflower midge, *Contarinia schulzi* Gagne. Infestation ratings, larval counts, and damage ratings were used to quantify midge resistance in seven sunflower hybrids grown at three locations. Six of the seven hybrids possessed midge resistance. Each hybrid was further categorized with respect to infestation resistance, antibiosis, and tolerance. Two bud characteristics, percent open and size, were examined for their contribution to infestation resistance. The percentage of time that buds of each hybrid were open was negatively correlated with infestation (indicating a preference for the open characteristic). Infestations were higher in buds greater-than-or-equal-to 2.5 cm in diameter. In artificial infestation trials, damage caused by first and second instars was very low due to high larval mortality. When sunflower buds were artificially infested with midge adults, moderate larval infestations occurred. Artificially infesting plants with adults confirmed the presence of antibiosis in two resistant and one susceptible hybrid.

HYBRIDIZATION EVIDENCE SUPPORTING SEPARATE SPECIES STATUS FOR *ANOPHELES-ALBITARSUS* AND *ANOPHELES-DEANEORUM* (DIPTERA, CULICIDAE) IN BRAZIL

KLEIN, TA; LIMA, JB; TANG, AT. 1991

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 cytotype. 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 GENETIC-CONTROL OF MATING-BEHAVIOR IN SCREWORM (DIPTERA, CALLIPHORIDAE) MALES THROUGH DIALLEL CROSSES AND ARTIFICIAL SELECTION

MANGAN, RL. 1991

The mode of genetic control of male screwworm (Diptera: Calliphoridae) mating behavior was examined using diallel cross and artificial selection. Diallel crosses showed strong dominance effects, with hybrids being uniformly more successful in copulation than their more inbred parental strains. Weaker additive and reciprocal effects were also noted. Environmental (replicate) effects were highly significant. Regression of array variances and covariances indicated that epistatic interactions or unequal allele distribution during gametogenesis may have occurred and that high courtship propensity polygenes show dominance over low propensity genes. Artificial selection on males from outbred strains from Guatemala and Belize resulted in a decreased number of mating attempts for lines selected for reduced activity, but mating attempts in lines selected for high mating activity did not increase. A combination of inbreeding during the selection cycles as well as selection for recessive traits would explain this response. The two types of experiments were in general agreement, indicating significant dominance and environmental influence on male mating behavior with weaker additive and possible maternal effects.

ANOPHELES-ANNULIPES WALKER (DIPTERA, CULICIDAE) AT GRIFFITH, NEW-SOUTH-WALES .1. 2 SIBLING SPECIES IN SYMPATRY

FOLEY, DH; BRYAN, JH. 1991

The existence of 2 sibling species of *An. annulipes* at Griffith, NSW is confirmed. The 2 species have been designated as species A and species G. In laboratory produced hybrids, the larval polytene chromosomes are largely asynaptic. The 2 species differ at the malic enzyme and the alpha-glycerophosphate dehydrogenase loci. No hybrid chromosomes were detected in the offspring of 103 wild-caught females from an area of sympatry and 98.5% of 6630 specimens from the same area were identified as either species A or species G by electrophoresis.

TEMPORAL VARIATION IN MORPHOLOGICAL AND GENETIC-CHARACTERISTICS WITHIN A HYBRID POPULATION OF CULEX-PIPIENS (DIPTERA, CULICIDAE)

PRYOR, SC; DALY, J. 1991

Samples of mosquitoes in the *Culex pipiens* L. complex from Memphis, Tenn., were collected from June to November 1985 and examined in regard to allozyme frequencies and ratios of the two arms of the phallosome of the male genitalia (DV/D). The dominant allozymes of hexokinase (Hk(A)) and 6-phosphogluconate dehydrogenase (PgD(F)) significantly increased in frequency during this period as did the mean DV/D ratio. An analysis of gene frequencies by species group designated by DV/D ratios revealed no significant differences among *Cx. pipiens*, *Cx. quinquefasciatus* Say, and intermediates. The lack of association of gene frequencies with the taxa determined by the DV/D ratio indicated that although allozyme frequencies were correlated temporally with the DV/D ratio in the population, they were not associated with subspecies. These results are consistent with previous work that has shown latitudinal association and thermal stability differences in the major allozymes of these enzymes in the *Cx. pipiens* complex.

SPECIES COMPOSITION OF THE ANOPHELES-GAMBAIE COMPLEX (DIPTERA, CULICIDAE) AT 2 SITES IN WESTERN KENYA

PETRARCA, V; BEIER, JC; ONYANGO, F; KOROS, J; ASIAGO, C; KOECH, DK; ROBERTS, CR. 1991

At two sites in the Kisumu area of western Kenya, the species composition of the *Anopheles gambiae* complex was determined by analysis of ovarian polytene chromosomes. Of 1,915 females, 26.1% were *An. arabiensis* Patton and 73.9% were *An. gambiae* Giles; one *arabiensis* x *gambiae* hybrid was identified. No major differences in the proportions of *An. arabiensis* and *An. gambiae* were observed between sites or between years. The ratio of *An. arabiensis* / *An. gambiae* was 6.7:1 (n = 231) in cow-baited traps, 0.2:1 (n = 1,525) in indoor resting samples, and 0.5:1 (n = 145) in all-night human bait catches. The proportion of *An. arabiensis* decreased progressively from 50.0% to 8.3% (n = 1,129) during 11 wk from September to November 1987; this change was correlated negatively with night temperature and positively with temperature range. In cow-baited traps, 97.4% (n = 194) of *An. arabiensis* were cow-fed and 95.8% (n = 1,054) of *An. gambiae* from indoor resting collections were human-fed. In indoor collections, 37.2% (n = 215) of *An. arabiensis* were cow-fed and 23.1% (n = 26) of *An. gambiae* from cow traps were human-fed. This demonstrates post-blood-feeding endophily by *An. arabiensis* and suggests post-blood-feeding exophily by *An. gambiae*. Malaria infection rates were higher for *An. gambiae* than for *An. arabiensis* by a ratio of 3:1 in 1986 (by *Plasmodium falciparum* ELISA) and 2.3:1 in 1987 (by dissection). Despite the higher proportion of infective *An. gambiae*, both species in this area serve as efficient vectors through their remarkably stable contact with the human population as demonstrated by their blood feeding and resting behavior.

IDENTIFICATION OF HUMAN BLOOD IN MOSQUITOS (DIPTERA, CULICIDAE) USING NONRADIOACTIVE DNA DOT BLOT HYBRIDIZATION

SATO, C; FURUYA, Y; HARADA, M; SUGURI, S. 1992

A dot blot hybridization procedure was developed to detect human blood meals in engorged mosquitoes. A biotinylated DNA probe allowed the detection of 10-100 ng of human DNA, discriminated well between human and nonhuman sources of blood, and cross-reacted only with monkey DNA. Results showed that this method was a specific and sensitive technique for the identification of blood meals up to 100 h after ingestion. The nonisotopic label offers easy handling without the problems inherent in the use of radioisotopes, and it can be adapted for use in routine field tests.

PATTERNS OF DIVERGENCE WITHIN THE GENUS UROPHORA INFERRED FROM MTDNA RESTRICTION FRAGMENTS

MANN, W; BRANDL, R. 1992

The mtDNA from nine species of the gall-forming phytophagous fly genus *Urophora* was isolated and processed with restriction enzymes. Based on the fragment pattern the percentage of nucleotide divergence between species was estimated as between 2 and 11%. The tree generated from the divergence matrix between species was not congruent with a tree derived from morphological data. This incongruence may be explained by hybridization events, lineage sorting, and/or saturation effects.

STUDIES ON THE REPRODUCTIVE-BIOLOGY OF WHITE YAM (DIOSCOREA-ROTUNDATA POIR)

SEGNOU; FATOKUN, CA; AKORODA, MO; HAHN, SK. 1992

Investigations were conducted on the identification and distribution of yam natural pollinators, flowering pattern and hybridization of white yam (*Dioscorea rotundata* Poir.) at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, in order to provide informations for producing hybrid seeds needed for yam genetic improvement. Insects belonging to the Coleoptera (48.0%), Diptera (20.4%), Hymenoptera (20.0%), Hemiptera (5.8%), and Thysanoptera (5.8%) were caught around yam plants and presumed to be pollinators: they entered open-receptive-flowers and their presence on yam plants coincided with the duration of the flowering period. Studies on the flowering pattern revealed that: (i) Male and female clones differed in their periods of flower initiation as well as the flowering duration; (ii) Seed production and conventional breeding methods are feasible if staggered planting, water and soil fertility could be managed to ensure that flowering periods of males and females nick and also extend flowering. Artificial pollinations within white yam and between white yam and yellow yam (*D. cayenensis* Lam) using the camel hair

brush method were successful, but resulted in a relatively low fruit set (31.8% maximum). This method was more effective than natural pollination (19.2%) and can thus supplement it for the production of hybrid seeds for introducing genetic variation in yam breeding populations.

THE GENOME OF THE MEDITERRANEAN FRUIT-FLY CERATITIS-CAPITATA - LOCALIZATION OF MOLECULAR MARKERS BY INSITU HYBRIDIZATION TO SALIVARY-GLAND POLYTENE CHROMOSOMES

ZACHAROPOULOU, A; FRISARDI, M; SAVAKIS, C; ROBINSON, AS; TOLIAS, P; KONSOLAKI, M; KOMITOPOULOU, K; KAFATOS, FC. 1992

We hybridized cloned DNA segments to salivary gland polytene chromosomes of the medfly, *Ceratitis capitata*, and thus established molecular markers for 24 sites on 6 out of 10 autosomal arms. An additional marker identified a medfly repetitive element that hybridizes to approximately 100 autosomal sites as well as a granular network that is thought to represent the X chromosome. Some of the markers correspond to 9 characterized transcription units, while 17 remain anonymous; at least 3 of the latter are restriction fragment length polymorphism (RFLP) markers. The characterized transcription units document that chromosomal arm 5L of *C. capitata* is homologous to the *Drosophila melanogaster* X chromosome, in agreement with previous inferences based on the extensive conservation of linkage groups in Diptera.

TRICHOME-BORNE AND ARTIFICIALLY APPLIED ACYLSUGARS OF WILD TOMATO DETER FEEDING AND OVIPOSITION OF THE LEAFMINER LIRIOMYZA-TRIFOLII

HAWTHORNE, DJ; SHAPIRO, JA; TINGEY, WM; MUTSCHLER, MA. 1992

Oviposition and adult feeding of the leafminer *Liriomyza trifolii* (Burgess) (Diptera, Agromyzidae) on *Lycopersicon pennellii* (Corr.) D'Arcy and its F1 hybrid with *Lycopersicon esculentum* (Mill.) was significantly less than that on the cultivated tomato, *L. esculentum*. The resistance of *L. pennellii* and the F1 was reduced following rinsing of foliage with ethanol. Resistant attributes of *L. pennellii* were transferred to *L. esculentum* through appression of *L. pennellii* foliage to *L. esculentum* leaflets. Application of purified 2,3,4-tri-O-acylglucoses (the principal component of type IV glandular trichome exudate of *L. pennellii*) to *L. esculentum* significantly decreased feeding and oviposition on *L. esculentum* leaflets by 61-99%. Therefore the principal mechanism of resistance to this leafminer by *L. pennellii* is the secretion of these acylglucoses. Dose response analysis of acylglucoses applied to *L. esculentum* shows that dosages as low as 10% those found on *L. pennellii* provide large reductions (91%) in leaf punctures and mines.

THE DROSOPHILA-MELANOGASTER RIBOSOMAL-PROTEIN L17A-ENCODING GENE

NOSSELLI, S; VINCENT, A. 1992

The structure and sequence of the gene encoding the *Drosophila melanogaster* homolog of the human and yeast large-subunit ribosomal protein L17A (rpL17A) is presented. The deduced amino acid (aa) sequence of 140 residues exhibits 87% and 77% identity to that of the human (140 aa) and yeast (137 aa) rpL17As, respectively. The *D. melanogaster* rpL17A gene is single copy and maps at 58F6-59A3, a chromosome region encompassing a previously characterized Minute locus, M(2)l. Despite this extensive homology in their protein products, the *D. melanogaster* and yeast rpL17A genes display different exon-intron structures, with the first *D. melanogaster* intron mapping within the 5'-untranslated mRNA leader. The rpL17A gene gives rise to a single 600-nucleotide transcript present throughout development, and is located close to another similarly expressed gene. The 5' end of the *D. melanogaster* rpL17A mRNA contains a polypyrimidine tract displayed by several mammalian rp genes and involved in translational control of their expression.

TEMPORAL ANALYSIS OF TONE PULSES WITHIN THE COURTSHIP SONGS OF 2 SIBLING DROSOPHILA SPECIES, THEIR INTERSPECIFIC HYBRID, AND BEHAVIORAL MUTANTS OF DROSOPHILA-MELANOGASTER (DIPTERA, DROSOPHILIDAE)

BERNSTEIN, AS; NEUMANN, EK; HALL, JC. 1992

Temporal analyses were applied to the tone pulses within the courtship songs of *Drosophila melanogaster*, *D. simulans*, their interspecific hybrid, and behavioral mutants of *D. melanogaster*. Linear regression was performed on various parameters of the song pulses (cycles per pulse, absolute peak amplitude, intrapulse frequency, number of peaks in fast Fourier transform, width of the primary frequency peak, and interpulse interval), as a function of their positions within pulse trains. Significant differences in the slope values of these two species and of the mutant genotypes allowed for discriminative quantification of temporal changes within trains. These results are discussed in relation to previous kinds of temporal analyses of *Drosophila* courtship songs and also with regard to the mechanisms of song production.

MITOCHONDRIAL AND RIBOSOMAL DNA VARIATION AMONG MEMBERS OF THE ANOPHELES-QUADRIMACULATUS (DIPTERA, CULICIDAE) SPECIES COMPLEX

MITCHELL, SE; NARANG, SK; COCKBURN, AF; SEAWRIGHT, JA; GOLDENTHAL, M. 1992

The extent of intra- and inter-specific variation in mitochondrial DNA and nuclear ribosomal RNA gene restriction sites was determined for the four sibling species of the *Anopheles quadrimaculatus* complex. Individual mosquitoes were identified by allozyme analysis according to previously published keys, and the total genomic DNA of these same individuals was then cleaved with restriction enzymes. Restriction maps of mitochondrial DNA, including the positions of variable sites, were constructed for each species. No evidence for interspecific hybridization was found in the populations surveyed. There was little variation in restriction patterns within any given species, but differences occurred among the four. Three restriction enzymes (AvaI, HindIII, and PvuII) yielded species-specific DNA restriction patterns for the mitochondrial DNA, while AvaI and HindIII produced diagnostic patterns for the ribosomal DNA. Thus, restriction patterns were very useful for detecting cryptic species but less appropriate than isozymes for studying genetic structure of populations within species.

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.

INFLUENCE OF LIFE-HISTORY DIFFERENCES OF 2 TACHINID PARASITIDS OF *HELICOVERPA-ZEA* (BODDIE) (LEPIDOPTERA, NOCTUIDAE) ON THEIR INTERACTIONS WITH GLANDULAR TRICHOME METHYL KETONE-BASED INSECT RESISTANCE IN TOMATO

FARRAR, RR; KENNEDY, GG; KASHYAP, RK. 1992

The effects of glandular trichome/methyl ketone (2-tridecanone and 2-undecanone) -based insect resistance in the wild tomato, *Lycopersicon hirsutum* f. *glabratum* C.H. Mull, accession PI 134417, on *Archytas marmoratus* (Townsend) and *Eucelatoria bryani* (Sabrosky) (Diptera: Tachinidae), both parasitoids of *Helicoverpa* (= *Heliothis*) *zea* (Boddie) (Lepidoptera: Noctuidae), were investigated in the laboratory. *A. marmoratus* deposits larvae (planidia) on the foliage of its host's food plant; planidia attach to passing hosts, penetrate the cuticle, and develop in the host pupae. *E. bryani* larviposits posits directly into its host; its larvae develop in the host larva. *A. marmoratus* planidia are killed by glandular trichomes of PI 134417 and also by trichomes of hybrid lines with no methyl ketones. The methyl ketones are toxic to planidia, but at least part of the effect is due to other factors, possibly physical entanglement. Both species can be affected indirectly by methyl ketones in the diet of the host. 2-Undecanone reduces the percentage of *A. marmoratus* larvae that reach pupation. This effect is evidently due to premature death and desiccation of the host pupa caused by 2-undecanone. 2-Tridecanone in host diets had no effect on *A. marmoratus*. In *E. bryani*, 2-tridecanone in the diet of the host reduced the number of parasitoids yielded by each parasitized host, although not the overall percentage of hosts parasitized. 2-Undecanone in the diet of the host had no effect on *E. bryani*.

SENSITIVITY TO 2,4-D IN SUNFLOWER AS AN INDICATOR OF TOLERANCE TO THE SUNFLOWER MIDGE (DIPTERA, CECIDOMYIIDAE)

ANDERSON, MD; BREWER, GJ. 1992

Nine sunflower hybrids were evaluated for their sensitivity to 2,4-dichlorophenoxyacetic acid (2,4-D) as measured by the production of ethylene. Sensitivity to 2,4-D was then compared with the degree of field tolerance to the sunflower midge, *Contarinia schulzi* Gagne, exhibited by each hybrid. For the hybrids evaluated, ethylene production-increased with 2,4-D concentration and sensitivity to 2,4-D was inversely proportional to midge tolerance. The procedure may be useful in identifying midge tolerant germplasm.

SPATIAL AND TEMPORAL DISTRIBUTION OF RESTING FEMALE MOSQUITOS (DIPTERA, CULICIDAE) IN THE COASTAL-PLAIN OF NORTH-CAROLINA

IRBY, WS; APPERSON, CS. 1992

The spatial and temporal distribution of 28 species of female mosquitoes resting in natural (swamp, woods, and swamp-woods ecotone) and human-made (beneath bridges) habitats in blackwater stream-associated ecosystems in Duplin County, N.C., was determined by sampling with a D-Vac vacuum aspirator during 1984 and 1985. Two types of resting distributions were evident. In type one, species including *Aedes atlanticus* Dyar & Knab, *Ae. canadensis* (Theobald), *Ae. triseriatus* (Say), and *Psorophora ferox* (von Humboldt) rested predominantly on vegetation and were not collected beneath bridges. In type two, species including *Anopheles punctipennis* (Say), *Culex erraticus* (Dyar & Knab), *Cx. peccator* Dyar & Knab, *Cx. pipiens* L. and *Cx. quinquefasciatus* Say and their hybrids, *Cx. restuans* Theobald, *Cx. territans* Walker, and *Uranotaenia sapphirina* (Osten Sacken) rested on vegetation, in natural shelters in swamp habitats, and beneath bridges. Differences in the proportion of gravid mosquitoes among habitats were evident for *Ae. canadensis*, *An. punctipennis*, *Cx. pipiens* and *Cx. quinquefasciatus* and their hybrids, *Cx. restuans*, *Cx. territans*, *Ps. ferox*, and *Ur. sapphirina*, indicating that gonotrophic condition may influence resting site selection.

SYSTEMATICS AND MODES OF REPRODUCTIVE ISOLATION IN THE HOLARCTIC *DROSOPHILA-TESTACEA* SPECIES GROUP (DIPTERA, DROSOPHILIDAE)

GRIMALDI, D; JAMES, AC; JAENIKE, J. 1992

Reproductive isolation and morphological differences among allopatric populations of the Holarctic species *Drosophila testacea* v. Roser indicate that this taxon is actually a complex of three morphocryptic species: *D. testacea*, from Europe and continental Asia; *D. orientacea*, n.sp., from Japan; and *D. neotestacea*, n.sp., from North America. Diagnostically important morphological variation is presented, along with distributional data for these three species and *D. putrida* Sturtevant, the only other member of the *testacea* species group. Both pre- and postmating barriers to reproduction were observed in various interspecific crosses. Premating isolation is strongly asymmetric between *D. testacea* and *D. neotestacea*. Modes of postmating isolation include lack of sperm transfer and failure of hybrid eggs to hatch. The revised taxonomy of this group should facilitate analyses of interesting aspects of the evolutionary ecology of these species.

RESISTANCE OF SELECTED INTERSPECIFIC *LYCOPERSICON* HYBRIDS TO *LIRIOMYZA-TRIFOLII* (DIPTERA, AGROMYZIDAE)

ERB, WA; LINDQUIST, RK; FLICKINGER, NJ; CASEY, ML. 1993

Lycopersicon interspecific hybrids and species inbreds were examined for resistance to the leafminer *Liriomyza trifolii* (Burgess) in three separate greenhouse experiments. Two *L. pennellii* Correll interspecific hybrids (ICR.13 x LA1735 and ICR.13 x LA716) had the highest levels of resistance. A second generation of leafminers was not able to be produced on these hybrids. Other highly resistant germplasm was an interspecific hybrid of *L. cheesmanii* Riley (ICR.13 x LA1401) and one of *L. hirsutum* f. *glabratum* Muller ('Tropic' x PI126449). Larval antibiosis occurred in all the interspecific hybrids examined, and adult antibiosis and antixenosis for feeding was partially a result of the tomato plant's trichome exudates. For breeding purposes, the resistance in *L. cheesmanii* has the most immediate value because it can be reciprocally hybridized with *L. esculentum* Miller, and it produces orange fruit.

GENETIC-ANALYSIS BY DNA-FINGERPRINTING IN TSETSE-FLY GENOMES

BLANCHETOT, A; GOODING, RH. 1993

Genomic DNA from tsetse flies (Diptera : Glossinidae: Glossina Wiedemann) was analyzed by hybridization using the whole M13 phage as a probe to reveal DNA fingerprinting (DNAfp) profiles. Intrapopulation variability, measured by comparison of DNAfp profiles of tsetse flies from a large colony of *G. brevipalpis*, showed a high degree of polymorphism similar to that found in other animal species. Different lines of *G. m. morsitans*, *G. m. centralis*, *G. m. submorsitans*, *G. p. palpalis* and *G. p. gambiensi* established from small colonies displayed less genetic variability than the *G. brevipalpis* population. The analysis of pedigree relationships within an inbred line of *G. m. centralis* conformed to a Mendelian inheritance pattern. In the pedigree presented no mutations were observed, one fragment was linked to the X chromosome, and three fragment sets were linked, but most fragments showed independent segregation. M13 revealed no characteristic DNAfp profile differences between the subgenus *Glossina* and the subgenus *Nemorhina*, but a conserved distribution pattern was found in the laboratory colonies within each subspecies. M13 also revealed line specific DNA fragments that may be useful as genetic markers to expand the present linkage map of *G. m. morsitans*.

EVALUATION OF THE POLYMERASE CHAIN-REACTION METHOD FOR IDENTIFYING MEMBERS OF THE ANOPHELES-GAMBIAE (DIPTERA, CULICIDAE) COMPLEX IN SOUTHERN AFRICA

PASKEWITZ, SM; NG, K; COETZEE, M; HUNT, RH. 1993

Chromosomally and electrophoretically identified field samples of members of the *Anopheles gambiae* Giles complex from southern Africa and colony material were used to test the polymerase chain reaction (PCR) method of identification. The samples included *An. arabiensis* Patton, *An. quadriannulatus* Theobald, and *An. merus* Donitz. Only one of the 217 specimens examined was disputed. Twenty specimens (9%) were reamplified because of the presence of multiple bands or lack of amplification after the first assay. Specimens of *An. merus* from this region displayed both the diagnostic *An. merus* PCR band as well as the *An. quadriannulatus* band, possibly because of interspecific hybridization or random variation within the population. This work extends the geographical range over which the PCR technique has been verified to southern Africa.

GENETIC-ANALYSIS OF STERILITY IN HYBRIDS FROM CROSSES OF GLOSSINA-MORSITANS SUBMORSITANS AND GLOSSINA-MORSITANS-CENTRALIS (DIPTERA, GLOSSINIDAE)

GOODING, RH. 1993

When genetically marked *Glossina morsitans submorsitans* Newstead were mated to *Glossina morsitans centralis* Machado, viable offspring were obtained when using *G. m. submorsitans* females but not when using *G. m. centralis* females. The maternally inherited sterility factor, from *G. m. submorsitans*, that causes this asymmetry was inactivated or replaced during recurrent backcrossing to *G. m. centralis*. F1 hybrid males were sterile but most F1 hybrid females were fertile. There was little evidence for differential transmission of *G. m. submorsitans* and *G. m. centralis* chromosomes by hybrid females. Almost all backcross males were sterile if they had an X and a Y chromosome from two different taxa; the exceptional males had recombinant X chromosomes. The X chromosome locus for X/Y compatibility lies closer to the locus for esterase-X than to the locus for glucose-6-phosphate dehydrogenase. Heterozygosity in linkage group II is also a factor in causing hybrid male sterility; the locus for compatibility is closer to the locus for octanol dehydrogenase than to the locus for esterase-1. Among the backcross males that had an X and a Y chromosome from the same taxon, 12 % of those obtained by backcrossing to *G. m. centralis* were fertile and 65 % of those obtained by backcrossing to *G. m. submorsitans* were fertile. Backcrossing F1 hybrid females to *G. m. submorsitans* produced females that were equally likely to be fertilized by *G. m. submorsitans* and *G. m. centralis*. However, backcrossing to *G. m. centralis* produced females that had a much lower probability of being fertilized by *G. m. submorsitans* than by *G. m. centralis*.

SHORT-TERM CHANGES IN THE ABUNDANCE AND PARITY RATE OF ANOPHELES-QUADRIMACULATUS SPECIES-C (DIPTERA, CULCIDAE) IN A CENTRAL FLORIDA SWAMP

JENSEN, T; KAISER, PE; BARNARD, DR. 1993

Updraft CDC traps baited with dry ice were used to monitor changes in the abundance and parity rate of *Anopheles quadrimaculatus* Say complex mosquitoes in an intermittently flooded swamp in Central Florida during an 18-d period. Mosquitoes collected each day were identified to species using DNA hybridization and isozyme electrophoretic techniques and were dissected to determine follicular maturation and parity. Of 1,178 *An. quadrimaculatus* mosquitoes identified to species, 4% were species A and 96% were species C. Dissections of females of both species indicated that 98% were nonblood fed and nongravid with ovariole development at Christophers' stage II. Overall parity rates were 0.19 and 0.51 for populations of species A and species C, respectively. The duration of the gonotrophic cycle for species C females was estimated to be 5 d based on the interval between peaks in the number of nulliparous females collected on days 1 and 6 and peaks in the number of parous females collected 5 d later. Significant trends were observed in the number of parous species C females collected each day, indicating that the parity rate could not be used as an unbiased estimator of survivorship.

BIOCHEMICAL AND GENETIC-STUDIES ON ALKALINE-PHOSPHATASE OF CERATITIS-CAPITATA

BOURTZIS, K; MARMARAS, VJ; ZACHAROPOULOU, A. 1993

Two forms of alkaline phosphatase exist in the integument of the "white pupae" (wp) and dark pupae (dp) mutant strains of *ceratitis capitata*, during transition from larvae to pupae. They were separated by DEAE-cellulose chromatography. Both isoenzymes have a molecular weight of approximately 180,000 and two pH optima, at 9.4 and at 11.0. The isoenzymes of the "dark pupae" mutant catalyze the hydrolysis of phosphotyrosine and beta-glycerophosphate but not phosphoserine, phosphothreonine, ATP, and AMP. In contrast, the isoenzymes of the white pupae mutant hydrolyze all the substrates tested. The ALPase 1 of the dark pupae mutant was inhibited by L-tyrosine, but L-phenylalanine had no effect on either isoenzyme. The effects of divalent cations, EDTA, temperature, urea, and 2-mercaptoethanol were also investigated. Electrophoretic analysis did not reveal any variants of the larval and pupal isoenzymes, but ALPase A, an adult stage-specific isoenzyme, was found to be polymorphic. The electrophoretic variants were shown to be controlled by three codominant alleles located on the third chromosome of *Ceratitis capitata*. Since we found no hybrid enzyme, we conclude that ALPase A is monomeric.

GENETIC-VARIABILITY AND SEGREGATION ANALYSIS IN GLOSSINA-MORSITANS MORSITANS (DIPTERA, GLOSSINIDAE) USING DNA-FINGERPRINTING

BLANCHETOT, A; GOODING, RH. 1994

DNA hybridization, using the M13 sequence as a probe, was used to analyze the genetic variability in four inbred lines of the tsetse fly *Glossina morsitans morsitans* Westwood. An average of 11.2 bands (ranging from 2 to 10 kb) were found per fly. An average of nine loci were detected in each line; 40% of the loci were polymorphic and the mean heterozygosity per locus varied from 0.098 to 0.29. Averaging the data across the four inbred lines, the band sharing estimates were 82.5% in males and 81.2% in females, and the mean band frequency estimates were 0.71 and 0.70 for males and females, respectively. Segregation of fragments, determined in "three generation" pedigrees, conformed to expected Mendelian ratios and two of seven fragments studied were linked to an X chromosome marker gene, *ocra* (body color).

HYBRID DYSGENESIS-LIKE PHENOMENA IN THE MEDFLY, CERATITIS-CAPITATA (DIPTERA, TEPHRITIDAE)

TORTI, C; MALACRIDA, AR; YANNOPOULOS, G; LOUIS, C; GASPERI, G. 1994

A syndrome of abnormal genetic effects, which resembles *Drosophila* hybrid dysgenesis, is described in *Ceratitis capitata*. This syndrome includes high frequency of partial or complete female gonadal sterility, chromosomal rearrangements (bridges and fragments) at male meiosis, and instabilities at the white eye locus. It was observed in hybrids of *C. capitata* when strains of different origin were mated. The morphology of the undeveloped ovaries recovered in the medfly is apparently very similar to the gonadal distrophy which in *D. melanogaster* is associated with the P-M and hobo mediated dysgenic syndromes. The amount of gonadal sterility that

can be observed in medfly hybrids depends on the parental strains used, which exhibit specific differences in their inducing abilities. In the considered interstrain combinations, there appears to be quantitative variation in the effect of temperature on GD sterility. The highest level of sterility occurs at 25 degrees C. The pattern of abnormal traits observed in medfly hybrids appears to be the phenotypic expression of a rather complex interacting dysgenic system of inducer and suppressor effects; probably more than one system is activated in the considered crosses.

ASSOCIATION OF CLASS-II SEQUENCES ENCODING DR1 AND DQ5 SPECIFICITIES WITH HYPERSENSITIVITY TO CHIRONOMID ALLERGEN CHI-T I

TAUTZ, C; RIHS, HP; THIELE, A; ZWOLLO, P; FREIDHOFF, LR; MARSH, DG; BAUR, X. 1994

A panel of 188 unrelated Caucasian subjects who were exposed to the larvae of *Chironomus thummi* (Diptera, nonbiting midges) was HLA-typed by polymerase chain reaction amplification of the second exons of the DRB, DQA1, and DQB1 genes followed by dot-blot hybridization with sequence-specific oligonucleotide probes. Type I sensitization to the allergen Chi t I and a large number of other inhalant allergens was determined by RAST and skin testing. Sixty-one individuals were found to be sensitized to Chi t I, of whom 24 were sensitive to this allergen and to no other allergens tested. Statistical analyses showed that only in the latter group were the HLA-D genes DRB1*0101, DQA1*0101, and DQB1*0501 associated with IgE-responsiveness to Chi t I. These results suggest that HLA associations responsiveness to certain allergens may be more striking in monosensitized subjects.

GEOGRAPHICAL VARIATION IN HABITAT CHOICE AND HOST SUITABILITY IN THE PARASITOID ASOBARA-RUFESCENS

KRAAIJEVELD, AR; VOET, S; VANALPHEN, JJM. 1994

In the Netherlands, *Asobara rufescens* (Forster) (Hymenoptera: Braconidae) is a parasitoid of drosophilid larvae in decaying plant material. In several places in the Mediterranean, parasitoids looking very similar to *A. rufescens* were collected on fermenting substrates a hybridization experiment showed that the parasitoids were indeed *A. rufescens*. In an olfactometer Portuguese *A. rufescens* do not have a preference for either the odour of yeast or decaying leaves, while their Dutch conspecifics prefer the odour of decaying leaves. The survival probability of Portuguese *A. rufescens* in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), a species typical for fermenting substrates, is much higher than the survival probability of Dutch *A. rufescens* in this host species. It is hypothesized that decaying plant material may be unsuitable for drosophilid larvae during part of the year in the Mediterranean, forcing *A. rufescens* there to broaden its microhabitat choice. The use of fermenting substrates brings *A. rufescens* in contact with its sibling *A. tabida* Nees, a species typical for fermenting substrates in most of Europe. Portuguese *A. rufescens* appear to be genetically isolated from *A. tabida*. In the Netherlands, where the two species occupy different microhabitats, there is only a premating barrier.

EXPLOITATION OF SORGHUM NONPREFERENCE RESISTANCE TO SORGHUM MIDGE (DIPTERA, CECIDOMYIIDAE) USING MIXED PLANTINGS OF RESISTANT AND SUSCEPTIBLE SORGHUM HYBRIDS

TEETES, GL; ANDERSON, RM; PETERSON, GC. 1994

The effectiveness of sorghum resistance to sorghum midge, *Contarinia sorghicola* (Coquillett), was improved in mixed plantings of resistant and susceptible sorghum, *Sorghum bicolor* (L.) Moench, hybrids in 1989 and 1992. Mixed plantings drew sorghum midges from the resistant to the susceptible plants, and damage to resistant plants was lessened. Adult sorghum midge abundance averaged 5.5 and 1.6 sorghum midges per panicle during the flowering period in 1989 and 1992, respectively. Mean sorghum midge damage ratings for susceptible and resistant plants were 5.9 and 2.4, and 7.7 and 1.6 in 1989 and 1992, respectively. Damage to resistant plants decreased and per panicle yield increased as the composition of susceptible sorghum plants in mixed plantings increased. Total grain yield (yield of resistant and susceptible plants combined) was highest when the proportion of susceptible plants was from 10 to 30% in 1989 and was 20 and 30% in 1992 and declined when percentage of susceptible plants was increased further. Total grain yields were less when all plants were resistant than when 10 to 30% and 20% of the plants were susceptible in 1989 and 1992, respectively.

CYTOGENETIC VARIATION IN NATURAL-POPULATIONS OF THE OLD-WORLD SCREWORM FLY CHRYSOMYA-BEZZIANA (DIPTERA, CALLIPHORIDAE)

BEDO, DG; SPRADBERRY, JP; MAHON, RJ. 1994

The existence of sibling species in the Old World screwworm fly *Chrysomya bezziana* would raise serious problems in eradicating this pest if it entered Australia. Cytogenetic variation in *C. bezziana* was investigated by analyzing pupal trichogen polytene chromosomes. Natural populations of *C. bezziana* spanning its range from southern Africa to Papua New Guinea were examined as well as hybrids between a New Guinea laboratory strain and natural populations. No evidence of sibling species was found. All populations exhibited the same basic banding pattern as the standard sequence established from a Papua New Guinea strain. Extensive asynapsis of chromosome homologues was found in some hybrid crosses and was therefore measured in all populations and hybrids to detect systematic variation. Asynapsis levels in most hybrids could not be statistically distinguished from those present in the parent populations except for crosses between populations at the ends of the range. This result does not permit asynapsis levels to be used in establishing the origin of introduced flies by estimating their distance from known populations. One inversion polymorphism and six band polymorphisms spread over three chromosomes were analyzed. Populations in each sampled region had characteristic combinations of band polymorphisms. This may offer a diagnostic method for determining the origin of flies accidentally introduced to Australia.

EFFECT OF CYTOPLASMIC MALE-STERILITY IN SORGHUM ON HOST-PLANT INTERACTION WITH SORGHUM MIDGE, CONTARINIA-SORGHICOLA

SHARMA, HC; VIDYASAGAR, P; ABRAHAM, CV; NWANZE, KF. 1994

Sorghum midge, *Contarinia sorghicola* Cog. (Diptera: Cecidomyiidae) is one of the most important pests of grain sorghum worldwide. We studied the reaction of midge-resistant and midge-susceptible genic-cytoplasmic male-sterile (A-lines) and their maintainers (B-lines), and the effect of resistant and susceptible restorers on sorghum midge. Midge damage and adult emergence were significantly lower on the B-lines of midge-resistant genotypes (PM 7061 and PM 7068) than their corresponding A-lines, while the reverse was true for the midge-susceptible genotypes (296A and ICSA 42). Differences in midge damage and the number of midges emerged were not significant between the midge-resistant and midge-susceptible A-lines when infested without pollination (except midge emergence on PM 7061A). Pollination with a midge-resistant restorer (DJ 6514) reduced midge emergence significantly in one of two seasons. Source of pollen did not influence midge emergence on the highly-resistant A-line, PM 7061A. The implications of these observations in the development of midge-resistant hybrids were discussed.

LARVAL DIAPAUSE, SIZE, AND AUTOGENY IN THE MOSQUITO AEDES TOGOI (DIPTERA, CULICIDAE) FROM TROPICAL TO SUB-ARCTIC ZONES

SOTA, T. 1994

Larval development of nine populations of *Aedes togoi* (Theobald) collected from tropical to subarctic zones was studied under photoperiods of 16 h light (L) : 8 h dark (D) and 10 h L : 14 h D at 15 degrees C. Larvae that did not pupate within 60 days were regarded as diapausing. Larval diapause did not occur in all populations at

16 h L : 8 h D. At 10 h L : 14 h D, four populations from tropical and subtropical zones included no diapausing larvae, whereas 60-99% of individuals from five northern populations of Japan and Canada were diapausing. Adult size increased with latitude of original locality. The proportion of autogenous females among those developed at 16 h L : 8 h D and 15 degrees C decreased with original latitude, whereas that among females from nondiapausing larvae reared at 10 h L : 14 h D and 15 degrees C was consistently high throughout the latitudes. At 10 h L : 14 h D and 15 degrees C, larval developmental characters of F-1 and F-2 hybrids between Malaysian (no diapause) and Japanese (diapausing) populations were intermediate between the parental populations. Expression of autogeny of the hybrids was similar to that of the Malaysian strain. A Canadian population, which might have been introduced from Japan, showed developmental traits similar to those of two populations

CHARACTERIZATION OF THE CULEX-PIPIENS COMPLEX (DIPTERA, CULICIDAE) IN MADAGASCAR

URBANELLI, S; SILVESTRINI, F; SABATINELLI, G; RAVELOARIFERA, F; PETRARCA, V; BULLINI, L. 1995

Morphological analysis of phallosome and multilocus electrophoresis were used to characterize populations of the *Culex pipiens* L. complex from Madagascar. Samples phenotypically and genetically corresponding to *Cx. p. quinquefasciatus* Say were found on the east and west coasts, whereas, on the high plateau, 1 sample was composed mostly of phenotypical *Cx. p. pipiens* L., genetically introgressed with *quinquefasciatus* at some loci (Hbdh, Aat-e, and Hk-1). A hybrid zone between the 2 taxa was detected on the plateau on a genetic basis, whereas at the morphological level a predominance of *Cx. p. quinquefasciatus* specimens and deficit of intermediates was observed. Accordingly, morphological analysis failed to describe satisfactorily the hybridization phenomena. Despite the high level of gene exchange, a complete mixing of the 2 gene pools apparently does not occur, possibly because of differential selective pressures in the climatically heterogeneous environment of the Madagascar plateau.

DISTRIBUTION AND HYBRIDIZATION BETWEEN CULEX-PIPIENS AND CULEX-QUINQUEFASCIATUS (DIPTERA, CULICIDAE) IN ARGENTINA

ALMIRON, WR; HUMERES, SG; GARDENAL, CN. 1995

To better understand the distribution of *Culex pipiens* and *Cx. quinquefasciatus* in Argentina, samples were collected from six localities situated in a North-South line from Castelli (Chaco Province) to Puerto Madryn (Chubut Province). Identification was based on the morphology of male genitalia. Only *Cx. quinquefasciatus* was found in Castelli and Esperanza, while in Rosario, 95.3% belonged to this species and 4.7% represented hybrid forms. Southern samples included only *Cx. pipiens*. With the purpose of verifying if *Cx. pipiens* and *Cx. quinquefasciatus* hybridize, different crosses between the two species were performed. All crosses produced viable egg rafts. Hatching ranged from 70 to 100%, except in one cross, female *Cx. pipiens* x male *Cx. quinquefasciatus*, where a high incompatibility was observed (11.1% hatch). The F-1 hybrids obtained from all crosses were fertile. The finding of hybrid forms in nature can be interpreted as evidence for subspecific status of *Cx. pipiens* and *Cx. quinquefasciatus* in Argentina.

Field application of the polymerase chain reaction (PCR) to the detection and characterization of trypanosomes in *Glossina longipalpis* (Diptera: Glossinidae) in Cote d'Ivoire

Solano, P; Argiro, L; Reifenberg, JM; Yao, Y; Duvallet, G. 1995

The Polymerase Chain Reaction (PCR) technique was used for the identification of natural trypanosome infections in *Glossina longipalpis* (Diptera: Glossinidae) in Cote d'Ivoire. A total number of 139 flies were examined microscopically for the presence of trypanosomes. Out of them 50 were detected positive and were subsequently prepared for the PCR using primers specific for *Trypanosoma* (*Nannomonas*) *congolense* of Savannah, Riverine-Forest, Kilifi, and Tsavo types, *T. (N.) simiae*, *T. (Duttonella) vivax* and *Trypanozoon*. Almost 90% of the infections detected by the PCR were attributed to *Nannomonas*, especially *T. congolense* Savannah and Riverine-Forest types, with many infections in which both of these two types were present. *T. simiae* and *T. vivax* were also detected in some flies. The sequence specificity of the PCR products was confirmed by hybridization with parasite-type specific DNA probes. Differences between parasitological and PCR results are discussed.

SSP GENES AND SPORE OSMOTOLERANCE IN BACILLUS-THURINGIENSIS ISRAELEMENSIS AND BACILLUS-SPHAERICUS

CUCCHI, A; DERIVAS, CS. 1995

It was shown previously that spores and vegetative cells of *Bacillus sphaericus* (Bf) and *Bacillus thuringiensis israelensis* (Bti) are very sensitive to osmotic variations. Since spore osmotolerance has been associated with their SASP (small acid soluble spore proteins) content coded by *ssp* genes, hybridization assays were performed with *sspE* and *sspA* genes from *B. subtilis* as probes and showed that Bti and Bf strains could lack an *sspE*-like gene. The *B. subtilis* *sspE* gene was then introduced into Bti 4Q2 strain; spores were obtained and showed a 65 to 650 times higher level of osmotolerance to NaCl, without affecting other important properties: hypoosmotic resistance in vegetative cells, spore UV resistance, and larvicidal activity against diptera larvae.

BEHAVIORAL REPRODUCTIVE ISOLATION IN DROSOPHILA-SILVESTRIS, D-HETERONEURA, AND THEIR F1 HYBRIDS (DIPTERA, DROSOPHILIDAE)

PRICE, DK; BOAKE, CRB. 1995

We investigated the role that courtship and aggressive interactions may have for the maintenance of reproductive isolation between *Drosophila silvestris* and *D. heteroneura*. We examined the behavioral bases of reproductive isolation between the parental species and we examined the courtship success of each sex of both reciprocal F-1 hybrids when paired with the parental species. We found reduced copulation success among heterotypic parental pairs compared to homotypic pairs, which was primarily due to the lack of courtship initiation between *D. silvestris* males and *D. heteroneura* females. When hybrid males from both reciprocal crosses were paired with parental females their copulation successes were not significantly different from that of parental males. In contrast, hybrid females from both crosses had reduced copulation success with *D. silvestris* males, which in turn was primarily due to a reduced success of reaching later stages of courtship. The time spent in copulation by hybrid males was intermediate between the two parental males. We studied aggression by observing the interactions of males of heterotypic pairs, both between the parental species and between the hybrids and parental males. A lack of aggressive interactions between *D. silvestris* males and *D. heteroneura* males in addition to the lack of courtship suggests that *D. silvestris* males do not respond to *D. heteroneura* individuals of either sex. Hybrid males were equally successful in winning fights with both *D. silvestris* and *D. heteroneura* males. These results indicate that the behavioral isolation between *D. silvestris* and *D. heteroneura* may be largely a consequence of the earliest stages of interactions. The two species may differ either in activity levels or in morphological or chemical traits that are important for species and mate recognition. The relatively high copulation and aggressive success of hybrids indicates that sexual selection against hybrids alone is unlikely to be a sufficient force to reduce gene flow and maintain species distinctions.

SIMPLE NONRADIOACTIVE DNA HYBRIDIZATION METHOD FOR IDENTIFICATION OF SIBLING SPECIES OF ANOPHELES DIRUS (DIPTERA, CULICIDAE) COMPLEX

AUDTHO, M; TASSANAKAJON, A; BOONSAENG, V; TPIANKIJAGUM, S; PANYIM, S. 1995

A simple method was developed for species identification of mosquitoes in the *Anopheles dirus* complex found in Thailand using horseradish peroxidase-labeled DNA probes and a chemiluminescent detection system. Species-specific DNA probes for *Anopheles dirus*, species B, C, and D detected 1-5 ng of target DNA, a sensitivity that was comparable with the radioisotopic detection system. Identification of individual mosquitoes was performed by dot-blot analysis of crude mosquito DNA. The method allowed identification using third or fourth instars as well as the adult head, thorax, or abdomen. This technique successfully identified the sibling species of the *A. dirus* complex in field collections.

NEW DISCRIMINATING TRAITS BETWEEN FEMALES OF 2 SIBLING SPECIES - DROSOPHILA-MELANOGASTER AND DROSOPHILA-SIMULANS (DIPTERA, DROSOPHILIDAE)

MORETEAU, B; PETAVY, G; GIBERT, P; MORIN, JP; MUNOZ, A; DAVID, JR. 1995

Females of the two sibling cosmopolitan species *D. melanogaster* and *D. simulans* are generally considered as very similar and impossible to distinguish, especially under field conditions. Various traits have been proposed to separate the species but the only completely diagnostic character is the length of egg filaments. This requires, however, either isolating each female in a culture vial, or dissecting its ovaries. We have found two new traits which provide complete discrimination between laboratory grown flies, i.e. the pattern of abdominal pigmentation and the wing/thorax ratio. These two traits can also be used for sorting out the two types of females in wild living samples. However the discrimination, which is efficient, does not provide absolute certainty, because of a much greater phenotypic variability. Interspecific hybrids are intermediate between parents and cannot be readily identified with these characters. For wing and thorax lengths and ratio, a dominance of *D. simulans* was observed in hybrids, as well as an important increase of individual variability.

DEVELOPMENT OF DNA PROBES FOR THE IDENTIFICATION OF SIBLING SPECIES-A OF THE ANOPHELES-CULICIFACIES (DIPTERA, CULICIDAE) COMPLEX

GUNASEKERA, MB; DESILVA, BGDNK; ABEYEWICKREME, W; SUBBARAO, SK; NANDADASA, HG; KARUNANAYAKE, EH. 1995

Three highly repetitive DNA sequences Rp36, Rp217 and Rp234, have been isolated from *Anopheles culicifacies* Giles sensu lato. The cloned DNA sequences were found at a higher copy number in species B and C, than in species A of the *A. culicifacies* complex. These sequences may therefore be used as DNA probes to distinguish species A from the other two species, using a 200-fold dilution of a single mosquito DNA extract in a dot-blot hybridization assay. Rp36 and Rp217 have been completely sequenced. Internal repeats were absent in Rp36. Two related core sequences of 13 and 16 bp were found tandemly repeated in Rp217. These probes enable the rapid detection of species A of *A. culicifacies* in field investigations.

INTERACTIONS BETWEEN 3 SPECIES OF MUSHROOM CECIDS (DIPTERA, CECIDOMYIIDAE) AND 3 HYBRID STRAINS OF THE CULTIVATED MUSHROOM AGARICUS-BISPOROUS

CLIFT, AD; TERRAS, MA. 1995

The effect of three species of paedogenetic cecidomyiid larvae, at two infestation times, on the yields of three hybrid *Agaricus bisporus* strains is reported. The densities of larvae that developed, time of larval swarming and rates of pupation were recorded. *Mycophila barnesi* was the most damaging species, followed by *Heteropeza pygmaea* and *Henria psalliotae*. Time of infestation was important, with earlier infestations of all species more damaging, both in direct yield loss and by larvae contaminating sporophores. The hybrid strains also responded differently, with the U3 hybrid most susceptible, followed by U1 and the Intermediate hybrid. The exception was *M. barnesi* infesting the Intermediate hybrid at spawning, contaminating over 90% of the sporophores.

HOST RELATIONSHIPS OF DASINEURA-INGERIS - OVIPOSITION PREFERENCES AND JUVENILE SURVIVAL ON CLONES OF SALIX-VIMINALIS, OTHER SALIX SPECIES AND HYBRIDS

AHMAN, I; LOVGREN, L. 1995

In the present study we assessed the extent to which oviposition preferences and larval survival rates of the gall midge *Dasineura ingeris* Sylven & Lovgren (Diptera: Cecidomyiidae) can explain variation in its use of *Salix viminalis* phenotypes as hosts. Furthermore, based on oviposition preferences and juvenile survival rates we estimated the host potential of various *Salix* species and species hybrids. Egg distribution patterns explained 67% of the intraspecific variation in damage to *S. viminalis* shoots in the field. Juvenile survival, on the other hand, was not related to field damage or egg distribution patterns among the *S. viminalis* clones studied. Clones of complete or partial *S. viminalis* origin ranked highest in suitability for *D. ingeris* survival, and four out of these six clones also received the highest egg rankings. Species belonging to the subgenus *Salix* were not oviposited on at all, whereas others belonging to the same subgenus as *S. viminalis*, i.e. *Vetrix*, were intermediate in terms of oviposition rates. Although there was a significant correlation between *D. ingeris* egg distributions and larval survival rates on the clones in the *Salix* species/hybrid set, mismatches were evident.

MOLECULAR CHARACTERIZATION OF AN 18 KB SEGMENT OF DNA PUFF C4 OF BRADYSIA-HYGIDA (DIPTERA, SCIARIDAE)

MONESI, N; FERNANDEZ, MA; FONTES, AM; BASSO, LR; NAKANISHI, Y; BARON, B; BUTTIN, G; PACOLARSON, ML. 1995

The data presented here are an extension of the molecular characterization of DNA puff C4 of *Bradysia hygida*. A cDNA related to a gene amplified in this puff and expressed when puff C4 expands was cloned and sequenced. Analysis of the amino acid sequence deduced from the open reading frame present in the cDNA indicate that the encoded protein is secreted and comprises mostly alpha-helical coiled-coil. An 18 kb genomic segment containing the transcription unit of this gene was also cloned and the structure and expression of the 1.4 kb mRNA was determined. Quantitative slot blot hybridization of DNA complementary to the transcription unit shows that this gene is amplified about 21 times in the salivary gland, confirming data previously obtained. Fragments upstream of the 5' end, and beyond the 3' end, of the gene transcription unit were also analysed and shown to be amplified at least eight and five times, respectively. Based on these data we discuss how amplification could occur at DNA puffs.

HYBRID DYSGENESIS IN THE MEDFLY CERATITIS-CAPITATA (DIPTERA, TEPHROTIDAE)

MALACRIDA, AR; TORTI, C; YANNOPOULOS, G; LOUIS, C; GASPERI, G. 1995

COMPARISON OF DIET IN COLLARED FLYCATCHER (*FICEDULA-ALBICOLLIS*) AND PIED FLYCATCHER (*FICEDULA-HYPOLEUCA*) NESTLINGS IN A HYBRID ZONE

BURES, S. 1995

A comparison of collared (*Ficedula albicollis*) and pied flycatcher (*Ficedula hypoleuca*) diet was conducted on the basis of simultaneous sampling of nestling diet taken by the ligature method in oak-beech forests in central Moravia. Diet in the collared flycatcher contained 657 prey items and that of the pied flycatcher 747. No difference in average body length of prey was found. Differences were found in diet composition, both in total and during sunny and rainy periods. In total, the diet of the collared flycatcher contained more Coleoptera and Heteroptera and less Diptera. The collared flycatcher compensated for reduced intake of Araneida, Bibionidae and Coleoptera during rainy periods, with an increased uptake of Formicidae, whereas the pied flycatcher hunted more Formicidae, Heteroptera, Limoniidae and Syrphidae. The results show that species specific prey selection and responses to changing prey availability can reduce food competition during nestling period.

Cytotaxonomy of Chironomidae (Diptera) from Lake Shabla (Bulgaria): Cytogenetic evidence for introgressive hybridization

Michailova, PV. 1996

Twenty-two chironomid species were found in Lake Shabla. Five species (*Chironomus bonus*, *Dicrotendipes lobiger*, *Kiefferulus tendipediformis*, *Cricotopus suspiciosus* and *Cricotopus sp.*) are new for Bulgaria. Their karyotypes are described for the first time. Cytogenetic studies of two closely related chironomid species, *Glyptotendipes glaucus* and *G. pallens*, show that in one area of sympatry (Lake Shabla, Bulgaria) about 2.5% of the naturally occurring individuals are hybrids. In most cases, in this hybrid, 1st, 11nd and 111rd chromosomes correspond fully to the chromosomes of *G. glaucus*. The IVth chromosome is a hybrid, with one homologue of the species *G. glaucus*; the other homologue is derived from *G. pallens*. Introgressive hybridization is of particular importance to the production of these combinations. Despite hybridization, natural selection appears to maintain the essential integrity of each separate gene pool.

Evolution of multiple families of non-LTR retrotransposons in phlebotomine sandflies

Booth, DR; Ready, PD; Smith, DF. 1996

In this paper we report on the diversity and distribution of a set of non-LTR retrotransposon (RTP) reverse transcriptase (RT) sequences isolated from phlebotomine sandflies, and their potential for investigating the evolutionary histories of members of this subfamily of flies (Diptera: Psychodidae, Phlebotominae). The phlebotomine RT sequence families derived from one species were as different from each other as they were from RT sequences derived from other species. When each was used to probe Southern blots of sandfly genomic DNA they hybridized only to the species of source and, usually, to others of the same subgenus, but not to DNA from other subgenera - a hybridization pattern consistent with vertical evolution. There was considerable intraspecific variation in hybridization pattern, suggesting the RTs were part of non-LTR RTPs that are (or were recently) subject to flux in genomic position and copy number. Most of the RT families detected in phlebotomines are monophyletic with respect to previously described RTs, and all are monophyletic with RTs of the F/Jockey (*Drosophila melanogaster*) type of RTP. Orthologous sequences were isolated from the closely related species *Phlebotomus perniciosus* and *P. tobbi* (subgenus *Larrousius*), and different populations of *P. perniciosus*. The level of sequence divergence among these orthologous RTs, the subgeneric distribution of each RT family, and the intraspecific variation in hybridization pattern of many of them, indicate this class of sequence will provide genetic markers at the sub-generic level.

Species, sub-species and hybrid populations of the blowflies *Lucilia cuprina* and *Lucilia sericata* (Diptera: Calliphoridae)

Stevens, J; Wall, R. 1996

The blowflies *Lucilia cuprina* Wiedmann and *Lucilia sericata* Meigen (Diptera: Calliphoridae) are facultative ectoparasites of warm blooded vertebrates, particularly domestic sheep. Despite being similar in morphology and ecology, the two species and different populations of each species, are known to vary in their importance as pests in different regions of the world. To elucidate the genetic basis of these species and population level differences, flies were collected from sites in Africa, Europe, Australasia, North America and the islands of Hawaii, and examined using a combination of morphology, the random amplified polymorphic DNA technique and a complementary mitochondrial DNA analysis. The results confirm the species integrity of *L. sericata* and *L. cuprina* and support the existence of intra-specific genetic variation in *L. cuprina*, but not *L. sericata*.

Cost/benefit analysis of a sorghum hybrid resistant to sorghum midge (Diptera: Cecidomyiidae)

Ervin, RT; Khalema, TM; Peterson, GC; Teetes, GL. 1996

The economic benefits resulting from the use of sorghum cultivars resistant to sorghum midge, *Stenodiplosis sorghicola* (Coquillett), were compared with the research and development costs. In 1984 dollars, the cost to develop the technology was approximately \$2,720,000. For each dollar invested in research and development of a sorghum midge-resistant sorghum hybrid, the value of benefits to increased crop yields from the use of the resistant hybrid ranged from \$24.2 at a 5% discount rate (the interest rate used to determine the present value of annual benefits and/or costs collected over time) when no insecticide was used to \$2.7 at a 20% discount rate when insecticide was applied five times. The average value of benefits to increased crop yields at zero, three, and five insecticide applications at discount rates of 5, 10, 15, and 20% was \$9.9 for each dollar spent to develop the technology. The intangible benefits that accrue to society as a result of the development and use of insect-resistant sorghum were not estimated. Important benefits from the development and deployment of sorghum midge-resistant sorghums include reduced crop production costs and greater yield, as well as enhanced sustainability of the sorghum production system and the conservation of natural and biological resources.

Intra-specific heterogeneity of the rDNA internal transcribed spacer in the *Simulium damnosum* (Diptera: Simuliidae) complex

Tang, JM; Toe, L; Back, C; Unnasch, TR. 1996

The internal transcribed spacer (ITS) of the rRNA gene cluster has been used as a model for the study of the action of concerted evolution and molecular drive on repeated sequence families. In contrast to this general finding, preliminary DNA sequence analysis of cloned representatives of the ITS from the West African black fly species complex *Simulium damnosum* s.l. demonstrated extensive intra-individual and intra-specific polymorphisms. Variability in the ITS was primarily confined to the ITS1 domain. The degree and type of intra-individual and intraspecific variability within the ITS was further characterized using gel electrophoresis, DNA hybridization, and heteroduplex analysis of the PCR products generated from the ITS1 domain. ITS1 copies from individual *S. damnosum* s.l. differed in length and sequence composition. These results, when taken together, demonstrate that a large degree of intra-individual and intra-specific heterogeneity exists in the ITS of *S. damnosum* s.l. The intra-individual heterogeneity was greater in the savanna-dwelling than forest-dwelling sibling species of *S. damnosum* s.l. This heterogeneity may be due in part to inter-breeding among sympatric sibling species, coupled with disturbance of *S. damnosum* s.l. populations resulting from intensive vector control efforts.

Evaluation of a laboratory bioassay for determining resistance levels to sorghum midge *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in grain sorghum

Franzmann, BA. 1996

The level of resistance to the sorghum midge, *Contarinia sorghicola*, in a range of grain sorghum hybrids was assessed: in the laboratory, by measuring oviposition (eggs/spikelet and % spikelets infested); in the glasshouse, by measuring oviposition and seed set; and in the field, by measuring panicle weight loss per ovipositing female per day. The levels of oviposition and seed set determined in the laboratory and glasshouse trials were significantly correlated with the field parameter. Number of eggs per spikelet was the laboratory parameter most highly correlated with the field result ($r = 0.93$, $P < 0.01$). Studies on the rate of oviposition under the conditions of the bioassay confirmed 6 h as an appropriate interval to expose panicles to midges. A laboratory method based on estimating the number of eggs produced by 5 midge females on 50 flowering spikelets in 6 h at 25 degrees C and 75%rh is suggested as a practical method of testing for level of resistance in those sorghum hybrids showing ovipositional antixenosis.

Development of insecticide resistance in *Piophilidae* (Diptera: Piophilidae) strains selected with low doses of deltamethrin

Rossi, E; Presciuttini, S. 1996

Two populations of the cheese skipper, *Piophilidae* (L.), were sampled. The 1st was from a sheep farm where no chemical was ever applied; the 2nd was present in a ham factory where chemical treatment with pyrethroids was applied in the past against house flies, *Musca domestica* L., and Dermestid beetles. A substantial difference between their resistance to deltamethrin was observed (LC(50) = 11.56 versus 68.08 $\mu\text{g/cm}^2$ for females and 1.11 versus 4.20 $\mu\text{g/cm}^2$ for males, respectively). Laboratory strains were established from both populations and were selected at constant rates for up to 20 generations (2, 4, and 7.3 ppm for the strains derived from the 1st population and 40 ppm for that selected from the 2nd population). Males and females of both strains showed an increase in LC(50) (tested at the 5th and 19th or 10th generation), except when females were selected at the lowest rate (2 ppm). At the end of the trial, slopes of logit regressions were substantially steeper in strains selected at higher rates, suggesting that the effect of the insecticide was to reduce resistance variance. Crosses between unselected and selected strains were done. Results of survival analysis in F-1 hybrids were analogous for males and females and were similar in both reciprocal crosses. LC(50)s were intermediate between those of the parental strains.

Genetics of hybridization of *Glossina swynnertoni* with *Glossina morsitans morsitans* and *Glossina morsitans centralis*

Gooding, RH. 1997

Reciprocal crosses were performed with *Glossina swynnertoni* and *Glossina morsitans morsitans* and with *G. swynnertoni* and *Glossina morsitans centralis*, using strains that carried marker genes in all three linkage groups. *Glossina swynnertoni* males can inseminate, but not fertilize, *G. m. morsitans*; all other crosses produced some fertile females. Hybridization did not cause sex ratio distortion among F-1 flies. Most F-1 and backcross females were fertile, but all F-1 males were sterile. Sterility among backcross males was also high (99% in Bx(1), 85% in Bx(2), and about 50% in Bx(3) to Bx(6)). Chromosome transmission by hybrid females usually conformed to Mendelian expectations, but genetic recombination was lower than observed in *G. m. morsitans*. The reduction in fertility among backcross females was not associated with heterozygosity in any linkage group. Sterility among hybrid and backcross males was associated with heterozygosity of sex chromosomes and probably autosomes. The results support the systematic placement of *G. swynnertoni* closer to *G. m. centralis* than to *G. m. morsitans*.

Field morphological variation and laboratory hybridization of *Culicoides variipennis sonorensis* and *C. v. occidentalis* (Diptera: Ceratopogonidae) in southern California

Velten, RK; Mullens, BA. 1997

Two field populations of *Culicoides variipennis* (Coquillett) from southern California, *C. v. occidentalis* Wirth & Jones from the Salton Sea and *C. v. sonorensis* Wirth & Jones from a dairy wastewater pond in the Chino Basin, were sampled monthly from February to July (6-7 mo). Morphometric analyses of slide-mounted adults reared from field-collected larvae and pupae indicated that females of the 2 forms were indistinguishable. Two of the standard characters, ying length and mandibular teeth, were correlated with seasonal temperature changes. Males of *C. v. sonorensis* were distinguishable by the presence of spicules on the aedeagus, which were entirely lacking in *C. v. occidentalis*. Two populations of *C. v. occidentalis* (Salton Sea and Bolsa Chica Marsh) and a laboratory strain of *C. v. sonorensis* hybridized successfully in the laboratory and were maintained for 6 generations. Differential hybrid viability (F-1) was observed in reciprocal crosses. Males of *C. v. occidentalis* mated with females of *C. v. sonorensis* resulted in a lower egg hatch (7.4%) than did the reciprocal cross (75.6%). Hybrid males displayed spicules on the aedeagus (a character of *C. v. sonorensis*), but the number of spicules was sometimes reduced compared with parental *C. v. sonorensis* (AA strain). Spicules in a field population of *C. v. sonorensis* were similar in number to the laboratory *C. v. sonorensis*-*C. v. occidentalis* hybrids. Based on successful hybridization, the 2 forms should be considered closely related. The 2 forms are separated ecologically by the nature and distribution of their larval habitats.

Development of a polymerase chain reaction assay for differentiation between *Culex pipiens pipiens* and *Cx-p-quinquefasciatus* (Diptera: Culicidae) in North America based on genomic differences identified by subtractive hybridization.

Crabtree, MB; Savage, HM; Miller, BR. 1997

Culex pipiens is a complex of mosquitoes that are involved in the transmission of pathogens, including St. Louis encephalitis virus in North America. The 2 major taxa in the complex, *Cx. p. pipiens* and *Cx. p. quinquefasciatus*, are nearly identical morphologically, making identification of field-collected specimens difficult, and attempts at differentiation based on biochemical and molecular techniques have been unsuccessful. We report here the use of genomic subtractive hybridization to identify a region of nucleic acid heterology between the genomes of *Cx. p. pipiens* and *Cx. p. quinquefasciatus* and the development of a polymerase chain reaction (PCR) assay to discriminate between them. PCR primers based on the nucleic acid sequence of a *Cx. p. pipiens*-unique DNA fragment were used to differentiate *Cx. p. pipiens* and *Cx. p. pipiens/quinquefasciatus* hybrids from *Cx. p. quinquefasciatus* by using extracted individual mosquito genomic DNA, crude DNA preparations from a mosquito head or legs, and DNA from triturated mosquito pools.

Californian hybrid zone between *Culex pipiens pipiens* and *Cx p quinquefasciatus* revisited (Diptera: Culicidae)

Urbanelli, S; Silvestrini, F; Reisen, WK; DeVito, E; Bullini, L. 1997

Phallosome morphology (DV/D ratio) and allozyme variation were used to re examine the transition from *Culex pipiens pipiens* L. to *Cx. p. quinquefasciatus* Say, detected in California from the northern Central Valley to the Mexican border of the United States of America. Significant deficiency of heterozygotes was observed at the diagnostic locus Mdhp-2 in populations from the central part of the hybrid zone. Long tails of introgression were detected: populations from both north and south ends of the transect were not genetically pure *Cx. p. pipiens* or *Cx. p. quinquefasciatus*, respectively, as previously considered, but included approximate to 5% introgressed individuals. A narrow reversed dine from the Delta area into the Sacramento Valley, characterized by increasing frequencies of *Cx. p. quinquefasciatus* alleles proceeding to the north, was confirmed. Both these dines appear to be related mainly to temperature gradients. Over the last 50 yr, an increase in the proportion of *Cx. p. pipiens* DV/D phenotypes was detected proceeding north to south along the main latitudinal dine, as well as in the narrow reversed dine.

Accordingly, the center of the main latitudinal hybrid zone has apparently moved approximate to 100 km to the south. This phenomenon is only partially paralleled by the differentiated locus Pgm of the 3 for which comparison was possible. Similarities to and differences from previous studies are discussed, also in relation with comparable data from another hybrid zone between *Cx. p. pipiens* and *Cx. p. quinquefasciatus* recently detected in Madagascar. Hybrid index scores based on differentiated allozymes and the diagnostic locus Mdhp-2 prove to be better descriptors than the DV/D ratio of hybridization and introgression occurring between *Ca. p. pipiens* and *Ca. p. quinquefasciatus*. This seems to be caused mainly by the influence of temperature on male genitalia development, and the weaker association found between genetic markers and DV/D phenotypes in hybrid populations.

Multiplex polymerase chain reaction for detection of *Dirofilaria immitis* (Filarioidea : Onchocercidae) and *Wuchereria bancrofti* (Filarioidea : Dipetalonematidae) in their common vector *Aedes polynesiensis* (Diptera : Culicidae)

Nicolas, L; Scoles, GA. 1997

In French Polynesia, *Aedes polynesiensis* (Marks) is the vector of the human filarial parasite *Wuchereria bancrofti* (Cobbold) and dog heartworm, *Dirofilaria immitis* (Leidy). A multiplex polymerase chain reaction (PCR) assay was designed to screen pools of field-collected *Ae. polynesiensis* for the presence of both parasites simultaneously using primers specific for each parasite. The sensitivity of detection on purified DNA was 1 and 10 pg, equivalent to 0.1 and 1 L3 larva per pool for *W. bancrofti* and *D. immitis*, respectively. Codetection was performed at an hybridization temperature of 58 degrees C to avoid competition between heterologous DNA and primers that was observed at 55 degrees C. In addition, *D. immitis* was detected by PCR in the blood of infected dogs.

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.

Genetic analysis of hybrid sterility in crosses of the tsetse flies *Glossina palpalis palpalis* and *Glossina palpalis gambiensis* (Diptera: Glossinidae)

Gooding, RH. 1997

Reciprocal crosses of *Glossina palpalis gambiensis* Vanderplank and *Glossina palpalis palpalis* (Robineau-Desvoidy) were carried out using flies that had four marker genes on the X chromosome, two in linkage group II and one in linkage group III. The results of the reciprocal crosses conformed to Haldane's rule: F-1 males were sterile and most F-1 females were fertile. F-1 females mated to *G. p. gambiensis* were more likely to be fertilized than females that were mated to *G. p. palpalis*. In three of the four experiments, the fertility of backcross females was not significantly different from that of F-1 females, and there was little evidence that specific chromosomal combinations influenced the fertility of backcross females. Intrachromosomal recombination was lower in hybrid females than in *G. p. palpalis*. The major genetic factor associated with sterility among backcross males was the presence of sex chromosomes from two subspecies; a minor factor was the number of heterozygous autosomes, but interactions between sex chromosomes and autosomes from different taxa did not contribute to hybrid male sterility. Evidence is presented that a major factor causing hybrid male sterility lies between the loci ran (an eye color) and Est-t (testicular esterase) on the X chromosome. The use of differences between the fertility of males produced by backcrossing F-1 females to the two parental subspecies as indicators that other X chromosome loci have a role in hybrid sterility is discussed.

The ercepeae complex: New cases of insular speciation within the *Drosophila ananassae* species subgroup (melanogaster group) and descriptions of two new species (Diptera: Drosophilidae)

Lemeunier, F; Aulard, S; Arienti, M; Jallon, JM; Cariou, ML; Tsacas, L. 1997

Two new species of the *Drosophila ananassae* subgroup are described. *D. comorensis* Tsacas sp. n. and *D. merina* Tsacas sp. n. are endemic to the Indian Ocean islands Comores and Madagascar, respectively. Comparisons with 2 other species, also endemic to Indian Ocean islands and belonging to the *D. ananassae* subgroup, *D. earpiece* Tsacas & David from La Reunion and *D. vallismaia* Tsacas from Seychelles, lead to the definition of a 3rd complex, ercepeae, within this subgroup. Relationships among the 4 species of the ercepeae complex are based on morphological data (the only data available for *D. comorensis*), mitotic and polytene chromosome analyses, behavioral interactions, interspecific hybridizations, chemical analyses of cuticular hydrocarbons, and amylase polymorphism. Within this complex, *D. ercepeae* and *D. merina* are distinguished from *D. vallismaia* and *D. comorensis*. AU characters show close affinities between *D. ercepeae* and *D. merina*. The geographic distribution of the species of the 3 complexes of the *D. ananassae* subgroup is also discussed.

Extrusion of yolk masses by hybrid embryos of two cryptic species of *Anastrepha fraterculus* (Diptera, Tephritidae)

Selivon, D; Perondini, ALP. 1997

Embryos of two cryptic species of *Anastrepha fraterculus* (type I and type II) differ in the frequencies at which masses of yolk are extruded from their extremities. In embryos from type I flies, the elimination of masses from the anterior pole occurs at a lower frequency than in type II embryos. No significant differences in extrusion at the posterior pole were found. In hybrid embryos produced by reciprocal crosses between type I and type II flies, extrusion of yolk masses at the anterior pole occurred at a frequency intermediate between those observed for the embryos of the parental populations. At the posterior pole there were no alterations. These results add evidence that genetic control might be involved in the phenomenon of extrusion of yolk masses by the embryos of *Anastrepha* species.

Analysis of the *Anopheles* (*Anopheles*) *quadrimaculatus* complex of sibling species (Diptera : Culicidae) using morphological, cytological, molecular, genetic, biochemical, and ecological techniques in an integrated approach

Reinert, JF; Kaiser, PE; Seawright, JA. 1997

The *Anopheles quadrimaculatus* complex of 5 cryptic species (i.e., *An. diluvialis* Reinert, new species; *An. inundatus* Reinert, new species; *An. maverlius* Reinert, new species; *API. quadrimaculatus* Say; *Ali. smaragdinus* Reinen, new species) is analyzed using multiple techniques, including morphological, cytological, molecular, genetic, biochemical, and ecological procedures. All life stages (egg, 4th-instar larva, pupa, and female and male adults) are described using morphological features, and pertinent stages or structures are illustrated. A neotype for *An. quadrimaculatus* is designated, and the synonymy of *An. annulimanus* Van der Wulp is confirmed. Several new morphological features are described. New, and summarized data from published literature on hybridization, cytological electrophoretic, molecular, and cuticular hydrocarbon studies are included. Immature and adult bionomics are given. The geographic distribution for each species is listed and shown on maps. Procedures for collecting, processing, and rearing specimens are described. Keys using morphological characters are included for the eggs, 4th-instar larvae, pupae, adult females, and male genitalia. Also, a biochemical key for the 5 species is included. Color and pattern variations of larvae and pupae are discussed.

Inheritance of the photoperiodic response controlling larval diapause in the blow fly, *Calliphora vicina*

McWatters, HG; Saunders, DS. 1997

Larvae of the blow fly *Calliphora vicina* R-D. (Diptera: Calliphoridae) display a diapause in response to the exposure of their parents to short photoperiods. Due to geographic variation in photoperiodic response, flies from a southern, English population show a long-day response to the fixed photoperiod of L:D 15.5:8.5 whilst flies from a northern population from Finland show a short-day response to the same photoperiod. Crosses between these strains have shown previously that diapause incidence is a maternal characteristic; here we demonstrate that the hybrid female offspring of such crosses are not intermediate between the two parental strains but show a photoperiodic response biased towards their maternal line. Thus not only are males unable to influence directly the diapause incidence among their offspring but the indirect effects of inheritance down the male line are weaker than down the female. Diapause duration, in contrast, is influenced by each parent in a similar manner. Diapause lasts longer in larvae with a greater admixture of northern genes regardless of whether they were maternal or paternal. (C) 1997 Elsevier Science Ltd.

Incipient reproductive isolation between two morphs of *Drosophila elegans* (Diptera: Drosophilidae)

Hirai, Y; Kimura, MT. 1997

Drosophila elegans is a flower-breeding species occurring in tropical and subtropical regions of Asia. Two morphs, brown and black, are known in this species. The brown morph is recorded from southern China, Philippines, Indonesia and New Guinea, while the black morph is from the Okinawa islands and Taiwan. The present crossing experiment suggests that the difference of body colour between them was due to alleles on a single locus or closely linked loci on an autosome; F-1 hybrids exhibited intermediate body colour. Female choice tests revealed asymmetrical premating isolation between the brown and black morphs; isolation indices ranged from 0.55 to 0.83 in the tests using females of the black morph (deviation from random mating was significant), but from -0.03 to 0.50 in the tests using females of the brown morph (deviation from random mating was insignificant). However, body colour was not used as a criterion of mate choice by females. A weak and asymmetrical postmating isolation was also observed between the brown and black morphs; viability was lowered in F-2 progenies of crosses between females of the brown morph and males of the black morph. No premating or postmating isolation was observed between geographic strains of each morph. Under irradiation, body temperature was higher in the black morph than in the brown morph. On the other hand, no significant difference was observed in tolerance to cold, heat and desiccation between the brown and black morphs. (C) 1997 The Linnean Society of London.

Rearing the decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) in imported fire ants (Hymenoptera: Formicidae) from the United States

Porter, SD; Williams, DF; Patterson, RS. 1997

The South American phorid fly *Pseudacteon tricuspis* Borgmeier was imported into quarantine facilities in Gainesville, FL, to study its life history and determine if it could be reared on imported fire ant workers from North America. The found that this fly developed successfully on *Solenopsis invicta* Buren workers from Florida and hybrid *Solenopsis richteri* Forel x *S. invicta* fire ants from Mississippi. It also was reared on *S. richteri* and *S. invicta* fire ants from Argentina. This fly, like its congener *Pseudacteon litoralis* Borgmeier, had the peculiar habit of decapitating its living host and using the ant's empty head capsule as its pupal case. We were able to rear this fly through 1 complete generation in the laboratory, indicating that mass rearing for inoculative releases in the United States may be possible.

New allele at a locus affecting developmental time in Mediterranean fruit fly (Diptera: Tephritidae) and its potential use in genetic sexing at the egg stage

Pizarro, JM; Manso, FC; Cladera, JL. 1997

The use of the chromosome 2 linked eye color mutation *sw-z*, which causes slow development during the larval and pupal stages, has been proposed as a way to separate males from females in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). But rearing costs could be greatly reduced if females were eliminated at an earlier stage. A new mutation, *sw-y*, showing slow embryonic development compared with wild type has been isolated. We compared the duration of the embryonal, larval, and pupal stages at 2 different temperatures among the mutants and hybrids between them. The results showed that *sw-y* and *sw-z* are significantly slower in embryonic, larval, and pupal development than the wild-type control, with *sw-y* being the slowest. These differences are greater at low than at high temperature. The complementation test indicates that *sw-z* and *sw-y* are 2 alleles at the same locus. It is concluded that separation at the egg-larva transition will be possible with *sw-y*.

Resurrection of *Aedes* (*Halaedes*) *ashworthi* Edwards 1921: Morphological characterisation and separation from its sibling *Aedes* (*Halaedes*) *australis* (Erichson) 1842 (Diptera: Culicidae)

Brust, RA; Mahon, RJ. 1997

The result of population crosses of members of the *Ae. australis* complex and the examination of the male genitalia of these populations have shown that *Ae. ashworthi* Edwards 1921 should be reinstated as a valid species. The parent females laid viable eggs when cross mated to males of different species, but the resulting F-1 females and males were largely sterile. When F-1 females were blood-fed and examined 3-8 d later, usually fewer than 10 ovarioles per ovary developed recognisable follicles and none of these formed eggs. F-1 males in nearly all cases possessed half-size testes, and there was no spermatid development in the reduced testes. A few males had normal-sized testes, but the spermatids were mostly tailless. The male of *Ae. ashworthi* can be separated from *Ae. australis* by the shape of the gonocoxite and the length of the gonostylus of the genitalia. The larvae of both species are identical, and the females differ only in the colour of some scale patches and the integument, which may prove to be too variable to be useful when more populations are compared. The morphological similarity of the two mosquitoes as larvae and adults supports the proposal that they should be recognised as sibling species, with *Ae. ashworthi* as the derived member of the *Ae. australis* complex. It is clear from morphological evidence, the viability of the F-1 hybrids and the unusual habitat occupied by both species, that they have diverged recently.

Cytogenetic analysis of a hybrid, *Glyptotendipes pallens* Mg. x *Glyptotendipes glaucus* Mg. (Diptera, Chironomidae): evolutionary considerations

Michailova, P. 1998

The karyotype of experimentally obtained hybrids between the two closely related species *Glyptotendipes pallens* and *Glyptotendipes glaucus* is described. Hybridization was successful in one direction only (*G. pallens* female x *G. glaucus* male). The polytene chromosomes AB and EF of the hybrid show a more or less intimate pairing throughout their length. In the chromosomes CD in which an inversion occurs the characteristic loop is formed. The homologues of chromosome G are almost completely asynaptic. The localization of centromere heterochromatin was also studied. Centromere heterochromatin as well as intercalary heterochromatin could be observed in all chromosomes. By C banding analyses it could be shown that *G. pallens* has a telomeric chromosome G while in *G. glaucus* it is acrocentric. According to karyotype similarity it can be assumed that these two species have quite recently derived from a common ancestor since they still share much of their genomic organization. On the Black Sea coast (southeast part of Bulgaria) a natural hybridization zone between the sympatric species *G. pallens* and *G. glaucus* has been detected. The idea that hybridization between the two species might finally proceed to the formation of a new species by hybrid origin and introgression is discussed.

Identification of the geographical source of adults of the Old World screw-worm fly, *Chrysomya bezziana* Villeneuve (Diptera : Calliphoridae), by multivariate analysis of cuticular hydrocarbons

Brown, WV; Morton, R; Lacey, MJ; Spradbery, JP; Mahon, RJ. 1998

The cuticular hydrocarbon compositions of male and female *Chrysomya bezziana* from different locations are qualitatively similar but show quantitative differences. The qualitative similarity and lack of marked quantitative differences over its whole range is consistent with *C. bezziana* being a single species. This accords with the results of studies on hybridization, morphology, allozymes, and chromosomes. Canonical variate analysis of quantitative differences has enabled grouping of locations by geographical regions. Components insensitive to age and sex have been identified that would permit the location by geographical region of a fly of unknown age and sex. However, greater precision is obtained if the sex of the fly be known. (C) 1998 Elsevier Science Inc.

Mitochondrial DNA polymorphism in a black fly, *Simulium vittatum* (Diptera : Simuliidae)

Zhu, X; Pruess, KP; Powers, TO. 1998

Mitochondrial DNA (mtDNA) was extracted from pooled field-collected samples representing six species of black flies (*Cnephia dacotensis*, *Simulium bivittatum*, *S. johansenni*, *S. luggeri*, *S. piperi*, *S. vittatum*) and compared by restriction fragment length polymorphism (RFLP) analysis. Morphospecies were molecularly distinct, with few shared restriction fragments. Eleven populations of *S. vittatum* were found that appeared to be homogeneous for a single mitochondrial haplotype. Ten other populations of *S. vittatum* showed extensive mitochondrial heterogeneity. In part, these samples contained mixtures of two cytologically recognized siblings: IIIL-1 and IS-7. About 70% of the mitochondrial genome of a population pure for sibling IIIL-1 was cloned as five HindIII fragments, which were used as hybridization probes to examine individual black flies. Thirteen mtDNA haplotypes involving permutations of 10 HindIII restriction sites were identified in individual black flies examined from 26 populations. DNA from 168 larvae cut with both EcoRI and HindIII revealed five additional haplotypes. One HindIII haplotype was present in 84% of 390 larvae examined and predominated in every population examined from New York to California and in both the IIIL-1 and IS-7 siblings. Nebraska populations had individuals with nearly all known haplotypes. The most common haplotype was usually the only form present in warm, silty streams with organic enrichment. Rarer haplotypes were found in cool, spring-fed streams but without clear geographic or phylogenetic components.

Molecular systematics, morphological analysis, and hybrid crossing identify a third taxon, *Aedes (Halaedes) wardangensis* sp.nov., of the *Aedes (Halaedes) australis* species-group (Diptera : Culicidae)

Brust, RA; Ballard, JWO; Driver, F; Hartley, DM; Galway, NJ; Curran, J. 1998

Phylogenetic and morphological analyses, male morphology, and hybrid crossing indicate that a population from Wardang Island, South Australia, is distinct from the monophyletic series of populations of *Aedes (Halaedes) australis* (Erichson) 1842 from Victoria, Tasmania, New South Wales, and New Zealand. The name *Aedes (Halaedes) wardangensis* has been assigned to the new species. Phylogenetic analysis of DNA sequences from the cytochrome oxidase II and internal transcribed spacer loci support the resurrection of *Aedes (Halaedes) ashworthi* Edwards, 1921 (Brust and Mahon. 1997). *Aedes ashworthi* is known only from Western Australia and was found to be infertile when crossed with *Ae. wardangensis* from South Australia and *Ae. australis* from New Zealand. The hybrid of *Ae. australis* from New South Wales x *Ae. australis* from New Zealand was fertile for three generations, documenting these as conspecific.

Spikelet flowering time: Cause of sorghum resistance to sorghum midge (Diptera : Cecidomyiidae)

Diarisso, NY; Pendleton, BB; Teetes, GL; Peterson, GC; Anderson, RM. 1998

Resistance of sorghum, *Sorghum bicolor* (L.) Moench, to sorghum midge, *Stolodiplosis sorghicola* (Coquillett), has not been well understood. In this study, possible causes of resistance were examined by comparing time of oviposition with times spikelets of resistant and susceptible sorghums flowered and thus were vulnerable to damage. Spikelets of resistant genotypes began flowering during the night, at 0100 hours (CDT), and most were closed and no longer vulnerable by the time sorghum midges began to oviposit in the field at 0800 hours. Peak flowering of resistant sorghums was between 0300 and 0700 hours. Most spikelets of susceptible sorghums, however, were flowering after daylight, at 0800 hours, when sorghum midge abundance began to increase. At 1000 hours, the time most sorghum midges (2.5 per panicle) were in the field, percentages of spikelets penetrable by a pin the diameter of a sorghum midge ovipositor were 32 and 62% for resistant and susceptible sorghums, respectively. Damage to panicles of resistant sorghums averaged 29.6%, whereas susceptible sorghums were more than twice as damaged (68.2%). Delaying for 5 h the daily flowering period of a resistant hybrid to coincide with time of peak sorghum midge abundance in the field resulted in 3.2 times as many sorghum midges emerging and 2.9 times as much damage as to panicles of the resistant hybrid that flowered at the normal time. Damage and numbers of sorghum midges emerging were not significantly different between normal and changed panicles of a susceptible hybrid. Most spikelets of resistant sorghums flowered and closed early and thus evaded damage before ovipositing sorghum midges were in the field. These results support the hypothesis that resistance is caused by asynchrony between time of sorghum spikelet flowering and presence of sorghum midge.

Genetic basis of photoperiodic control of summer and winter diapause in geographic ecotypes of the rice stem maggot, *Chlorops oryzae*

Takeda, M. 1998

Chlorops oryzae Matsumura (Diptera: Chloropidae) is bivoltine in northern Japan but trivoltine in the southern part of the country. Larvae of the bivoltine strain (Akita, 39 degrees N) develop without delay under L14:D10 but enter summer diapause in the mature larval stage under L15:D9. In contrast, larvae of the trivoltine strain (Aichi, 35 degrees N) develop quickly under L15:D9 but enter summer diapause under L14:D10. The genetic basis of their different summer-diapause behavior was analyzed by reciprocal crossing between the two ecotypes and backcrossing to the hybrids. Although a polygenic system seems to be involved, there is a single gene

on the X-chromosome exerting a major effect on summer diapause. The northern bivoltine ecotype has a longer critical photoperiod for the induction of winter diapause in the first larval stage and remains in diapause longer than the southern trivoltine ecotype. The differences in the critical photoperiod and the winter diapause duration between the two ecotypes seem to be controlled by a polygenic system but again a sex-linked or closely linked gene (or genes) plays a major role. The two geographic ecotypes differ significantly in summer- and winter-diapause traits, both of which are characterized by sex-linked inheritance. Despite this, no apparent sterility or inviability in hybrids between the ecotypes occurs.

Relationship between cold stupor and cold tolerance in *Drosophila* (Diptera : Drosophilidae)

Hori, Y; Kimura, MT. 1998

Cold stupor (knock-down temperature) was studied in relation to cold tolerance (lethal temperature) in adult flies of cool-temperate, warm-temperate, and subtropical species belonging to the *Drosophila melanogaster* species group. Knock-down temperature was strongly correlated with lethal temperature ($r = 0.91$). The cool-temperate species had lower knock-down and lethal temperatures, the subtropical species had higher ones, and the warm-temperate species had intermediate ones. Little intraspecific variation was observed in knock-down temperature. In a cool-temperate species, individuals in reproductive diapause had lower knock-down and lethal temperatures than reproducing ones. In addition, knock-down and lethal temperatures were lower in flies reared at a lower temperature. When rearing temperature was shifted, knock-down temperature changed with a logarithmic function of acclimation period. The rate of acclimation did not differ among the cool-temperate, warm-temperate, and subtropical species. Measurements on hybrids between *D. lutescens* Okada (a warm-temperate species) and *D. takahashii* Sturtevant (a subtropical species) suggested that loci responsible for the difference in knock-down temperatures between these 2 species were located on both autosome(s) and the X chromosome.

Estimation of genetic divergence and gene flow between *Culex pipiens* and *Culex quinquefasciatus* (Diptera : Culicidae) in Argentina

Humeres, SG; Almiron, WR; Sabattini, MS; Gardenal, CN. 1998

Allele frequencies at seven polymorphic loci controlling the synthesis of enzymes were analyzed in six populations of *Culex pipiens* L. and *Cx. quinquefasciatus* Say. Sampling sites were situated along a north-south line of about 2,000 km in Argentina. The predominant alleles at *Mdh*, *Idh*, *Gpdh* and *Gpi* loci presented similar frequencies in all the samples. Frequencies at the *Pgm* locus were similar for populations pairs sharing the same geographic area. The loci *Cat* and *Hk-1* presented significant geographic variation. The latter showed a marked latitudinal cline, with a frequency for allele b ranging from 0.99 in the northernmost point to 0.04 in the southernmost one, a pattern that may be explained by natural selection ($F_{ST} 0.46$; $p < 0.0001$) on heat sensitive alleles. The average value of $F_{ST}(0.088)$ and $N_m(61.12)$ indicated a high gene flow between adjacent populations. A high correlation was found between genetic and geographic distance ($r = 0.83$; $p < 0.001$). The highest genetic identity ($I-N = 0.988$) corresponded to the geographically closest samples from the central area. In one of these localities *Cx. quinquefasciatus* was predominant and hybrid individuals were detected, while in the other, almost all the specimens were identified as *Cx. pipiens*. To verify the fertility between *Cx. pipiens* and *Cx. quinquefasciatus* from the northern and southernmost populations, experimental crosses were performed. Viable egg rafts were obtained from both reciprocal crosses. Hatching ranged from 76.5 to 100%. The hybrid progenies were fertile through two subsequent generations.

Hybrid syndrome-induced postzygotic reproductive isolation: A second reproduction barrier in *Chironomus thummi* (Diptera, Chironomidae)

Hagele, K. 1999

The frequency of the occurrence of hybrids between *Chironomus thummi thummi* and *Chironomus thummi piger* is estimated to be 0.047% in the wild. The rare hybridization events are the consequence of the sexual isolation mechanism of different swarming behavior of *thummi* and *piger*. Under laboratory conditions hybrids are easily obtained. The reciprocal hybrids show two different dysgenesis syndromes depending on the direction of crosses. The *Rud* syndrome reduces the number of fertile *thummi* (f) x *piger* (m) hybrids to less than 2% by rudimentary gonads, and the *HLE* syndrome the number of Fertile *piger* (f) x *thummi* (tn) hybrids to 12% by a reduction of the hatching frequency of the hybrid larvae. The overall frequency of the occurrence of hybrids in the wild that are able to reproduce is estimated to be 0.0033%. In backcrosses of the hybrids with the parental strains a further fertility reduction of 40% occurs. Thus, the two syndromes operate postzygotically as an effective second reproductive isolation mechanism that evolved most probably as an important prerequisite of speciation.

The population genetics of the apple maggot fly, *Rhagoletis pomonella* and the snowberry maggot, *R. zephyria*: implications for models of sympatric speciation

Feder, JL; Williams, SM; Berlocher, SH; McPheron, BA; Bush, GL. 1999

True fruit flies belonging to the *Rhagoletis pomonella* (Diptera:Tephritidae) sibling species complex possess several attributes consistent with a history of sympatric divergence via host plant shifts. Here, we investigate whether hybridization and genetic introgression is occurring between two members of the group, *Rhagoletis pomonella* (Walsh), whose primary hosts are domestic apples (*Malus pumila*) and hawthorns (*Crataegus* spp., and *R. zephyria* (Snow) whose host is snowberries (*Symphoricarpos* spp.). These flies are important because they appear to be at a transition stage between taxa reproductively isolated solely on the basis of host plant-related adaptations and those that have evolved additional non-host dependent sterility and inviability. Observing extensive hybridization and introgression between *R. pomonella* and *R. zephyria* would have major repercussions for current models of sympatric speciation. In a survey of allozyme and mitochondrial DNA (mtDNA) variation for 1105 flies collected throughout the northern United States we found two results suggesting that low level hybridization occurs between *R. pomonella* and *R. zephyria*. (1) Two flies reared from snowberries and one fly reared from hawthorns had genotypes indicative of them being *R. pomonella* and *R. zephyria*, respectively. *Rhagoletis pomonella* and *R. zephyria* adults map therefore occasionally frequent each others host plant, providing the opportunity for hybridization, (2) Four flies collected from hawthorns and one from snowberries had genotypes that made them likely to be F1 hybrids. Likelihood analysis revealed the data were also consistent with an hypothesis of shared ancestral alleles (i.e., the pattern of genetic variation could also be explained by *R. pomonella* and *R. zephyria* sharing alleles/haplotypes whose origins date to a common ancestor). We estimated that, in the absence of interspecific mating, random assortment of genes within *R. pomonella* and *R. zephyria* populations would produce an average of 5.4 flies with genotypes suggesting they were F1 hybrids - a number equivalent to the 5 putative F1 hybrids observed in the study. Our results therefore underscore the difficulty in distinguishing between hypotheses of low level introgression and shared ancestral polymorphism. But even if hybridization is occurring, the data suggest that it is happening at a very low and probably evolutionarily insignificant level (perhaps 0.09% per generation), consistent with sympatric speciation theory. Future tests are discussed that could help resolve the hybridization issue for *R. pomonella* and *R. zephyria*.

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.

Interspecific mating of introduced, sterile *Bactrocera dorsalis* with wild *B-carambolae* (Diptera : Tephritidae) in Suriname: a potential case for cross-species sterile insect technique

McInnis, DO; Rendon, P; Jang, E; Van Sauers-Muller, A; Sugayama, R; Malavasi, A. 1999

Copulation with sperm transfer was observed between the oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the carambola fruit fly, *B. carambolae* (Drew & Hancock), in outdoor field cages in Suriname. Sterilized oriental fruit flies from Hawaii were shipped to Suriname for testing against wild carambola fruit flies to investigate whether the oriental fruit fly could be used in a potential sterile insect program against the carambola fruit fly. Of 217 total observed copulations on 5 test dates, 17 (7.8%) were interspecific, and 10 (4.6%) were of the crucial type, sterile oriental fruit fly male x wild carambola fruit fly female. Interspecific copulations tended to be of short duration, especially when the male was an oriental fruit fly (i.e., average time in copula was 148.0 min for carambola fruit fly male x oriental fruit fly female, whereas only 4.67 min for oriental fruit fly male x carambola fruit fly female at a 5:1 oriental fruit fly:carambola fruit fly ratio). Homogamic (like with like) pairings tended to be longer, especially for carambola fruit fly, with average times in copula of 115.7 and 481.4 min for oriental fruit fly male x oriental fruit fly female and carambola fruit fly male x carambola fruit fly female, respectively. With both sexes present, oriental fruit flies began mating earlier in the evening (approximate to 2,500-lux), whereas carambola fruit flies started mating at approximate to 300 lux. With only sterile male oriental fruit flies released, the proportion of interspecific pairs (oriental fruit fly male x carambola fruit fly female) rose to 1/3 of the total, at a sterile male: wild male ratio of 5:1. This was 3-fold higher than for the release of bisexual oriental fruit fly. An index of mating isolation, relative isolation index, was high (>100, where random mating = 1) because of high assortative mating in the case of bisexual oriental fruit fly. Approximately 70% of carambola fruit fly females that copulated with carambola fruit fly males had sperm in their spermathecae. By comparison, 0% (0/6 flies) were mated in the case of carambola fruit fly female mated to oriental fruit fly male when both oriental fruit fly sexes were present, whereas 50% (2/4 flies) were mated when males-only oriental fruit fly were released.

A 24 kDa parasitism-specific protein from the Caribbean fruit fly, *Anastrepha suspensa*: cDNA and deduced amino acid sequence

Shi, XZ; Gomez, SP; Lawrence, PO. 1999

A 24 kDa parasitism-specific protein (PSP24) was previously reported from the hemolymph of the Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae) after parasitization by the wasp *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). This study was designed to sequence the open reading frame of PSP24 and to determine whether it is encoded by the wasp, fruit fly host or by the entomopoxvirus D1EPV which is normally injected into the host with the wasp's egg. Utilizing an existing partial amino acid sequence of PSP24, we obtained two cDNAs by reverse transcription-polymerase chain reaction, from the host hemolymph 48 h post parasitization. The smaller cDNA has an open reading frame (ORF) that encodes 85 amino acids (aa) with a molecular mass of 9711.33 Da and the larger encodes 203 aa with a molecular mass of 23 076 Da. Both cDNAs share a common N-terminus with a signal peptide predictive of secreted proteins, a characteristic that agrees with the observed nature of PSP24. The mature proteins have 39 and 157 aa with deduced molecular masses of 4286.86 Da and 17 651 Da, respectively. Western blots of host hemolymph probed with the anti-PSP24 serum reveal proteins of 0.10 and 0.24 kD, respectively. The discrepancy between the deduced and the observed molecular masses may be explained by their predicted O-linked glycosylation. The amino acid sequences are not homologous with any protein in the available databases. Southern blot hybridization experiments revealed that the proteins are encoded by both the host and the parasite. Furthermore, injection of D1EPV into healthy fruit fly puparia induces the two proteins. Thus, in surprising contrast to an earlier hypothesis that D1EPV encodes PSP24, these results clearly demonstrate that the PSP24 proteins are encoded by wasp and fruit fly but not D1EPV genes. However, their expression is D1EPV induced. (C) 1999 Elsevier Science Ltd. All rights reserved.

Cytogenetic evidence for a species complex within *Anopheles pseudopunctipennis* Theobald (Diptera : Culicidae)

Coetzee, M; Estrada-Franco, JG; Wunderlich, CA; Hunt, RH. 1999

Anopheles pseudopunctipennis was collected from Acapulco, Mexico and Saltee River, Grenade, West Indies and used in cross-mating experiments. Larvae from the cross, Mexico female X Grenada male, died in the third instar. However, adult progeny were obtained from the reciprocal cross Grenada female X Mexico male. These hybrid males had testes with apparently normal appearance but some without viable sperm. Polytene chromosomes obtained from hybrid females exhibited extensive asynapsis of the X chromosomes. Previously undescribed fixed inversion differences between the two populations were noted on the X chromosome. It is concluded that the two populations belong to different species. The Grenada population is designated *An. pseudopunctipennis* species C, since it is the third taxon recognized in this species complex.

Distinguishing between two sympatric species, *Bactrocera occipitalis* and *B.philippinensis* (Diptera : Tephritidae), based on aedeagal length

Iwahashi, O. 1999

A simple method based on the length of aedeagus was applied to identify fruit fly males on Guimaras Island, Philippines, where 2 species of *Bactrocera* are sympatric. Males with aedeagal length of <2.81 mm are *Bactrocera occipitalis* (Drew & Hancock), and those of >2.89 mm are *B. philippinensis* (Drew & Hancock). It was notable that the length of the aedeagus is not strongly affected by the body size. The relationship between descriptive scores based on 6 characters and aedeagal length indicated that the males that have scores of 6.0-3.0 have an aedeagal length of <2.81 mm (i.e., *B. philippinensis*); males with scores of 2.0-0 have aedeagal lengths of >2.89 mm (i.e., *B. occipitalis*); and those between 2.81 and 2.98 are considered hybrids of the 2 species, although its rate was very low (1.0%). However, males scored at 2.5 were composed of both species. Ultimately, 95% of *B. occipitalis* and 89% of *B. philippinensis* could be correctly identified using 2 characters, the lateral marks on tergite 4 and the depth of the wing's costal band.

Selection of *Anopheles dirus* for refractoriness and susceptibility to *Plasmodium yoelii nigeriensis*

Somboon, P; Prapanthadara, L; Suwonkerd, W. 1999

Two lines of the Oriental malaria vector mosquito *Anopheles dirus* species A (Diptera: Culicidae), one fully refractory and one fully susceptible to *Plasmodium yoelii nigeriensis* (an African rodent malaria parasite), were established after 17 generations of mass selection, followed by single female selection for one or two generations. Prior to selection, the stock colony of *An. dirus* was 17% refractory. Both lines of *An. dirus* produced abundant ookinetes that started to invade the midgut within 24h post-infection, as seen in histological sections. In most of the refractory mosquitoes, oocysts stopped development <12h post-invasion, indicating a rapid defence mechanism. Dead *P. y. nigeriensis* parasites were apparently localized as small melanized spots (2-5 µm) seen in wet preparations of mosquito

midguts dissected 5-7 days post infective bloodmeal. In some refractory *An. dirus* females, apart from the spots, a small number of totally encapsulated oocysts (c. 10 μ m) were also present. These larger melanized parasites predominated in a few females: they appeared 2-3 days post-infection as a secondary delayed defence mechanism. The progeny of reciprocal matings between susceptible and refractory lines had similar to 50% susceptibility. Backcrosses of F-1 hybrids with susceptible or refractory lines increased or decreased the susceptibility of backcross progeny accordingly. Overall, these results suggest polygenic control of susceptibility to *P. y. nigeriensis* infection. The refractory line of *An. dirus* showed normal susceptibility to natural infections of the human malaria *P. falciparum* and *P. vivax* from local patients.

Haldane's rule and other aspects of reproductive isolation observed in the *Anastrepha fraterculus* complex (Diptera : Tephritidae)

Selivon, D; Perondini, ALP; Morgante, JS. 1999

Some aspects of reproductive isolation between allopatric populations of two closely related species of the *Anastrepha fraterculus* complex (*A. fraterculus* sp. 1 and sp. 2) were evaluated in laboratory conditions. Most of the crosses were fertile in each species as well as between sp. 2 females and sp. 1 males. In the reciprocal cross only 41.7% of the matings yielded viable progeny. Egg hatching occurred at similar rates within the two species, but was significantly lower in the crosses between the species. Adult emergence did not differ significantly among crosses. The sex ratio of adult progeny within each species, as well as in the hybrid progeny derived from sp. 1 females crossed to sp. 2 males, did not differ from the expected 1:1 ratio. However, in the crosses between sp. 2 females to sp. 1 males, a significant deviation in the sex ratio in favor of females was observed, according to the Haldane's rule. The results reinforce previous data which indicated that *A. fraterculus* sp. 1 and *A. fraterculus* sp. 2 are distinct biological entities.

Evidence of multiple mating and hybridization in *Simulium damnosum* s.l. (Diptera : Simuliidae) in nature

Boakye, DA; Back, C; Brakefield, PM. 2000

Beakers with aeration were used to rear individual (single) egg batches of *Simulium damnosum* (Theobald) s.l. to larvae and adults. Chromosomal analysis of the progeny of individual females indicated that multiple mating occurred within the *S. damnosum* complex and that hybridization occurred between *S. damnosum* s.s. and *S. sirbanum* (Vajime & Dunbar) in nature. These results indicate that possibly more extensive hybridization may be taking place in some areas of the Onchocerciasis Control Program than previously reported. Dramatic changes in the rates of hybridization among members of the *S. damnosum* complex possibly may lead to the formation of new cytotypes.

Hybridization asymmetries in tsetse (Diptera : Glossinidae): Role of maternally inherited factors and the tsetse genome

Gooding, RH. 2000

Among the morstians-group of tsetse there are several pairs of taxa in which there is a marked hybridization asymmetry (HA), i.e., one cross produces significantly more offspring than does the reciprocal cross. To investigate the relative contribution of maternally inherited factors (MIF) and chromosomal factors to HA, three hybrid lines were established in which flies have MIF from one taxon and chromosomes from another. HA was then compared among crosses of the parental taxa and crosses of each parental taxon with the appropriate hybrid line. The results indicate that HA in reciprocal crosses of *Glossina morsitans morsitans* Westwood and *Glossina swynnertoni* Austin and in reciprocal crosses of *G. m. morsitans* and *Glossina morsitans centralis* Machado are caused by chromosomal factors, not MIF. Reciprocal crosses of *G. m. centralis* and *G. swynnertoni* do not display HA, and none developed as a result of a novel combination of MIF and tsetse chromosomes.

Egg size and hybrid syndrome-dependent embryo mortality in *Chironomus* hybrids (Diptera : Chironomidae)

Hagele, K; Kasper-Sonnenberg, M. 2000

Female hybrids of the cross *Chironomus t. thummi* female x *Ch. t. piger* male which are largely affected by the sterility inducing Rud syndrome were backcrossed with males of both parental strains. The aim of the study was to provide information about those egg volumes that are insufficient for a normal embryogenesis and to ascertain whether in the hybrids the lethally small egg size represents a new abnormal trait of the Rud syndrome. The egg masses obtained contain eggs of very different sizes with volumes ranging from 0.5 nl to 3.49 nl. Embryo mortality is unusually frequent in those eggs of the backcrosses and of the parental strains that have volumes smaller than 1.5 nl. An egg volume of 1.5 nl represents in *Ch. thummi* the lower limit for those volumes that are sufficient for a normal embryogenesis. Mortality increases with decreasing egg size, reaching 100% in backcross eggs with volumes of 0.99 nl and smaller. Small egg size is a new trait of the Rud syndrome affected *thummi* female x *piger* male hybrids. This trait is part of a postzygotic reproductive isolation barrier between *thummi* and *piger* and manifests first in the backcrosses. Most backcross eggs show volumes between 1.5 nl and 2.99 nl. Within this volume range the amount of mortality does not depend upon egg volume. Here, embryo death is great in the backcrosses but normal in the parental strains. The high frequency of embryo death in the backcrosses must be predominantly due to the action of the Rud syndrome and a second hybrid syndrome, called HLE syndrome. Since further characteristic traits of these syndromes could be detected in surviving backcross individuals, the study demonstrates the occurrence of the syndromes in this generation also. Therefore, the postzygotic reproductive isolation mechanism of both hybrid syndromes is effective in the hybrids and in their progeny as well.

Evidence of the specific status of *Anopheles flavirostris* (Diptera : Culicidae)

Somboon, P; Tuno, N; Tsuda, Y; Takagi, M. 2000

Anopheles flavirostris (Ludlow) from Lombok Island, Indonesia, was crossed with *An. minimus* species A (Chi strain) from Thailand and species E (ISG strain), a new sibling species, from Japan, to determine genetic compatibility. We also compare the scanning micrographs of female cibarial armature of these three species. Both *An. minimus* CM and ISG females crossed with *An. flavirostris* males produced eggs without embryos. One *An. flavirostris* female crossed with an *An. minimus* CM male deposited unhatchable egg with an 18% embryonation rate. The scanning micrographs of the cibarial armature clearly show significant differences among *An. flavirostris* and the two sibling species of the *An. minimus* complex. These results support the specific status of *An. flavirostris*. Previous records of *An. minimus* Theobald in this country are also discussed.

Identification and evaluation of root maggot (*Delia* spp.) (Diptera : Anthomyiidae) resistance within Brassicaceae

Dosdall, LM; Good, A; Keddie, BA; Ekuere, U; Stringam, G. 2000

Current varieties of canola/oilseed rape, *Brassica napus* L. and *Brassica rapa* L., are susceptible to infestation by the root maggots *Delia radicum* (L.) and *Delia floralis* (Fallen) (Diptera: Anthomyiidae) in western Canada. Crop losses from root maggot infestations can be very significant, and infestation levels have increased

substantially from 1983 to present. Although cultural and chemical strategies exist to reduce crop losses caused by root maggots, these methods are either not sufficiently effective or pose unacceptable environmental risk. This paper reports progress achieved to develop canola cultivars resistant to attack by root maggots as the most environmentally sustainable strategy for management of these pests. Twelve species of Brassicaceae, and many genotypes within species, were evaluated for levels of root maggot resistance, in addition to many intergeneric hybrids produced by crosses of *Sinapis alba* x *B. napus*. *Sinapis alba* had greatest resistance to infestation by root maggots among the Brassicaceae species evaluated, and five intergeneric hybrids had levels of resistance to root maggots that were similar to *S. alba* and were maintained from year to year. Low amounts of *S. alba* DNA were detected in the hybrids using genomic slot-blot analysis. These results indicate that a genetic basis for resistance to root maggot infestation exists in the Brassicaceae, and our research represents the first step toward introgression of genetic sources of root maggot resistance from related species into commercial canola varieties. (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.

Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri*

Porter, SD; Briano, JA. 2000

Matching biotypes of potential biocontrol agents to target host populations can greatly improve the effectiveness of control. This study was designed to determine if the fly *Pseudacteon curvatus* Borgmeier from Las Flores, Buenos Aires Province, Argentina prefers its natural host, the black fire ant, *Solenopsis richteri* Forel. We found that *P. curvatus* strongly preferred *S. richteri* from Argentina, *S. richteri* from the United States, and hybrid (*S. richteri* x *S. invicta*) fire ants from the United States when each was tested against *S. invicta* from the United States. The time to pupation of developing parasitoids was 10% and 21% longer in hybrid and red fire ants than in black fire ants. Parasitism rates, however, were not significantly different among these ant hosts in no-choice parasitism tests.

Evaluation of planting date, sorghum hybrid, and insecticide treatment on sorghum midge (Diptera : Cecidomyiidae) management in northeast Louisiana

Castro, BA; Riley, TJ; Leonard, BR. 2000

The combined effect of planting date, insecticide treatment, and host-plant resistance was studied in northeast Louisiana for management of the sorghum midge, *Stenodiplosis sorghicola* (Coquillett), during 1994 and 1995. Significantly higher numbers of sorghum midges were observed visiting flowering spikelets of the midge-susceptible sorghum hybrid (Delta and Pine Land 'DP1552') than those of the midge-resistant sorghum hybrid (DeKalb 'DK-60'). Numbers of midges averaged 1.2 and 0.6 per flowering panicle in the susceptible and resistant sorghum hybrids, respectively, in 1994 and 1.8 and 1.0, respectively, in 1995. Midge densities increased significantly as the sorghum flowering season progressed. Sorghum midge reached peak densities during the first half of August in 1994 and 1995. The length of the flowering period in the early-planted (mid-March) sorghum was significantly longer compared with the flowering periods in the mid-April, mid-May, or mid-June planted sorghums. This resulted in prolonged exposure of flowering panicles to ovipositing midges and increased midge damage in the early-planted (mid-March) sorghum. Damage by sorghum midge was significantly higher in the early-planted (mid-March) sorghum hybrids than in the late-planted (mid-June) sorghum hybrids. The midge-susceptible hybrid produced highest yields when planted in mid-April and mid-May (optimum period) and lower yields when planted very early (i.e., mid-March) or late (i.e., mid-June). No significant differences were observed in yields for the resistant hybrid at any planting date in 1994. However, in 1995, significantly lower yields were recorded in resistant sorghum planted in mid-June. Levels of sorghum midge damage and sorghum seed yields in the untreated resistant hybrid were not significantly different than those observed in the insecticide-treated susceptible hybrid. Numbers of adult midges captured on sticky traps were positively correlated to numbers of visual estimates of ovipositing midge females visiting flowering spikelets.

Comparison of two alpha-cyano pyrethroids when impregnated into bednets against a pyrethroid resistant and susceptible strain of *Anopheles stephensi* (Diptera : Culicidae) and their F-1 progeny

Kolaczinski, JH; Curtis, CF. 2000

The two alpha-cyano pyrethroid insecticides lambda-cyhalothrin and alpha-cypermethrin were tested as bednet treatments at a target dose of 20 mg m⁻². To establish their efficacy, female pyrethroid resistant and susceptible *Anopheles stephensi* Liston, and the F-1-hybrids were allowed to fly freely in a room with a human subject under an impregnated net. Both treatments provided good personal protection by significantly reducing the number of blood fed mosquitoes compared to an untreated control net. Mortality after 24 h was significantly higher for the alpha-cypermethrin treated net when compared to lambda-cyhalothrin. For each insecticide there were no significant differences in the proportion of susceptible homozygotes and F-1-hybrids found dead after a 24 h holding period, which suggests that there would be no selection for pyrethroid resistant heterozygotes by either of the insecticides.

The resolution of Californian populations of *Liriomyza huidobrensis* and *Liriomyza trifolii* (Diptera : Agromyzidae) using PCR

Morgan, DJW; Reitz, SR; Atkinson, PW; Trumble, JT. 2000

Liriomyza trifolii (Burgess) and *Liriomyza huidobrensis* (Blanchard) are important vegetable pests in California. Populations of each species differ in their impact in central and southern regions. This difference may be explained by geographical or host plant differences in each of the regions. We used random amplified polymorphic DNA polymerase chain reactions to assess genetic differences between two laboratory populations of each species collected from central and southern California. Individual *L. trifolii* from the two regions could be discriminated by the presence/absence of PCR products. No such qualitative differences were apparent in PCR products amplified from *L. huidobrensis* individuals, but the origins of individuals could be differentiated using a bootstrap analysis of marker frequencies. Marker primers were used to compare held and laboratory individuals. No evidence was found for the existence of further populations or of hybrid populations in central and southern California. The distribution of populations of *L. huidobrensis* was explained completely by geographical differences. As a consequence of the absence of leafminer infestations on the same host plant varieties in both regions, factors governing *L. trifolii* population distribution differences were less apparent. The presence of the same host plant varieties at both sites suggests that the two *L. trifolii* populations differ in host plant preference.

Cytogenetics of the *Anopheles gambiae* complex in Sudan, with special reference to *An. arabiensis*: relationships with East and West African populations

Petrarca, V; Nugud, AD; Ahmed, MAE; Haridi, AM; Di Deco, MA; Coluzzi, M. 2000

The species composition of malaria vector mosquitoes belonging to the *Anopheles gambiae* complex (Diptera: Culicidae) from > 40 localities in Sudan, representing most ecological situations, was determined by analysis of ovarian polytene chromosomes. Of 2162 females, 93% were identified as *An. arabiensis* Patton and 7% were *An. gambiae* Giles sensu stricto. No hybrids were found between the two species. *Anopheles arabiensis* occurred in all but two sites, whereas *An. gambiae* s.s. was effectively limited to the southernmost, more humid localities. For chromosomal paracentric inversions, the degree of polymorphism was low in *An. gambiae* s.s. (inversions 2La, 2Rb and 2Rd), higher in *An. arabiensis* (inversions Xe, 2Ra, b, bc, d1, s; 3Ra, d). *Anopheles gambiae* samples from Sudan were all apparently panmictic, i.e. they did not show restricted gene flow such as observed among West African populations (interpreted as incipient speciation). Chromosomal inversion patterns of *An. gambiae* in southern Sudan showed characteristics of intergrading Savanna/Forest populations similar to those observed in comparable eco-climatic situations of West Africa. *Anopheles arabiensis* was polymorphic for inversion systems recorded in West Africa (2Ra, 2Rb, 2Rd1, 3Ra) and for a novel 2Rs polymorphism, overlapping with inversion systems 2Rb and 2Rd1. Samples carrying the 2Rs inversion were mostly from Khashm-el-Girba area in central-eastern Sudan. In the great majority of the samples all polymorphic inversions were found to be in Hardy-Weinberg equilibrium. Sudan populations of *An. arabiensis* should therefore be considered as generally panmictic. *Anopheles arabiensis* shows more inversion polymorphism in west than in east African populations. Sudan populations have more evident similarities with those from westwards than those from eastwards of the Great Rift Valley. The possible influence of the Rift on evolution of *An. arabiensis* is discussed.

Experimental hybridization among *Anastrepha* species (Diptera : Tephritidae): Production and morphological characterization of F-1 hybrids

dos Santos, P; Uramoto, K; Matioli, SR. 2001

We report experimental interspecific hybridization between *Anastrepha fraterculus* (Wiedemann), *A. sororcula* Zucchi, and *A. obliqua* (Macquart), widespread and highly destructible crop pests in the Neotropical region. Prezygotic reproductive isolating mechanisms (such as different mating times and male calling songs) seem to be easily disrupted in no-choice crossing conditions, and hybrid flies can be obtained in both directions in most crosses. Crosses between *A. sororcula* females and *A. fraterculus* males and between *A. obliqua* females and *A. sororcula* males yield adult offspring of males and females. Crosses between *A. fraterculus* or *A. sororcula* females and *A. obliqua* males yield only females clearly obeying Haldane's rule (preferential inviability or sterility of heterogametic hybrids). Crosses between *A. obliqua* females and *A. fraterculus* males and between *A. fraterculus* females and *A. sororcula* males yield poor or no adult offspring of both sexes, with a few exceptions. Most F-1 hybrids are fertile and can be backcrossed to both parental species. The few morphological diagnostic characters of the parental forms appear intermediate in F-1 hybrids. In addition, we describe some morphological abnormalities found in hybrids, attributed to maternal or cytoplasmic effects, and the occurrence of gynandromorphs in the progeny of one cross.

Interspecific crosses of *Bactrocera tryoni* (Froggatt) and *Bactrocera jarvisi* (Tryon) (Diptera : Tephritidae) in the laboratory

Cruickshank, L; Jessup, AJ; Cruickshank, DJ. 2001

F-1 progeny from the cross between two species of Australian fruit flies, *Bactrocera tryoni* (Froggatt) and *Bactrocera jarvisi* (Tryon), mated with each other in the laboratory to produce viable F-2 offspring. F-1 and F-2 hybrids were morphologically distinct from their *B. tryoni* and *B. jarvisi* parents.

Schineria larvae gen. nov., sp nov., isolated from the 1st and 2nd larval stages of *Wohlfahrtia magnifica* (Diptera : Sarcophagidae)

Toth, E; Kovacs, G; Schumann, P; Kovacs, AL; Steiner, U; Halbritter, A; Marialigeti, K. 2001

Four bacterial strains were isolated from the fly larvae of an obligate parasitic fly, *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). These isolates were characterized by a polyphasic approach and represent a new lineage of gamma-Proteobacteria as their closest relative is *Xylella fastidiosa* (87.1% 16S rDNA similarity). The four strains are identical at the 16S rDNA level, the level of similarity between them, based on DNA-DNA hybridization, is high (97.8-102.5 %) and they are similar in their physiological and biochemical characteristics, although they differ in their utilization of different sole carbon sources. All produce chitinase. They are obligately aerobic: no growth is detected under anaerobic conditions, even in the presence of NO; as terminal electron acceptor. Their predominant respiratory quinone is Q-8. The G+C content of their DNA is 42 mol%. Their cell membrane contains phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and two unknown polar lipids. Their main fatty acids are C(18:1), C(16:0) and C(14:0). To accommodate these bacteria, a new genus, *Schineria* gen. nov., with the type species *Schineria* larvae sp. nov., is proposed.

Sex chromosome variation and cytotaxonomy of the onchocerciasis vector *Simulium squamosum* in Cameroon and Nigeria

Traore-Lamizana, M; Somiari, S; Mafuyai, HB; Vajime, CG; Post, RJ. 2001

On the basis of sex chromosome variation, three cytotypes of *Simulium squamosum* (Enderlein) (Diptera: Simuliidae) are described from Cameroon and Nigeria. *Simulium squamosum* A is the typical form as originally described by Vajime & Dunbar (1975) with chromosome I as the sex chromosome. It occurs throughout most of Cameroon and south-east Nigeria. A second cytotype, *S. squamosum* B, is described from the river Sanaga (Cameroon). It also has chromosome I as the sex chromosome, but the nature of the sex differential region is different. *Simulium squamosum* C has no sex-linked chromosomal rearrangements. It is widespread in Nigeria and occurs near Mount Cameroon, where it seems to hybridize with *S. squamosum* A.

Cytotaxonomic characteristics of the genus *Glyptotendipes* Kieffer (Chironomidae, Diptera) from fish and retention ponds (Silesia, southern Poland)

Michailova, P; Warchalowska-Sliwa, E; Kownacki, A. 2001

The cytotaxonomic characteristics of species of the genus *Glyptotendipes* (Chironomidae): *G. glaucus* Meigen, *G. paripes* Edwards, and *G. barbipes* (Staeger) are described. The studied material was collected from a fish pond at Golysz and a sewage retention pond at Chybie in Silesia. All the studied species have the chromosome set $2n = 8$, but with many specific structural and functional changes. The cytogenetic data of *G. glaucus* showed that the studied specimens of this species have been produced by the introgressive hybridization of two sibling species: *G. glaucus* x *G. pallens*, and subsequent crossing-over in the hybrid chromosome CD. Owing to this process, the band pattern of chromosome arm D coincided with those of *G. pallens*. New aberrations (homo- and heterozygous deletions in arm G as well as heterozygous inversions in chromosome arm B) were detected in *G. glaucus*. Specific band sequences were discovered in chromosomes of *G. paripes*. The band patterns established in chromosomes AB and G of this species were identical with those of Siberian populations. The banding patterns of the polytene chromosomes of *G. barbipes* do not differ from the standard. However, high frequency of pericentric inversion of chromosome AB was established. Many new puffs were found in the polytene chromosomes of all the studied species. Their nucleolar organizer was very sensitive to environmental changes. In *G. glaucus* it

appeared in three different states: very active, slightly active, and heterozygous state. The organic pollution existing in the sewage retention pond may contribute to possible mutations and chromosomal damage in *Glyptotendipes* species. Structural and functional rearrangements of the polytene chromosomes of these species mobilized their genomes and provide for survival under polluted conditions.

Development of a diagnostic DNA probe for the fruit flies *Ceratitis capitata* and *Ceratitis rosa* (Diptera : Tephritidae) using amplified fragment-length polymorphism

Kakouli-Duarte, T; Casey, DG; Burnell, AM. 2001

The AFLP technique (amplified fragment-length polymorphism) was employed to identify and isolate species specific markers in tephritids. We have found that the technique has good potential for this purpose, with the only difficult part being the reamplification of AFLP fragments from silver stained gels. Cloning of putative species-specific markers and genomic dot blot hybridizations resulted in the development of diagnostic probes for tephritid identification. A repetitive DNA sequence from the genome of *Ceratitis capitata* (Wiedemann) was isolated. This sequence rapidly and reliably identified *C. capitata* and *C. rosa* Karsch in a collection of closely related and outgroup species tested in this study. Although this probe has been developed for *C. capitata* and *C. rosa*, the proposed methodology can be applied to any group of organisms.

Genetics, experience, and host-plant preference in *Eurosta solidaginis*: Implications for host shifts and speciation

Craig, TP; Horner, JD; Itami, JK. 2001

Host-associated mating is crucial in maintaining the partial reproductive isolation between the host races of *Eurosta solidaginis* (Diptera: Tephritidae), a fly that forms galls on *Solidago altissima* and *S. gigantea*. (We refer to flies reared from *S. gigantea* as *gigantea* flies and those reared from *S. altissima* as *altissima* flies.) We measured the host preference of males and females of both host races, F1 hybrids between the host races, F2, and backcrosses to both host races. Male and female *altissima* flies and female *gigantea* flies had high host fidelity, whereas male *gigantea* flies had low host fidelity. This result suggests that there may be gene flow between the host races due to nonassortative mating that occurs when male *gigantea* mate with *altissima* females on *S. altissima*. This indicates assortative-mating mechanisms in addition to host-associated mating are required to produce the partial reproductive isolation between the host races that has been observed. Nongenetic factors had no influence on host preference. Larval conditioning did not influence host preference: reciprocal F1 hybrids reared in *S. altissima* and *S. gigantea* both preferred *S. gigantea*. Adult experience had no impact on host preference: females preferred their natal host plant regardless of which host they encountered first as an adult. The hypothesis that maternal effects influence preferences was rejected because male and female flies did not show a consistent preference for the host plant of their mother. We also found no evidence that preference was a sex-linked trait because F1 and backcrosses to the host races with different combinations of X chromosomes from the two host races preferred *S. gigantea*. Our results indicate that host preference is not determined by a large number of genes because preference of hybrids did not correspond to the proportion of the genome derived from each host race. The strength of the ovipuncture preference for *S. gigantea* by *gigantea* females, the females of both reciprocal F-1 hybrids, the backcross to *gigantea*, and F(2)s indicates that preference is inherited nonadditively at a limited number of loci. The F1 female hybrids, however, had a weaker host preference for *S. gigantea* than the pure *gigantea* host race, indicating that there may be incomplete dominance or modifier loci. Males had different host preference patterns than females, with individual male *gigantea* and male F1 hybrids usually exhibiting preference exclusively for *S. gigantea* or *S. altissima*. One hypothesis explaining the difference in host preference between males and females is that the same gene influences both female and male host preference, but it is a sex-influenced gene. Thus, males carrying the gene for *S. gigantea* preference have an intermediate host preference, whereas females have a strong host preference to *S. gigantea*. In summary, we found that the host preference that produces host-associated mating is inherited nonadditively at a relatively small number of loci on autosomal genes. This mode of inheritance meets the assumptions of models of sympatric speciation, indicating that the host races could have evolved in sympatry.

Intraspecific hybridization of *Anopheles sinensis* (Diptera : Culicidae) strains from Thailand and Korea

Min, GS; Choochote, W; Jitpakdi, A; Kim, SJ; Kim, W; Jung, J; Junkum, A. 2002

Anopheles (*Anopheles*) *sinensis* [Wiedemann (1828)] is a member of the *hyrcanus* species group, and it has been incriminated as the natural or experimental malaria vectors in the Republic of Korea, Japan, China, and Indonesia. In Thailand, however, *An. sinensis* seems to be of little medical importance. Hybridization tests among the three iso-female lines (isolines) of *An. sinensis* [i.e., Form A (X, Y-1) and Form B (X, Y-2) (Thailand strain), and Form B (X, Y-2) (Korean strain)] were established based on two distinct types of metaphase chromosomes and geographical differences. The chromosomal form of the Korean strain was first identified from this study. Results of reciprocal and back crosses indicated that both karyotypic forms of the *An. sinensis* Thailand and Korean strains were genetically compatible, and provided viable progenies and completely synaptic polytene chromosomes. The sequences of the rDNA internal-transcribed spacer 2 (ITS2) and mitochondrial cytochrome c oxidase subunit 11 (COII) among the *An. sinensis* strains were nearly identical to each other, and the intraspecific sequence variability was very low (0.0-0.6%). Sequence comparisons among the cryptic inter-species (i.e., *An. sinensis*, *An. lesteri*, and *An. yatsushiroensis*), however, revealed extensive divergence, and the intraspecific variability ranged from 12.2 to 34.6%. Therefore, it is concluded from these results and previous vector ability studies that the *An. sinensis* Forms A and B exhibit cytological polymorphic races that have different vector abilities in their transmission of malaria, depending on their geographical locations.

Assessment of rDNA IGS as a molecular marker in the *Simulium damnosum* complex

Morales-Hojas, R; Post, RJ; Cheke, RA; Wilson, MD. 2002

For five cytospecies of the *Simulium damnosum* Theobald complex of blackflies (Diptera: Simuliidae) from West Africa, both ends of the intergenic spacer region (IGS) of the rDNA have been sequenced with the aim of developing specific molecular markers. No specific differences in these two regions were detected between *Simulium sanctipauli* V. & D., *Simulium sirbanum* V. & D., *Simulium soubrense* V. & D., *Simulium squamosum* Enderlein and *Simulium yahense* V. & D., except in the number of A subrepeats at the 5' end of the IGS (two in *S. squamosum* and four or five in the others) and in position 310 of the 3' end (a C in *S. squamosum* and a G in the others). However, genetic distances within and between species overlapped. These DNA sequences had no strong phylogenetic signal, and the trees obtained were mostly unresolved. Although most sequences from *S. squamosum* clustered together, a few of them were more similar to those in other cytospecies. These results could be explained either by hybridization with genetic introgression or by ancestral polymorphism and recent speciation.

On the possibility of hybridogenesis in the origin of midge *Chironomus usenicus* Loginova et Beljanina (Chironomidae, Diptera)

Polukonova, NV; Beljanina, SI. 2002

Hybridogenesis as a possible way of speciation in Chironomidae was considered with special reference to the species *Chironomus usenicus* resulting from hybridization between *C. plumosus* and *C. behningi*. The three species had $2n = 8$ and belonged to the thummi cytotaxon with chromosome arm combinations AB, CD, EF, and G. Arm G had a marker chromosome disk sequence (CDS) and was used to demonstrate the hybrid origin of *C. usenicus*. Most *C. usenicus* larvae were heterozygous in CDS of arm G. CDS use G2 proved to be identical to CDS beh G1 of *C. behningi* and CDS use G1, to CDS plu G1 of *C. plumosus*. It was assumed that *C. usenicus* results from hybridization between eurybiont *C. plumosus* and stenobiont *C. behningi* at the boundary of their species areas, in freshwater or brackish water bodies of the southern Saratov oblast and northern Kazakhstan. Morphologically and karyotypically, the hybrid was probably similar to *C. plumosus*. Crosses

with *C. plumosus* eliminated virtually all *C. behningi* chromosome sequences from the karyotype of the hybrid. Further chromosome divergence resulting in *C. usenicus* involved a number of chromosome rearrangements, including duplication of pericentric heterochromatin and other chromosome regions; inversion, which occurred in arm F (regions 13-16) and was fixed in the karyotype; and other paracentric inversions and deletions accumulated in heterozygote in the karyotype pool of the species. Since *C. behningi* was eliminated from the introgression zone and its species area reduced, the assimilation character was assumed for introgressive hybridization of *C. behningi* and *C. plumosus*.

Resistance to pre-dispersal seed predators in a natural hybrid zone

Campbell, DR; Crawford, N; Brody, AK; Forbis, TA. 2002

Plant hybrids can be more. less. or equally resistant to herbivores compared to their parental species. These patterns in resistance can be critical determinants of the fitness of plant hybrids and may also influence distribution of the herbivore. We examined resistance to a pre-dispersal seed predator by natural and experimental hybrids between *Ipomopsis aggregata* and *I. tenuituba*. These species and their hybrid offspring differed primarily in ability to avoid oviposition by *Hylemya* sp. (Diptera: Anthomyiidae) rather than in reducing damage to seeds by a developing larva. Plants of *I. tenuituba* had the lowest frequency of fly eggs and were thus the most successful at avoiding damage. Hybrids were either intermediate to or less resistant than both parental species. Because these patterns persisted in experimental arrays of interspersed potted plants, they cannot be attributed to ongoing differences in the environment between hybrid and parental sites. In experimental arrays, the frequency of fly eggs correlated positively with corolla width, a dimension of flower size that also influences the rate of pollination, suggesting seed predators can generate selection on reproductive traits of hybrids. Furthermore, in one of the arrays, oviposition on F2 hybrids exceeded the average for the F1 and the midparent. Our results underscore the need to consider genetic background of hybrids in assessing plant responses to herbivores.

Post-bloodmeal diuretic shedding of hepatitis B virus by mosquitoes (Diptera : Culicidae)

Blow, JA; Turell, MJ; Walker, ED; Silverman, AL. 2002

Persistence and diuretic shedding of hepatitis B virus (HBV) by mosquitoes (Diptera: Culicidae) was studied by using infectious blood feedings, intrathoracic inoculations, and detection of virus by polymerase chain reaction (PCR) and Southern hybridization. Results showed that both *Anopheles stephensi* Liston and *Ochlerotatus triseriatus* (Say) shed HBV during diuresis for up to 72 h after feeding on an HBV-positive serum drawn from a human donor. HBV did not persist in the bodies of either *An. stephensi* or *Oc. triseriatus* past 72 h by infectious feeding or intrathoracic inoculation of HBV suspension. Viral dissemination did not occur by infectious feeding in *An. stephensi* or *Oc. triseriatus*, or by intrathoracic inoculation in *An. stephensi*, *Oc. triseriatus*, or *Culex quinquefasciatus* Say. These results suggest that HBV could be transmitted to humans by a stercorarian route, especially if mosquitoes that fed on an HBV-positive human are interrupted during feeding and move to another person to resume feeding.

Differentially expressed midgut transcripts in *Culicoides sonorensis* (Diptera : Ceratopogonidae) following Orbivirus (Reoviridae) oral feeding

Campbell, CL; Wilson, WC. 2002

Understanding the vector insect's gene expression response to a virus infection may aid design of control measures for arbovirus diseases. *Culicoides sonorensis* is a vector of several agriculturally important pathogens, such as epizootic haemorrhagic disease virus (EHDV) that causes disease in ruminants. Two approaches, differential display and suppression subtractive hybridization, were used to identify 400+ *Culicoides* transcripts that were more abundant in midguts 1 day following an oral meal containing EHDV. Of these, quantitative PCR confirmed seven to be more abundant in virus-fed midguts than controls. One such transcript encodes a putative RNA editase, CsRED1, induced by dsRNA. Transcripts encoding putative receptors involved in cell differentiation included CsLAR, a protein tyrosine phosphatase, and CsFZ2, homologous to the wingless receptor in *D. melanogaster*. Transcripts encoding putative translation machinery components included CsEF3, CsEF5A and CsRPS6. Overall, the cDNA fragments identified in this study increased in the midgut at one day postfeeding; by 2 days postfeeding, increases in transcript levels shifted from the midgut to the remainder of the infected midge.

Population variability in *Chironomus* (Camptochironomus) species (Diptera, Nematocera) with a Holarctic distribution: evidence of mitochondrial gene flow

Martin, J; Guryev, V; Blinov, A. 2002

Phylogenetic analysis of DNA sequences from mitochondrial (mt) genes (Cytochrome b and Cytochrome oxidase I) and one nuclear gene (globin 2b) was used for the investigation of Nearctic and Palearctic populations representing four *Chironomus* species of the subgenus *Camptochironomus*, namely *C. biwaprimus*, *C. pallidivittatus*, *C. tentans sensu stricto* and *C. dilutus* (the last two species constitute Holarctic *C. tentans sensu lato*). Phenograms constructed on the basis of mt sequences were not congruent with trees based on nuclear genes, or with morphological and cytological data. The mt tree divided the populations by continental region, rather than by the species groupings recognized by the other data sets. The incongruence is explained by mt gene flow resulting from hybridization between the sympatric species on each continent. Calculation of divergence times, based on the sequence data, suggest that *C. tentans* (s.l.) and *C. pallidivittatus* have both been in North America for about 2.5 My.

Speciation and distribution of the members of the *Anopheles punctulatus* (Diptera : Culicidae) group in Papua New Guinea

Cooper, RD; Waterson, DGE; Frances, SP; Beebe, NW; Sweeney, AW. 2002

Mosquito collections were made throughout the mainland of Papua New Guinea to identify the members of the *Anopheles punctulatus* group present and to determine their distribution. Identification was made using morphology, DNA hybridization, and polymerase chain reaction (PCR)-RFLP analysis. Nine members of the group were identified: *An. farauti* s.s. Laveran, *An. farauti* 2, *An. koliensis* Owen, and *An. punctulatus* Donitz, were common and widespread; *An. farauti* 4 was restricted to the north of the central ranges where it was common; *An. farauti* 6 was found only in the highlands above 1,000 m; and *An. farauti* 3, *An. sp. near punctulatus* and *An. clowi* Rozeboom & Knight were uncommon and had restricted distributions. Identification of *An. koliensis* and *An. punctulatus* using proboscis morphology was found to be unreliable wherever *An. farauti* 4 occurred. The distribution and dispersal of the members of the *An. punctulatus* group is discussed in regard to climate, larval habitats, distance from the coast, elevation, and proximity to human habitation.

Detection of circulating hypodermin C: an antigen capture ELISA for diagnosis of cattle grub (Diptera : Oestridae) infestations

Panadero-Fontan, R; Lopez-Sanchez, C; Parra-Fernandez, F; Morrondo-Pelayo, P; Diez-Banos, P; Colwell, DD. 2002

An antigen capture assay for the detection of circulating hypodermin C was developed for diagnosis of hypodermosis. A murine monoclonal antibody to recombinant hypodermin C was raised using rapid immunization and a one-step hybridization-cloning technique. A highly reactive, specific monoclonal antibody was tested using

sera spiked with known quantities of purified, native hypodermin C or with recombinant hypodermin C. Sensitivity of 96.4% and specificity of 95.6% for the antigen capture assay was assessed using a panel of sera from animals unexposed to cattle grubs and from cattle with palpation proven cattle grub infestations. Data from this panel of sera was used to establish the cut-off OD for further testing. The kinetics of circulating hypodermin C was assessed using the assay in three groups of cattle artificially infested with 50, 100 or 200 first instar *Hypoderma lineatum*. Antigen was first detected approximately 6 weeks after infestation. The amount of antigen detected increased in each group of animals reaching peaks at different times in each group. Levels of antigen fell quickly following arrival of grubs at the back and completion of the molt to second instar.

Proventriculus-specific cDNAs characterized from the tsetse, *Glossina morsitans morsitans*

Hao, ZG; Aksoy, S. 2002

Peritrophic matrix (peritrophic membrane or PM) is an important structure in the gut of most insects at some stage in their development. It is composed of chitin, proteins and proteoglycans. Multiple roles for the PM ranging from partitioning of digestive enzymes and food to protection of gut epithelial cells from viral and parasitic invasion have been proposed. While most adult members of Diptera have a Type I PM synthesized in response to a blood meal, the medically and agriculturally important vector insect, tsetse has a sleeve-like Type 11 PM which is constitutively synthesized by cells in the proventriculus (cardia). Using a differential hybridization approach, we have identified three abundant cDNAs from a proventriculus cDNA library of *Glossina morsitans morsitans*: GmPro1, GmPro2 and GmPro3. DNA sequence analysis indicates that GmPro1 and GmPro2 share similarities with the peritrophin-15 family of larval PM proteins, while GmPro3 is a member of the serine protease family. Northern analysis indicates that transcripts for all three cDNAs are preferentially expressed in the proventriculus tissue. The expression profile of these genes in response to the presence of trypanosome indicates that transcription of GmPro1 is increased in the presence of parasites (immune sensitive), while the other two are not affected. Western analysis using antibodies developed against the recombinant GmPro2 shows its primary localization in the gut to be within the peritrophic matrix structure. We discuss the molecular characteristics of these proventriculus specific cDNAs and their products as well as their potential role for vector control studies. (C) 2002 Elsevier Science Ltd. All rights reserved.

Nonrandom chromosomal distribution of spontaneous breakpoints and satellite DNA clusters in two geographically distant populations of *Chironomus riparius* (Diptera : Chironomidae)

Bovero, S; Hankeln, T; Michailova, P; Schmidt, E; Sella, G. 2002

Two geographically distant populations of *Chironomus riparius* (syn. *C. thummi*) from two environmentally polluted sites (Santena, Italy and Varna, Bulgaria) show numerous somatic and inherited chromosomal aberrations (inversions, deletions and deficiencies). Fifty-five percent of the observed breakpoints occurred in at least two larvae from both populations. Breakpoints occurring twice or more were considered as 'common' structural chromosomal breakpoints. We tested whether such common breakpoints in larvae of the two polluted populations had a random chromosomal distribution or occurred preferentially in specific heterochromatic regions. Distribution of common breakpoints was not random, and proximal regions of first and third chromosome had significantly more common breakpoints than distal ones. By FISH we identified and mapped 56 chromosomal sections containing clusters of two tandem-repetitive satellite DNA families called Hinf and Alu elements. Like the common breakpoints, these repetitive DNA clusters appeared to be significantly more abundant in regions of constitutive heterochromatin such as the pericentromeric regions, while in distal sections of chromosomal arms they were rare or absent. Twenty-four out of 45 common breakpoints (i.e., 53.3%) occurred in cytogenetic sections where Alu and Hinf satellite DNA probes hybridized. The frequency of co-localization between common breakpoints and repetitive DNA hybridization signals was significantly higher than expected by chance. We hypothesize that spontaneous or induced breaks occur more frequently in sections containing blocks of repetitive DNA.

Spatial and habitat distribution of *Anopheles gambiae* and *Anopheles arabiensis* (Diptera : Culicidae) in Banambani Village, Mali

Edillo, FE; Toure, YT; Lanzaro, GC; Dolo, G; Taylor, CE. 2002

We studied the larval distribution and composition of *Anopheles arabiensis* Patton, *An. gambiae* s.s. Giles, and its forms, among local habitats; and their association with the adults between these habitats in Banambani village, Mali during the mid-rainy seasons of 1997-1999. For species and form identification we used polymerase chain reaction (PCR) and PCR-restriction fragment-length polymorphism (RFLP). Differences among species in the distribution of larvae were observed in 1998, but not in 1997 or 1999, although they were on the borderline of statistical significance. Differences among the M and S molecular forms were statistically significant in 1999 when rainfall was high, but not in the two prior, drier sampling periods. Combining all information into the Fisher multiple comparisons test, there were statistically significant differences between species and molecular forms during the 3-yr study period. Hybrid larvae between the M and S forms were observed (0.57%), the first such observation to our knowledge. In spite of differences among larval distribution, no differences of adult species composition were observed among habitats. Factors that influence the distributions of *An. gambiae* larval populations are discussed.

Factors affecting female remating frequency in the mediterranean fruit fly (Diptera : Tephritidae)

Vera, MT; Wood, R.J; Cladera, J.L; Gilburn, A.S. 2002

Mating and remating of two laboratory strains (Petapa and Guate), one wild population (Antigua) of *Ceratitis capitata* (Wiedemann) and one of the hybrids between them were studied under laboratory conditions. No evidence of sexual isolation at first mating was found among them. Remating frequency was higher under crowded conditions for the two laboratory strains. The probability of Petapa females remating depended more on the origin of the male and was negatively associated with the duration of the first mating, but these variables had no effect on remating tendency of Guate females. Matings by Petapa males were significantly less prolonged than those of Guate or hybrid males. With respect to remating, Petapa non-virgin females preferred Petapa to Guate males.

Potential for mating between *Bactrocera tryoni* (Froggatt) and *Bactrocera neohumeralis* (Hardy) (Diptera : Tephritidae)

Pike, N; Meats, A. 2002

We conducted a series of mating experiments in the laboratory and in a glasshouse to examine male calling and the potential for mating between *Bactrocera tryoni* and *B. neohumeralis*. Males of each species do make presumed courtship calls, but at entirely discrete periods and regardless of the presence of other males or females. Despite this, assortative mating tests in the glasshouse demonstrated the potential for rare interspecific matings. When F₁ hybrids were included in these assortative mating tests they commonly mated with *B. neohumeralis* during the middle of the day and with *B. tryoni* at dusk. Hybrids may facilitate bidirectional gene flow between these species.

Isoenzyme variation in *Aedes aegypti* correlated with *Dirofilaria immitis* infectability

Nayar, JK; Knight, JW. 2002

From the Vero Beach strain of the mosquito *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae), substrains were selected for susceptibility (SS) and refractoriness (RR) to the dog heartworm *Dirofilaria immitis* (Leidy) (Filarioidea: Onchocercidae). These two lines and their reciprocal F-1 hybrids were analysed for genetic variation at 14 enzyme loci, using polyacrylamide gel electrophoresis. Six of the enzyme loci showed variation (sample size 48 alleles/locus/line). Three of these were monomorphic in the refractory line but polymorphic in the susceptible, i.e. aconitase hydratase (Acoh), isocitrate dehydrogenase-1 (Idh-1) and phosphoglucomutase (Pgm). The other three loci, glucose-6-phosphate isomerase (Gpi), hexokinase-1 (Hk-1) and isocitrate dehydrogenase-2 (Idh-2), were polymorphic in both SS and RR lines and their hybrids. At two loci (Hk-1, Pgm) three alleles were detected, whereas the other polymorphic loci had only two alleles. For Hk-1, the most frequent allele was Hk-1(80) (0.563) in refractory and Hk-1(100) in the susceptible (0.521) and F-1 hybrids. For Pgm the most frequent alleles were Pgm(125) in the susceptible line (0.646) and Pgm(100) in the F-1 hybrids (0.563 and 0.604) and refractory line (1.000). The mean observed heterozygosity (H-o), the mean Hardy-Weinberg expected heterozygosity (H-e) and the mean number of alleles per locus in the refractory line were lower, but not significantly so, than in the susceptible line and their reciprocal F1 hybrids; the proportion of polymorphic loci was significantly lower in the refractory than in the susceptible line and their F1 hybrids. Within both lines all polymorphisms were in Hardy-Weinberg equilibrium, whereas significant departures from predicted frequencies were observed in SS x RR hybrids at four polymorphic loci (Acoh, Gpi, Hk-1, Pgm) and at three polymorphic loci (Acoh, Hk-1, Pgm) in RR x SS hybrids. The average Nei's and modified Rogers' genetic distances between the lines were 0.024 and 0.139, respectively. These electrophoretic data show that the refractory line (putatively lacking fi allele) can be distinguished from the susceptible line (fi/fi) and their hybrids (heterozygous fi) by isozyme marker frequencies, but it remains to be seen whether this difference is causal or chance linkage. In any case, this model system of *Ae. aegypti*/D. immitis provides opportunities to better understand and manipulate the molecular biology of filariasis transmission.

Mating and remating of medflies (Diptera : Tephritidae) in Guatemala: Individual fly marking in field cages

McInnis, DO; Rendon, P; Komatsu, J. 2002

The sterile insect technique (SIT) depends critically upon the ability of sterilized, released males to locate and mate with wild females. The overall efficiency of the method also depends upon the relative frequencies of remating by wild females following first matings to laboratory or wild males. Using a newly devised technique that individually marks the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), a field cage study was undertaken in a Guatemala coffee orchard to record individual fly mating behaviors between each of several laboratory strain and coffee-reared wild flies. Five laboratory strains were tested- a genetic sexing strain examined in sex ratios between 50%-100% sterile males, two standard bisexual strains, and two F1 hybrid strains. The marking technique revealed a substantial amount of information on individual fly mating and remating. Wild male flies significantly outcompeted each of the lab strains in the first matings with both wild and lab females. Approx. 22% and 3% of wild males and females, respectively, remated in the field cages during two consecutive morning observation periods, while 4-8% of lab males, and 2-8% of lab females remated, respectively. Male flies from each lab strain averaged significantly shorter copulation times than wild males. Female flies, either lab or wild, tended to remate more often if they first mated to a lab male, but the differences were not statistically significant. An index was devised to provide a measure of relative male mating quality. Wild males tended to have higher individual index values than lab strain males. Average values of the latter ranged from ca. half to roughly equal that of wild males.

Improving male mating competitiveness and survival in the field for medfly, *Ceratitis capitata* (Diptera : Tephritidae) SIT programs

McInnis, DO; Shelly, TE; Komatsu, J. 2002

The success of the sterile insect technique (SIT) depends critically upon mating between released sterilized males and wild females. In Hawaii, improvements in the efficiency of sterile males were attempted on two separate fronts - mating enhancement and survival improvement. In the former, two methods have been investigated selective breeding and aromatherapy. In the latter, flies which survived in field cages for several days were selected and bred to produce progeny with enhanced survival ability compared to control flies. Regarding mating selection, standard laboratory-reared males that successfully mated with wild females in field cages were allowed to breed. F1 offspring were inbred, then the selection procedure was repeated for four additional cycles. In the aromatherapy procedure, laboratory-reared males were exposed to ginger root oil for several hours 1 day prior to testing in field cages. Compared to controls, the selected flies improved the mating competitiveness of male flies ca. 3-fold, irradiation reduced this increase to ca. 2.5-fold. Exposing the selected, hybrid strain raised the fitness of the lab males to ca. 9-fold that of wild males. In the ongoing survival selection study, we have obtained lines in which the selected males survived ca. 2-fold better than laboratory control males over several days in an outdoor field cage, with food and water provided. The goal is to combine the traits of higher survival and mating ability into a single strain for SIT release.

Species associations of gall-formers on willows (*Salix* spp) at the floodplains of the Rhine River (Hymenoptera : Tenthredinidae : *Euura*, *Phyllocolpa*, *Pontania*; Diptera : Cecidomyiidae : *Dasineura*, *Iteomyia*, *Rabdophaga*)

Kopelke, JP; Amendt, J. 2002

The gall-former communities of willows (*Salix* spp) have been investigated in 1999 and 2000 at nine natural field sites along the floodplains of the Rhine River. 15 *Salix* species and two hybrids occurred at the willow stands studied, revealing great differences between the sites with regard to composition and abundances of the willow species. A total number of 1,277 willow individuals were mapped, checking the presence and densities of gall-forming species. A total of 43 gall-forming species have been registered, consisting of sawflies (Tenthredinidae: 29 spp) and gall midges (Cecidomyiidae: 14 spp). These gall-formers represented about 67% of the total of 64 potential gall-formers which may occur on the particular willow species studied. The distribution pattern of the gall-formers varied noticeable within a site as well as between the different sites. The maximum number of 7-8 gall-forming species has been recorded from *S. alba* (subgenus *Salix*), *S. caprea*, and *S. purpurea* (subgenus *Vetrix*), respectively. A complete utilization of the host plant resource by its particular gall-formers was uncommon at all sites. The gall-former densities varied between the individuals and sexes of the host plants as well as between the sites studied. Among the gall-former associations within a host plant population those consisting of only two species were most frequent. The relationship of densities of the associated gall-formers were analyzed at the level of shoots among their host plant species. The results indicate that species pairs of gall-formers co-occurring on the same host plant species tended to attack different willow specimens or different shoot length classes within the same willow individual.

The likely fate of hybrids of *Bactrocera tryoni* and *Bactrocera neohumeralis*

Pike, N; Wang, WYS; Meats, A. 2003

Bactrocera tryoni (Froggatt) and *B. neohumeralis* (Hardy) (Diptera: Tephritidae) are sympatric species which hybridise readily in the laboratory yet remain distinct in the field. *B. tryoni* mates only at dusk and *B. neohumeralis* mates only during the day, but hybrids can mate at both times. We investigated the inheritance of mating time in successively backcrossed hybrid stocks to establish whether mating with either species is more likely. The progeny of all backcrosses to *B. tryoni* mated only at dusk. The majority of the progeny of the first and a minority of the progeny of the second backcross to *B. neohumeralis* also mated at dusk, but the third successive *B. neohumeralis* backcross produced flies that mated only during the day. This trend towards dominance of the *B. tryoni* trait was also reflected in a diagnostic morphological character. We discuss the possible genetic background for these phenomena and propose that unidirectional gene flow might explain how the two species remain distinct in the face of natural hybridisation.

Mississippi areawide fire ant suppression program: Unique aspects of working with black and hybrid imported fire ants

Vogt, JT; Streett, DA; Pereira, RM; Callcott, AMA. 2003

Chemical and biological controls for imported fire ants (*Solenopsis invicta* Buren, the red imported fire ant, *S. richteri* Forel, the black imported fire ant, and their hybrid) are being investigated and demonstrated in a multistate, multiagency Areawide Management Program. The Mississippi component of the program offers the unique opportunity to implement the latest control technologies against monogyne (single queen) black and hybrid imported fire ant colonies because sites in the other participating states (Florida, South Carolina, Texas, and Oklahoma) are dominated by polygyne (multiple queen) red imported fire ant colonies. Several observed and potential differences were examined between populations of black/hybrid fire ants and populations of red imported fire ants. Lower nest density of monogyne black and hybrid fire ant populations (range = 19-52% of mean nest density at other sites) required modification of sampling protocols. *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae), a phorid fly collected from Las Flores, Argentina, where *S. richteri* is present, was established at the Mississippi sites because it preferentially attacks black and hybrid imported fire ants; at the other sites, *P. tricuspis*, a phorid that parasitizes red imported fire ants, has been released. Other potential differences in management of black, hybrid, and red imported fire ants are discussed.

Genetic delineation of sibling species of the pest fruit fly *Bactocera* (Diptera : Tephritidae) using microsatellites

Wang, Y; Yu, H; Raphael, K; Gilchrist, AS. 2003

Using a large set of microsatellites, the genetic relationships between three closely related Australian fruit fly species, *Bactocera tryoni* (Froggatt), *B. neohumeralis* (Hardy) and *B. aquilonis* (May) were investigated. *Bactocera tryoni* and *B. neohumeralis* are sympatric, while *B. aquilonis* is allopatric to both. The sympatric species, *B. tryoni* and *B. neohumeralis*, were found to be genetically distinct. It is likely that despite differences in mating time between these two species, some gene flow still occurs. In contrast, the sibling species *B. tryoni* and *B. aquilonis* were found to be closely related, despite allopatry. The level of genetic divergence was similar to that found within eastern Australian populations of *B. tryoni*. Consideration of all available genetic data suggests that this similarity is not due to recent (i.e. within the last 30 years) displacement of *B. aquilonis* by *B. tryoni* from the *B. aquilonis* region (north-western Australia). Instead the data suggests that, at least in the areas sampled, asymmetrical hybridization may have occurred over a longer timescale.

Analysis of the amplification and transcription of the C3-22 gene of *Rhynchosciara americana* (Diptera : Sciaridae) in transgenic lines of *Drosophila melanogaster*

Soares, MAM; Monesi, N; Basso, LR; Stocker, AJ; Paco-Larson, ML; Lara, FJS. 2003

Drosophila melanogaster was transformed with an 18 kb fragment of the C3 DNA puff of *Rhynchosciara americana*, including the C3-22 gene and the origins of replication that direct amplification. Different tissues and developmental stages of five independent transgenic lines were analyzed by quantitative Southern blot hybridization. No indication was found that the transformed fragment was amplified, strongly suggesting that factors involved in DNA puff amplification have not been conserved in *Drosophila*. Transcription of the C3-22 gene in the transgenic lines was found to be at a low and constitutive level throughout development. These results indicate that, unlike other DNA puff genes, the factors that regulate the C3-22 gene are not conserved in *Drosophila*.

Phorid flies in Alabama: A tale of two species

Graham, LCF; Porter, SD; Bertagnolli, VE. 2003

Two species of phorid fly have been released at 11 sites in Alabama and have been recovered from 9 sites. *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) was released in South Alabama in populations of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), and *Pseudacteon curvatus* Borgmeier was released in North Alabama in hybrid fire ant populations (*Solenopsis invicta* x *Solenopsis richteri* Forel). The number of mounds per hectare and mound size was recorded for all release sites and for control sites, if established. Data from the three oldest sites are presented. Mound numbers decreased at two release sites, but increased at the third. Further analysis and data collection are needed to determine the long-term effect of phorid flies on Alabama fire ants.

Comparative genomics of insect-symbiotic bacteria: Influence of host environment on microbial genome composition

Rio, RVM; Lefevre, C; Heddi, A; Aksoy, S. 2003

Commensal symbionts, thought to be intermediary amid obligate mutualists and facultative parasites, offer insight into forces driving the evolutionary transition into mutualism. Using macroarrays developed for a close relative, *Escherichia coli*, we utilized a heterologous array hybridization approach to infer the genomic compositions of a clade of bacteria that have recently established symbiotic associations: *Sodalis glossinidius* with the tsetse fly (Diptera, *Glossina* spp.) and *Sitophilus oryzae* primary endosymbiont (SOPE) with the rice weevil (Coleoptera, *Sitophilus oryzae*). Functional biologies within their hosts currently reflect different forms of symbiotic associations. Their hosts, members of distant insect taxa, occupy distinct ecological niches and have evolved to survive on restricted diets of blood for tsetse and cereal for the rice weevil. Comparison of genome contents between the two microbes indicates statistically significant differences in the retention of genes involved in carbon compound catabolism, energy metabolism, fatty acid metabolism, and transport. The greatest reductions have occurred in carbon catabolism, membrane proteins, and cell structure-related genes for *Sodalis* and in genes involved in cellular processes (i.e., adaptations towards cellular conditions) for SOPE. Modifications in metabolic pathways, in the form of functional losses complementing particularities in host physiology and ecology, may have occurred upon initial entry from a free-living to a symbiotic state. It is possible that these adaptations, streamlining genomes, act to make a free-living state no longer feasible for the harnessed microbe.

The ceratotoxin gene family in the medfly *Ceratitis capitata* and the Natal fruit fly *Ceratitis rosa* (Diptera : Tephritidae)

Rosetto, M; Marchini, D; de Filippis, T; Cioffi, S; Frati, F; Quilici, S; Dallai, R. 2003

Ceratotoxins (Ctxs) are a family of antibacterial sex-specific peptides expressed in the female reproductive accessory glands of the Mediterranean fruit fly *Ceratitis capitata*. As a first step in the study of molecular evolution of Ctx genes in *Ceratitis*, partial genomic sequences encoding four distinct Ctx precursors have been determined. In addition, anti-*Escherichia coli* activity very similar to that of the accessory gland secretion from *C. capitata* was found in the accessory gland secretion from *Ceratitis* (*Pterandrus*) *rosa*. SDS-PAGE analysis of the female reproductive accessory glands from *C. rosa* showed a band with a molecular mass (3 kDa) compatible with that of Ctx peptides, also slightly reacting with an anti-Ctx serum. Four nucleotide sequences encoding Ctx-like precursors in *C. rosa* were determined. Sequence and phylogenetic analyses show that Ctxs from *C. rosa* fall into different groups as *C. capitata* Ctxs. Our results suggest that the evolution of the ceratotoxin gene family might be viewed as a combination of duplication events that occurred prior to and following the split between *C. capitata* and *C. rosa*. Genomic hybridization demonstrated the presence of multiple Ctx-like sequences in *C. rosa*, but low-stringency Southern blot analyses failed to recover members of this gene family in other tephritid flies.

Diversification of sympatric *Sapromyza* (Diptera : Lauxaniidae) from Madeira: six morphological species but only four mtDNA lineages

Pestano, J; Brown, RP; Suarez, NM; Baez, M. 2003

A series of recent studies on speciation of insects within the Canary Islands have indicated considerable within-island diversification, similar to that described in the Hawaiian islands. Little work has yet been carried out on the neighboring Madeiran archipelago, which is also volcanic. This study examines relationships among all known Lauxaniid flies of the genus *Sapromyza* from Madeira (including six newly described morphological species) based on mitochondrial gene trees constructed from cytochrome c oxidase (subunit I) and 16S rRNA partial sequences. Phylogenies based on maximum likelihood distances, a Bayesian method based on Markov chain Monte Carlo sampling from the posterior probability distribution, and maximum parsimony show that eight of the nine Madeiran species comprise a single monophyletic group. This clade is also split into two subclades representing black- and yellow/orange-bodied forms. The latter mtDNA clade corresponds to only two species (*Sapromyza imitans* and *Sapromyza indigena*) which are not reciprocally monophyletic. Monophyly is strongly supported within four of the six black-bodied species but not for the species pair (*Sapromyza inconspicua*, *Sapromyza laurissilvae*). We discuss the double occurrence (at least) of introgressive hybridization/incomplete lineage sorting within this group and suggest that recent speciation is the most likely explanation. The remaining species on the island. *Sapromyza madeirensis*, is very divergent from the aforementioned group, occupying a more basal position in the tree than the other Atlantic island and continental *Sapromyza* that were included in the analysis. At least two speciation events for Madeiran *Sapromyza* appear to correspond to quite ancient periods relative to the age of the island, while others are more recent. This suggests that a combination of island colonization and within-island sympatric and/or vicariance-mediated speciation may explain the observed diversity. (C) 2003 Elsevier Science (USA). All rights reserved.

Field releases of the decapitating fly *Pseudacteon curvatus* (Diptera : Phoridae) for control of imported fire ants (Hymenoptera : Formicidae) in Alabama, Florida, and Tennessee

Graham, LC; Porter, SD; Pereira, RM; Dorough, HD; Kelley, AT. 2003

The little decapitating fly, *Pseudacteon curvatus* Borgmeier, was released at 11 sites in Alabama, Florida, and Tennessee as a potential self-sustaining biocontrol agent of imported fire ants. We used a biotype from Buenos Aires Province, Argentina that parasitizes black fire ants (*Solenopsis richteri* Forel). Generally, several thousand flies were released as larvae in parasitized ant workers over a 1-2 week period. *Pseudacteon curvatus* flies were successfully established on hybrid fire ants (*Solenopsis invicta* x *Solenopsis richteri*) at a site near Talladega, Alabama where they have persisted more than two years and expanded out 5-20 km from the original release site. Flies failed to establish in Florida and Tennessee although a few 1st-generation field-reared flies were recovered at four sites in Florida. This fly is only the second parasitoid species to be successfully released against imported fire ants or any other pest ant species. Possible reasons for failures at the other sites include insufficient vegetation cover, competition with another *Pseudacteon* species in Florida, severe winter kill of ants at a site in Tennessee, and the possibility that the biotype of *R. curvatus* released was not a viable parasitoid of red imported fire ants.

Effect of sorghum resistant to *Stenodiplosis sorghicola* (Coquillett) (Diptera : Cecidomyiidae) on oviposition and development of *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae)

Franzmann, BA; Scholz, BC. 2003

Helicoverpa armigera oviposition preference for, and larval development on sorghum hybrids with differing resistance to sorghum midge, *Stenodiplosis sorghicola*, were investigated. When *H. armigera* larvae were fed seed of resistant and susceptible hybrids in the laboratory there were no differences in larval and pupal sizes or the rate of development. The same result was recorded when larvae fed on panicles on plants in a glasshouse. On some sampling occasions, significantly more eggs were laid on panicles of resistant hybrids in the field. This occurred when plants were in plots and also in a mixed planting. Midge-resistance status did not affect levels of egg parasitism. In a field study using recombinant inbred lines between a midge-resistant and a midge-susceptible line, no relationship was found between level of resistance and oviposition of *H. armigera*. We conclude that, although midge-resistant hybrids are sometimes preferred for oviposition by *H. armigera*, the resistance per se does not determine this preference. Egg survival, larval survival, development and resultant damage are not significantly affected by the midge-resistance status of the host.

Parasitoids incidence and diversity on maize stem borers *Sesamia nonagrioides* Lefebvre and *Ostrinia nubilalis* Hubner in NW SPAIN

Monetti, L; Malvar, RA; Ordas, A; Cordero-Rivera, A. 2003

The most important insect pests on maize in Spain are the pink stem borer *S. nonagrioides* (Lefebvre) and the European corn borer *O. nubilalis* (Hubner). Parasitoid degree of control on stem borers and other maize pests in Spain is not known. The aim of this research was to evaluate the incidence and diversity of parasitoids on maize stem borers in the province of Pontevedra (NW Spain). Samplings of hybrid field maize plants (DMB-1570) were taken in four locations, from March 1999 to November 2000. Plants were dissected and stem borer overwintering and non-overwintering larvae were collected in separated plastic boxes. Larvae of *Mythimna* spp. and *Helicoverpa armigera* were also collected. Larvae were reared in the insectary and adult parasitoids obtained from them were determined. Diversity of parasitoids was in general very ton,. The most frequently found species on non-overwintering larvae was *Lydella thompsoni* (Herting) (Diptera: Tachinidae). Differences in percentage of parasitism (PP) between *O. nubilalis* and *S. nonagrioides* were significant. *Lydella thompsoni* was also the dominant parasitoid on overwintering larvae. Its PP was higher on *O. nubilalis* than on *S. nonagrioides*. The correlation between number of stem borer per plant and percentage of parasitised larvae was not significant, neither for *S. nonagrioides* nor for *O. nubilalis*. Shannon-Wiener index as a measure of parasitoid diversity was similar in the different hosts. In conclusion, a very low parasitoid natural control on maize borers, in hybrid corn crops grown at the province of Pontevedra was found. Low numbers of parasitoids and low percentage of parasitism in the studied area make difficult to draw definitive conclusions. Stem borers seem to be protected from parasitoids by several circumstances, specifically the development of their immature stages within refuges (the stalks), but other factors probably would contribute to the situation.

Kelp flies and species concepts - the case of *Coelopa frigida* (Fabricius, 1805) and *C-nebularum* Aldrich, 1929 (Diptera : Coelopidae)

Laamanen, TR; Petersen, FT; Meier, R. 2003

The beaches of the North Atlantic and North Pacific are home to kelp flies of the *Coelopa frigida*/nebularum complex, which consists of one to three different species depending on whether the two nominal species are accepted and a cryptic species proposed by Remmert is counted. The morphological differences between two populations of *C. frigida* (Fabricius, 1805) from the North Sea and the Baltic Region and two populations of *C. nebularum* Aldrich, 1929 from Alaska and Japan are described and discussed for small, medium, and large specimens. Crossing experiments are used to demonstrate that, under laboratory conditions, no isolation mechanisms between either population exist. *Coelopa frigida* and *C. nebularum* are therefore regarded as a single biological species, a conclusion that is congruent with the observation that the genetic distances based on Ef 1-alpha and 16S rDNA indicate lower levels of differentiation within *C. frigida*/nebularum than between undisputed *Coelopa* species. The substantial morphological, breeding and genetic information on the *C. frigida*/nebularum species complex is then applied to six different species concepts popular in the modern systematic literature. According to the Biological, Hennigian and Recognition Species Concepts, only a single species would be recognized. The Evolutionary Species Concept is too vague to be applicable and under two variants of the Phylogenetic Species Concept, *C. frigida* and *C. nebularum* would constitute separate species. This result confirms that Phylogenetic Species Concepts lead to a higher species number than concepts based

on reproductive isolation. Practical and theoretical problems with the various species concepts are briefly discussed.

Reverse transcriptase-related proteins in telomeres and in certain chromosomal loci of *Rhynchosciara* (Diptera : Sciaridae)

Gorab, E. 2003

The localization of reverse transcriptase-related proteins in polytene chromosomes of dipterans was investigated using previously characterized antibodies to a recombinant polypeptide containing conserved motifs of insect reverse transcriptases. The immunoreactions were carried out with polytene chromosome squashes of eight sciarids, one chironomid and three *Drosophila* species. Telomeric staining was regularly observed on chromosomes of the sciarid *Rhynchosciara americana* under normal growth conditions. Five of eight chromosomal tips were labelled except for the heterochromatic ends that are occasionally found associated forming a chromocentre in the salivary gland. Reverse transcriptase-related proteins were detected at chromosomal tips of young larvae and remained bound to the telomeres throughout larval development. As in salivary gland chromosomes, five non-telocentric ends of the chromosomes from Malpighian tubules of *R. americana* appeared clearly stained with anti-reverse transcriptase. The occurrence of telomeric reverse transcriptase in *R. americana* correlates with the presence of RNA in addition to an unusual enrichment with homopolymeric dA/dT DNA associated with the telomeric heterochromatin. The antibodies also reacted with a few interstitial sites in chromosomes of four *Rhynchosciara* species, one band overlapping the histone gene locus of three species in the *americana*-like group. The results provide evidence for a reverse transcriptase-related protein as a constitutive component in telomeres of *R. americana* and also in certain interstitial loci of *Rhynchosciara* species in which RNA was immunologically detected in the form of RNA:DNA hybrids.

KDR mutation, a genetic marker to assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera : Culicidae) in the tropical savannah area of West Africa

Abdoulaye, D; Thierry, B; Chandre, C; Roch, DK; Pierre, K; Robert, GT; Frederic, S; Pierre, G; Janet, H; Marc, HJ. 2003

A sodium channel 'kdr'-type mutation was identified in the M form of *Anopheles gambiae* from Burkina Faso in the tropical savannah area belt. The molecular M form of *An. gambiae* is found at high frequencies in the flooded rice cultivation area of Kou Valley, where the insecticide selection pressure is limited. The spread of the mutation in the M population is an ongoing process, as it increased from a frequency of 0.006 in 1999 to 0.02 in 2000. The S molecular form occurs in sympatry in our study village, with the M form at a relatively low frequency. The common 'kdr' mutation was previously detected in this area in the S form, and has probably invaded the M population through genetic introgression. This impacts on the question of actual levels of gene flow between the two molecular forms in tropical savannah areas. A hybrid M/S individual was identified during the course of this study, which was homozygous for the 'kdr' mutation. Pyrethroid resistant *An. gambiae* were caught in October and November, which is the time of year that the molecular S form migrates into this area as rain-fed breeding sites in the cotton fields dry out.

Tendency for upwind movement in the sibling fruit fly species, *Bactrocera tryoni* and *B. neohumeralis* and their hybrids (Diptera : Tephritidae): influence of time of day, sex and airborne pheromone

Pike, N; Meats, A. 2003

Natural incidence of fruit flies with character states intermediate to those of the sibling species *Bactrocera tryoni* (Froggatt) and *B. neohumeralis* (Hardy) (Diptera : Tephritidae)

Pike, N. 2004

Naturally occurring flies of intermediate form between *Bactrocera tryoni* and *B. neohumeralis* have been known since the 1950s, and their resemblance to laboratory hybrids has been repeatedly noted. It has been demonstrated that although intermediates may be hybrids, these may equally be the consequence of intraspecific variation within either parent species. This paper aims to provide insight into the true nature of field-collected intermediate flies by assessing their appearance, their mating behaviour and their incidence relative to the abundance of the parent species. Flies were reared from a variety of infested fruit that was collected over an 8 day period from coastal regions of south-eastern and central Queensland. The vast majority (93%) of the >4000 flies collected were *B. tryoni*. *B. neohumeralis* and intermediates, respectively, comprised only 5% and 2% of the population. The callus colour and patterning of intermediates did not change as flies matured. The majority of intermediates tended to have brownish calli and exhibited courtship behaviour during the day. Intercrossing of these intermediates led to a large number of flies that were morphologically identical to *B. neohumeralis*, although intermediate types did persist throughout the two subsequent generations studied. These data provide circumstantial evidence for the occurrence of interspecific gene flow, particularly in the direction of *B. neohumeralis*, but do not discount the possibility of a non-hybrid origin of intermediate flies.

Analysis of reproductive isolation between sibling species *Anopheles albitarsis sensu stricto* and *Anopheles deaneorum*, two malaria vectors belonging to the *Albitarsis* complex (Diptera : Culicidae)

Lima, JBP; Valle, D; Peixoto, AA. 2004

Complexes of sibling species are common among mosquitoes, and their existence within vector species can have important epidemiological consequences. *Anopheles albitarsis sensu stricto* and *Anopheles deaneorum* Rosa-Freitas are two putative vectors of malaria parasites belonging to the *Albitarsis* species complex (Diptera : Culicidae). Using an induced mating technique, we studied the reproductive isolation between these two closely related species and their reciprocal hybrids. Evidence for hybrid male sterility consistent with Haldane's rule was found. The results indicate that male hybrids show very low insemination rates, probably due to abnormalities in their reproductive organs. In addition, the data show that hybrid males carrying an X chromosome derived from *An. deaneorum* perform significantly worse than hybrid males carrying an *An. albitarsis* s.s. X chromosome.

Characterization of telomere DNA among five species of pteridophytes and bryophytes

Suzuki, K. 2004

Higher plant chromosomes possess tandem repeats of TTTAGGG as telomere DNA, while the alga *Chlamydomonas reinhardtii* contains tandem repeats of TTTTAGGG as telomere DNA. This study analysed the telomere composition in five species of mosses and ferns. Genomic Southern hybridization against a tobacco telomere probe indicated intense and highly diffuse signals in all three pteridophytes and the two bryophytes used here. DNAs from a moss *Barbula unguiculata*, a liverwort *Marchantia paleacea* var. *diptera* and a fern *Psilotum nudum* were examined further by kinetic DNA digestion by nuclease Bal31 followed by hybridization to the tobacco telomere probe. All three plants gave signals hypersensitive to the nuclease treatment confirming the telomeric location of the hybridization signals. The average size of the telomere repeat was 0.5 kbp in the liverwort. The liverwort telomeric fragments were cloned in a plasmid vector. Sequencing of the cloned DNA showed that the terminal sequences are composed of tandem repeats of TTTAGGG and the orientation of the repeats is the same as that in higher plants. Based on

these results, it was concluded that both the pteridophytes and the bryophytes have the higher-plant-type telomere sequences.

X-chromosome mapping experiments suggest occurrence of cryptic species in the tsetse fly *Glossina palpalis palpalis*

Gooding, RH; Solano, P; Ravel, S. 2004

Using flies from colonies of *Glossina palpalis palpalis* (Robineau-Desvoidy, 1830) (Diptera: Glossinidae) that originated in Nigeria and Bas-Zaire, the two microsatellite loci Gpg19.62 and Gpg55.3 have been added to the X-chromosome map, thus increasing to seven the number of loci mapped on that chromosome. During the mapping and other crossing experiments, sterile F-1 and backcross males were found. Similarities between the patterns of sterility found in the present study and those occurring during hybridization of some subspecies of tsetse suggest that the nominal taxon *G. p. palpalis* may contain cryptic taxa. Differences in the width of the postgonite head of males from the two colonies were consistent with this suggestion.

Characterization of Wolbachia infections and interspecific crosses of *Aedes (Stegomyia) polynesiensis* and *Ae. (Stegomyia) riversi* (Diptera : Culicidae)

Dean, JL; Dobson, SL. 2004

Prior studies have identified a complicated pattern of interspecific hybridization between members of the *Aedes (Stegomyia) scutellaris* (Walker) mosquito group, which includes medically important vectors of bancroftian filariasis and dengue. Here, we report that two members of the group, *Aedes polynesiensis* Marks and *Aedes riversi* Bohart & Ingram, are both infected with intracellular *Wolbachia* bacteria. Sequencing of the *Wolbachia* *wsp* gene demonstrates that the infections differ from each other and from *Wolbachia* infections previously reported in mosquitoes. *Aedes polynesiensis* is the first mosquito identified with a wMel *Wolbachia* type. Intraspecific crosses of infected and aposymbiotic lines generated via antibiotic treatment show that the *Wolbachia* infections in both species cause high levels of cytoplasmic incompatibility. Interspecific crosses show that the two species are reproductively isolated. However, repeating the interspecific crosses with aposymbiotic mosquito strains demonstrates that the *Wolbachia* infections play a role in preventing hybrid offspring. We discuss *Wolbachia* infections in relation to better defining the evolutionary relationships and causes of speciation within the group, understanding the basis for the observed east-to-west gradient in filarial refractoriness, and developing novel genetic control measures.

Molecular identification of two species of myiasis-causing *Cuterebra* by multiplex PCR and RFLP

Noel, S; Tessier, N; Angers, B; Wood, DM; Lapointe, FJ. 2004

The myiasis-causing flies *Cuterebra grisea* (Coquillett) and *Cuterebra fontinella* (Clark) (Diptera: Oestridae) are normally parasites of mice, predominantly of the genus *Peromyscus*. The morphological similarities of these species and the existence of intermediate morphotypes bearing characters of both species make the identification of adults problematic; furthermore the identification of larvae is apparently not possible. This study presents two molecular approaches to discriminate between these species using specific band patterns: (i) species-specific primers designed in the cytochrome oxidase II (COII) region used in multiplex polymerase chain reaction (PCR) and (ii) restriction fragment length polymorphism (RFLP) on amplified segments of cytochrome oxidase I (COI) gene. Both methods were tested on *Cuterebra* larvae and on adult museum specimens. The two techniques showed a clear difference between *C. grisea* and *C. fontinella*, although species-specific primers were more successful than RFLP for degraded DNA. No intraspecific variation in RFLP and species-specific amplifications were detected for the two species of *Cuterebra*. The results exhibit discrepancies between molecular and morphological identification, suggesting that some of the adults were misidentified.

Quantitative reconstruction of past salinity variations in African lakes: assessment of chironomid-based inference models (Insecta : Diptera) in space and time

Verschuren, D; Cumming, BF; Laird, KR. 2004

Faunal records of 20 common midge species (Diptera: Chironomidae) in 32 African surface waters with salinities ranging from 20 to 41 000 $\mu\text{S.cm}^{-1}$ were used to develop inference models for quantitative reconstruction of past salinity variations from larval chironomid fossils preserved in lake sediments. Weighted-averaging regression and calibration models using presence-absence data (P/A) and presence-absence data with tolerance down-weighting (P/A(tol)) produced bootstrapped coefficients of determination (r^2) of 0.78 and 0.81, respectively, and root mean squared errors (RMSE) of prediction of 0.42 and 0.39 log conductivity units. Historical conductivity data from African lakes are scarce. Therefore, model performance was tested in time by comparing chironomid-inferred conductivity estimates with the corresponding diatom-inferred estimates in sediment records of two fluctuating lakes in the Rift Valley of Kenya. A hybrid procedure in which presence-absence calibration models were applied to abundance-weighted fossil data yielded significantly higher correlation between chironomid- and diatom-inferred time series (Lake Oloiden AD 1880-1991, $r^2 = 0.76$ -0.78; Crescent Island Crater AD 900-1993, $r^2 = 0.56$ -0.61) than by applying the same models to presence-absence fossil data ($r^2 = 0.47$ -0.56 and 0.26-0.42, respectively). Overall, model performance confirms that Chironomidae are valuable bioindicators for natural and man-made changes in the water balance of African lakes.

Sandflies of the *Phlebotomus perniciosus* complex: mitochondrial introgression and a new sibling species of *P. longicuspis* in the Moroccan Rif

Pesson, B; Ready, JS; Benabdennbi, I; Martin-Sanchez, J; Esseghir, S; Cadi-Soussi, M; Morillas-Marquez, F; Ready, PD. 2004

The bloodsucking adult females of *Phlebotomus perniciosus* Newstead and *P. longicuspis* Nitzulescu (Diptera: Psychodidae) are important vectors of the protozoan *Leishmania infantum* Nicolle (Kinetoplastida: Trypanosomatidae) in western Mediterranean countries. The species status of the two phlebotomine sandflies was assessed, along with the epidemiological implications. Individual sandflies from three Moroccan Rif populations were characterized morphologically, isoenzymatically (by the isoelectrofocusing of alleles at the polymorphic enzyme loci of HK, GPI and PGM), and by comparative DNA sequence analysis of a fragment of mitochondrial Cytochrome b (mtDNA). By reference to the character profiles of specimens from other locations, including southern Spain and the type-locality countries, the Moroccan flies were placed in three lineages: first, the lineage of *P. perniciosus*, which contained two mtDNA sublineages, one (pnt) widely distributed and associated with the morphology of the male types from Malta, and the other (pna) associated with a *P. longicuspis*-like male morphology; second, the lineage of *P. longicuspis sensu stricto*, including typical forms from Tunisia; and third, a new sibling species of *P. longicuspis*. The mtDNA sublineage (pnt) of typical *P. perniciosus* was also found in some *P. longicuspis* from Morocco, indicating interspecific hybridization. The typical race of *P. perniciosus* occurs in Italy as well as in Malta, Tunisia and Morocco. It is replaced in southern Spain by the Iberian race (with the pni mtDNA sublineage). The discovery of interspecific gene introgression and a new sibling species mean that previous records of the two morphospecies do not necessarily reflect their true vectorial roles or geographical and ecological distributions.

Intraspecies differentiation in the chironomid *Glyptotendipes gripekoveni* (Diptera, Chironomidae)

Belyanina, SI; Durnova, NA. 2004

Glyptotendipes gripekoveni karyotypic forms are analyzed. The revealed morphological and ecological distinctions made it possible to level the karyoforms up to the rank of subspecies (*G. gripekoveni gripekoveni* and *G. gripekoveni paragripekoveni*). The numerous hybrids between the subspecies attest to a selective advantage of hybrid forms over parental ones.

The "wingbeat hypothesis" of reproductive isolation between members of the *Anopheles gambiae* complex (Diptera : Culicidae) does not fly

Tripet, F; Dolo, G; Traore, S; Lanzaro, GC. 2004

Recent advances have demonstrated that, in the absence of postmating barriers to hybridization, reproductive isolation between different forms of *Anopheles gambiae* sensu stricto is maintained by strong assortative mating. The forms of *An. gambiae* s.s. and the sister species *An. arabiensis* commonly form mixed swarms in which they mate. This raises the question as to how individuals recognize mates of their own species or form within swarms. It has been proposed that wingbeat frequency is used as a cue to discriminate potential mates. This has important implications for prospective genetic control programs. We used a photosensor to record the transient waveforms generated by individuals *An. arabiensis* and from the M and S molecular forms of *An. gambiae* s.s. as they flew through a beam of light. We found no significant between-species or between-form differences in the fundamental harmonic-equivalent to wingbeat frequency-either in males or females collected from sympatric populations in Mali, West Africa. However, there were significant differences in the amplitude of the first and third harmonics in females and of the first and second harmonics in males. Whereas these results suggest some morphological or behavioral differences between species and forms, the extensive overlap in the distributions of harmonic amplitudes does not point to them as reliable cues for assortative mating. Combining all waveforms parameters into a discriminant analysis did not yield characteristic scores either for males or females. Thus, our results do not support the wingbeat hypothesis of premating isolation in the *An. gambiae* complex.

Rapid assays for identification of members of the *Culex* (*Culex*) pipiens complex, their hybrids, and other sibling species (Diptera : Culicidae)

Smith, JL; Fonseca, DM. 2004

Mosquitoes in the *Cidex* (*Cidex*) pipiens complex of species, known as vectors of periodic filariasis and deadly encephalitides, have recently emerged as important vectors of West Nile virus in the United States. Highly conserved morphology but marked differences in potential vectorial capacity require the development of polymerase chain reaction (PCR)-based tests that unambiguously distinguish among the different species. We introduce and describe a series of PCR-based assays that use polymorphisms in the second intron of the acetylcholinesterase-2 (*ace-2*) locus for the identification of members of the *Cx. pipiens* complex (*Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. p. pallens*, *Cx. australicus*). two other species that are commonly mislabeled as *Cx. pipiens* (*Cx. torrentium* and *Cx. pervigilans*), as well as hybrids between *Cx. pipiens* and *Cx. quinquefasciatus*.

Species abundance and insecticide resistance of *Anopheles gambiae* in selected areas of Ghana and Burkina Faso

Yawson, AE; McCall, PJ; Wilson, MD; Donnelly, MJ. 2004

The Ghanaian National Malaria Control Programme has prioritized insecticide-treated materials as a key strategy for malaria control. We report on a survey of the distribution of the molecular forms of *Anopheles gambiae* Giles (Diptera: Culicidae) and insecticide resistance (the *kdr* mutation), carried out by sampling mosquitoes from 11 locations in Ghana and one additional site in Burkina Faso. The molecular M and S forms of *An. gambiae* were found to occur in sympatry in southern Ghana. The S form predominated throughout its distribution in the coastal savannah, except at one location in the strand and mangrove zone where rice was cultivated. The M form was the only form collected in northern Ghana and was the predominant form (97.5%) in Burkina Faso. No M/S hybrids were detected. The *kdr* mutation was observed at very high frequencies (98-100%) within the S form but reached a maximum of only 3.38% in the M form in one population at an irrigation scheme in the Ghanaian coastal savannah zone.

Clinal genetic variation and the 'rare allele phenomenon' in random mating populations of *Urophora cardui* (Diptera : Tephritidae)

Steinmetz, R; Johannesen, J; Seitz, A. 2004

In the present study we investigate a contact zone between two population groups of the tephritid fly *Urophora cardui*. We investigate scenarios that may have produced the genetic differentiation of the two groups, and we describe the 'rare allele phenomenon' from the contact zone. The rare allele phenomenon refers to alleles that are found at high frequency in contact zones but are rare or lacking outside the contact zone. The phenomenon is often observed in hybrid zones between subspecies of limited reproductive compatibility, but seldom in populations with random mating. Clinal genetic variation was observed at three loci in the contact zone. Three alleles at the locus Aat showed steep clines, between 20-70 km wide. A rare Aat-A allele occurred at high frequency in the centre of the contact zone. Two further loci, Hk and Pgd, showed less steep clinal genetic variation, the transition being in and slightly south of the centre of the Aat cline. Populations showed Hardy-Weinberg proportions and there was no evidence for linkage disequilibrium. These findings suggest random mating and gradual introgression between the population systems, which may originate from at least two range expansions. Aat's steep clines and rare allele may indicate selection on Aat alleles, although we presently can not quantify any agents. Because *U. cardui* experiences random mating in the contact zone with no apparent 'hybrid' incompatibility, mating experiments offer the possibility for future enquiries about the genetic basis of the rare allele phenomenon.

Fruit fly (Diptera : Tephritoidea) infestation in citrus in the state of Sao Paulo, Brazil

Raga, A; Prestes, DAO; Souza, MF; Sato, ME; Siloto, RC; Guimaraes, JA; Zucchi, RA. 2004

From February 1998 to May 2000, fruits of different citrus varieties and hybrids were collected in order to assess the fruit fly infestation levels and tephritoid/parasitoid complex in the state of Sao Paulo. A total of 12,239 fruits (1,416.93 kg) was collected in 25 municipalities. From all the samples 5,252 puparia and 3,039 adults of Tephritoidea were recovered. About 78.1% of all adults collected were Tephritidae [77.1% de *Anastrepha* sp. and 1.0% de *Ceratitis capitata* (Wied.)] and 21.9% were Lonchaeidae (*Neosilba* spp.). All females of *Anastrepha* were identified as *A. fraterculus* (Wied.). Adults of Braconidae, Diapriidae and Eucoliinae emerged from the citrus samples. Mean infestation indices were 0.4 puparium/fruit and 3.70 puparia/kg of fruits. Sweet oranges were the most susceptible to Tephritoidea infestations when the number of puparia/fruit was considered. In isolated samples, 'Cravo mandarin' (*Citrus reticulata*) and sour orange (*Citrus aurantium*) showed the highest infestation indices (3.4 and 2.4 puparia/fruit, respectively). Some samples of 'Kunquat' (*Fortunella* sp.) and 'Cravo' mandarin reached high levels of infestation (64.0 and 37.9 puparia/kg of fruits, respectively). The sweet oranges were the most susceptible to Tephritoidea infestations in the state of Sao Paulo. The parasitoid Opiinae *D. areolatus* was the most abundant braconid species.

Interspecific hybridization between *Liriomyza sativae* Blanchard and *L. trifolii* (Burgess) (Diptera : Agromyzidae)

Tokumaru, S; Abe, Y. 2005

We confirmed the occurrence of interspecific hybridization between *Liriomyza sativae* and *L. trifolii* on kidney bean (*Phaseolus vidgaris* L.) at 25 degrees C under a 15L-9D photoperiod regime. Among 48 replications of *L. sativae* females and *L. trifolii* males tested, females of 19 replications (39.6%) oviposited, but no eggs hatched. In contrast, among 115 replications of *L. trifolii* females and *L. sativae* males tested, females of 47 replications (40.9%) oviposited and progeny from 10 replications (8.7%) developed to adult eclosion. The hybrid offspring were obtained only when several *L. trifolii* females and several *L. sativae* males were placed together. The hatchability, Pupation rate, and adult emergence rate of hybrid flies were 14.9%, 94.9%, and 61.8%, respectively. All the hybrid adults were female (n = 127). When these females were backcrossed with males of either species, none oviposited. The mean adult longevity of a hybrid female was 19.2 d.

Visualization of chromosome territories in interphase nuclei of ovarian nurse cells in *Calliphora erythrocephala* Mg. (Diptera : Calliphoridae)

Anan'ina, TV; Vedernikov, AE; Wasserlauf, IE; Karamysheva, TV; Rubtsov, NB; Stegnii, VN. 2005

Analysis of localization of chromosomes 2, 3, and 6 of *Calliphora erythrocephala* Mg. in ovarian nurse cell nuclei with different chromatin structure has shown that the regions of DNA probe hybridization reduced with increasing chromatin compaction. Hybridization of DNA probes of chromosomes 3 and 6 to secondary reticular nuclei demonstrated that chromosomes retain their territories in the nuclei when the chromatin acquires a reticular structure. These results suggest regular organization of the chromosomal apparatus at all stages of the endomitotic cycle, including the stage of highly polyploid reticular nuclei. FISH of DNA probe of the chromosome 2 telomeric region to secondary reticular nuclei revealed a peripheral distribution of the signal. Zones of more intensive DNA probe hybridization have been distinguished. These zones probably are the regions of accumulation of telomeric and (or) centromeric chromosome regions.

A chromosome end satellite of *Rhynchosciara americana* (Diptera : Sciaridae) resembling nematoceran telomeric repeats

Madalena, CRG; Gorab, E. 2005

The characterization of chromosome end sequences of *Rhynchosciara americana* was initiated with the screening of a plasmid microlibrary made from a microdissected polytene chromosome tip. A 268 bp insert chosen for analysis hybridized specifically to non-telocentric chromosome ends in which reverse transcriptase had been previously identified. Southern-blot hybridization of *R. americana* genomic DNA cut with XbaI, the only restriction site identified in the 268 bp probe, showed a ladder composed of multimers of a band in the range of 400 bp indicating a tandem array of the repeat partially represented in the cloned fragment. The complete repeat unit obtained by inverse PCR is 414 bp long, 67% AT-rich, characterized by the restriction sites XbaI and Sall and displays features typical of a nematoceran telomeric satellite. The telomere-like repeat is apparently absent from the chromosomes of two other *Rhynchosciara* species, *R. baschanti* and *R. milleri*. Double staining for satellite hybridization and reverse transcriptase in *R. americana* suggests that the arrays composed of the telomeric-like satellite do not reach the chromosome ends. This may indicate that telomeric-like features of some nematoceran terminal satellites do not warrant their telomeric position.

Reproductive isolation among geographical populations of *Drosophila bipectinata* Duda (Diptera, Drosophilidae) with recognition of three subspecies

Matsuda, M; Tomimura, Y; Tobari, YN. 2005

Among *D. bipectinata* Duda, 1923, three subspecies, *bipectinata* from Southeast Asia (SEA) and Okinawa (OKN), *szentivanyi* stat. nov. from Papua New Guinea (PNG) (Mather & Dobzhansky, 1962) and *pacificiae* ssp. nov. from South Pacific Ocean (SPO), are recognized. The external morphology of the reproductive organs and the numbers of teeth per row in the sex combs are different between the three subspecies. Furthermore, the sterility of hybrid males between strains from the different regions confirms the subspecies status of each population from SEA, PNG and SPO, together with different gene arrangements in the geographical populations. Although males of the strains from OKN (Okinawa), the northernmost population, show significant differences in the number of teeth of sex combs from males of SEA (Southeast Asia) strains, hybrid males between them are fertile.

Mushroom host influence on *Lycoriella mali* (Diptera : Sciaridae) life cycle

O'Connor, L; Keil, CB. 2005

Lycoriella mali Fitch (Diptera: Sciaridae) infests mushroom crops early in the crop cycle. Recent observations in mushroom houses indicated a difference in emergence time and size of adult *L. mali* developing on various strains of commercial mushrooms. Samples of adult flies from isolated mushroom houses growing Portabella mushrooms were significantly heavier than those from oyster mushroom houses, whereas flies from shiitake mushroom houses were lightest in weight. Flies collected from isolated Portabella mushroom houses were reared on four strains and species of *Agaricus*. and *Pleurotus* mushrooms. After the adults emerged, females were weighed, mated, and allowed to oviposit. The number of eggs laid increased as the weight of the female increased. Flies collected from isolated Portabella mushroom houses were reared on eight strains and species of mushrooms. Flies were reared for four generations on each host mushroom mycelium then switched to different host Mushrooms. Overall, the hybrid strain of *Agaricus bisporus* (Lange) Imbach (Agaricales: Agaricomycetideae) was the most favorable host for *L. mali*, whereas the wild strain of *A. bisporus* was the least favorable host. Mushroom hosts influence developmental time, survivorship, weight, and reproduction of *L. mali*.

The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host shifts

Dambroski, HR; Linn, C; Berlocher, SH; Forbes, AA; Roelofs, W; Feder, JL. 2005

Rhagoletis pomonella (Diptera: Tephritidae) use volatile compounds emitted from the surface of ripening fruit as important chemosensory cues for recognizing and distinguishing among alternative host plants. Host choice is of evolutionary significance in *Rhagoletis* because these flies mate on or near the fruit of their respective host plants. Differences in host choice based on fruit odor discrimination therefore result in differential mate choice and prezygotic reproductive isolation, facilitating sympatric speciation in the absence of geographic isolation. We test for a genetic basis for host fruit odor discrimination through an analysis of F-2 and backcross hybrids constructed between apple-, hawthorn-, and flowering dogwood-infesting *Rhagoletis* flies. We recovered a significant proportion (30-65%) of parental apple, hawthorn, and dogwood fly response phenotypes in F2 hybrids, despite the general failure of F, hybrids to reach odor source spheres. Segregation patterns in F2 and backcross hybrids suggest that only a modest number of allelic differences at a few loci may underlie host fruit odor discrimination. In addition, a strong bias was observed for F-2 and backcross flies to orient to the natal fruit blend of their maternal grandmother, implying the existence of cytonuclear gene interactions. We explore the implications of our findings for the evolutionary dynamics of sympatric host race formation and speciation.

Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies

Simmons, AT; Gurr, GM. 2005

1 The cultivated tomato, *Lycopersicon esculentum*, is an economically important worldwide crop. Current pest management techniques rely heavily on pesticides but

trichome-based host-plant resistance may reduce pesticide use. 2 A review of the literature is provided on trichomes of wild *Lycopersicon* species and the effects of trichome-based host-plant resistance on arthropods. Solvents have been used to remove glandular trichome exudates and the resulting diminution of their effects quantified. Correlational approaches to assess the relationship between the different trichome types and effects on pests have also been used. 3 Most studies have focused on Lepidoptera and Hemiptera, although some work has included Coleoptera, Diptera and Acarina, and both antibiotic and antixenotic effects have been demonstrated. 4 Natural enemies are a cornerstone of international pest management and this review discusses how the compatibility of this approach with trichome-based host-plant resistance is uncertain because of the reported negative effects of trichomes on one dipteran, one hemipteran and several Hymenoptera. 5 For trichome-based host-plant resistance to be utilized as a pest management tool, trichomes of wild species need to be introgressed into the cultivated tomato. Hybrids between the cultivated tomato and the wild species *Lycopersicon hirsutum* f. *glabratum*, *Lycopersicon pennellii* and *Lycopersicon cheesmanii* f. *minor* have been produced and useful levels of resistance to Acarina, Diptera and Hemiptera pests have been exhibited, although these effects may be tempered by effects on natural enemies. 6 This review proposes that studies on genetic links between fruit quality and resistance, field studies to determine the compatibility of natural enemies and trichome-based host-plant resistance, and a strong focus on *L. cheesmanii* f. *minor*, are all priorities for further research that will help realize the potential of this natural defence mechanism in pest management.

Ribosomal intergenic spacer (IGS) length variation across the Drosophilinae (Diptera : Drosophilidae)

Mateos, M; Markow, TA. 2005

Background: The intergenic spacer of the ribosomal genes in eukaryotes (IGS) contains duplications of the core transcription promoter. The number of these duplicated promoters, as measured by the IGS length, appears to be correlated with growth rate and development time in several distantly related taxa. In the present study, we examined IGS length variation across a number of species of *Drosophila* to determine the amount of variation in this trait across different evolutionary time scales. Furthermore, we compared the usefulness of two methods commonly used to determine IGS length: Southern Blot Hybridization (SB) and Polymerase Chain Reaction (PCR). Results: Our results show broad variation in IGS length across the genus *Drosophila*, but closely related species had similar IGS lengths. Our results also suggest that PCR tends to underestimate the true IGS size when the size is greater than 5 kb, and that this degree of underestimation is greater as the IGS size increases. Conclusion: Broad variation in IGS length occurs across large evolutionary divergences in the subfamily Drosophilinae. Although average IGS length has been shown to evolve rapidly under artificial selection, closely related taxa generally have similar average IGS lengths. Our comparison of methods suggests that without previous knowledge of the DNA sequence of the IGS and flanking regions, both methods be used to accurately measure IGS length.

Integration of *Anopheles beklemishevi* (Diptera : Culicidae) in a PCR assay diagnostic for palaearctic *Anopheles maculipennis* sibling species

Kampen, H. 2005

A few years ago a PCR-based assay for a quick and reliable identification of six palaearctic sibling species of the *Anopheles maculipennis* complex was presented making use of differences in the nucleotide sequence of the ITS2 ribosomal mosquito DNA. *An. beklemishevi*, which is distributed in Scandinavia and Russia only, has now been integrated into this test after analysis of its ITS2 region which turned out to be much longer than those of the other sibling species. Three oligonucleotides putatively specific for *An. beklemishevi* were constructed and tested in combination with a universal genus-specific primer for the amplification of an *An. beklemishevi*-specific ITS2 DNA-fragment. Two of the three oligos generated accurate and specific PCR products, even when used in a multiplex PCR together with the specific primers for the other six sibling species. Cross-hybridization of the primers to heterologous culicid DNA was never observed. The amplicons that identify *An. beklemishevi* consist of 554 and 735 bp, respectively, and are easily distinguished from those specific for the other sibling species after gel electrophoresis.

Pollination of *Alocasia cucullata* (Araceae) by two *Colocasiomyia* flies known to be specific pollinators for *Alocasia odora*

Miyake, T; Yafuso, M. 2005

Two aroid congeners, *Alocasia odora* and *Alocasia cucullata*, grow on Okinawa Island, Japan. Two floricolous species of *Colocasiomyia* *alocasiae* and *Colocasiomyia xenalocasiae* (Diptera: Drosophilidae), previously known to be specific pollinators for *A. odora*, were found pollinating *A. cucullata*. We collected the floral volatiles of *A. odora* and *A. cucullata* and compared them using gas chromatography and mass spectrometry because floral volatiles act as attractive signals for these pollinators. The volatile compositions detected were similar and dominated by methyl salicylate, 4,8-dimethyl-1,3,7-nonatriene, beta-caryophyllene, bicyclogermacrene, methyl benzoate and alpha-humulene, which means it is likely that the flies cannot discriminate between the two plant species when they search for hosts. Interspecific hybridization did not occur when *A. odora* was hand pollinated with *A. cucullata* pollen, suggesting that the movement of flies from one host species to another will result in wastage of pollen. Adults of both *Colocasiomyia* flies emerged from *A. cucullata* inflorescences collected in the field, suggesting that their larvae can also develop on *A. cucullata*. We did not find any difference in larval performance of *C. alocasiae* on *A. cucullata* or *A. odora*. *C. alocasiae* does not appear to suffer any ill effects when using *A. cucullata* as a host plant. The partnership between *A. cucullata* and the two *Colocasiomyia* flies may be an example of exaptation, although other possibilities cannot be excluded.

Identification, mapping, and economic evaluation of QTLs encoding root maggot resistance in Brassica

Ekuere, UU; Dosdall, LM; Hills, M; Keddie, AB; Kott, L; Good, A. 2005

Commercial varieties of canola (*Brassica napus* L. and *B. rapa* L.) are susceptible to infestations by the root maggots *Delia radicum* (L.) and *Delia floralis* (Fallen) (Diptera: Anthomyiidae) in western Canada. Although cultural strategies can ameliorate crop damage from root maggot infestations, these methods are not sufficiently effective to prevent substantial economic losses. In this paper we report the development of germplasm for resistance to root maggot infestations and the introgression of genes from a resistant relative (*Sinapis alba* L.) to susceptible *B. napus*. The effectiveness of the conferred resistance to root maggot damage was validated by comparing different genotypes for pest damage and yield loss with and without insecticide applications. Yield of *B. napus* plants, susceptible to root maggot infestations, increased when insecticide was applied (by up to 24%), but no significant yield differences were observed among resistant intergeneric hybrids that were treated or not treated with insecticide. One hundred and thirty-five restriction fragment length polymorphisms (RFLPs) were used to construct a *B. napus* genetic linkage map and to identify quantitative trait loci (QTLs) associated with resistance to root maggot damage. Two QTLs, RM-G8 and RM-G4, were found to be associated with resistance to root maggot damage. Together, these two QTLs explain 54.6% of the total variation observed.

Species and populations of the *Anopheles gambiae* complex in Cameroon with special emphasis on chromosomal and molecular forms of *Anopheles gambiae* s.s.

Wondji, C; Simard, F; Petrarca, V; Etang, J; Santolamazza, F; Della Torre, A; Fontenille, D. 2005

We studied the geographical distribution of species, chromosomal, and molecular forms of the *Anopheles gambiae* Giles (Diptera: Culicidae) complex in 23 sites in Cameroon, Central Africa. Almost all the specimens collected in the four northern-most and sites were *Anopheles arabiensis*. *Anopheles melas* was found in a rural locality surrounded by mangrove swamps, on the Atlantic Coast. In total, 1,525 *An. gambiae* s.s. females were identified down to their molecular form, and inversion polymorphisms on polytene chromosomes were scored from 186 half-gravid females. The Forest chromosomal form, with standard arrangements almost fixed on both arms of chromosome-2, was the only one observed in the southern, more humid localities. Karyotypes typical of Savanna and Mopti were recorded northwards,

in the humid savannas of the Adamawa Province. The molecular forms M and S were widespread throughout Cameroon, and assort independently from the chromosomal forms. S-form populations were characterized by karyotypes typical of Forest and Savanna chromosomal forms, and M-form populations were characterized by karyotypes typical of Forest, Savanna, and Mopti. No M/S hybrid patterns were detected, although M and S mosquitoes were sympatric in 15 sites, providing further evidence for positive assortative mating within molecular forms. The observed ecogeographical distribution of M and S was peculiar: the ecological parameters involved in this distribution still need to be clarified as well as the possible role of competitive exclusion between chromosomally homosequential molecular forms. No difference was observed in host preference or in *Plasmodium falciparum* infection rates between sympatric M and S populations.

Unique phenotypes and variation in the sex comb patterns and their evolutionary implications in the *Drosophila bipectinata* species complex (Diptera : Drosophilidae)

Mishra, PK; Singh, BN. 2006

Understanding the genetic mechanisms of morphological evolution is one of the greatest challenges in evolutionary biology and for such studies sexually dimorphic traits in closely related species are of prime interest. In the *Drosophila bipectinata* species complex, which consists of four closely related species, namely *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoananassae*, the pattern of sex combs (a sexually dimorphic trait) is found to be highly diversified. The present investigation documents some unique and new sex comb phenotypes and demarcates intra- and interspecific variations in the sex comb pattern among the four species and their hybrids. There is remarkable similarity in sex comb pattern of *D. bipectinata* and *D. parabipectinata* but it differs from that of *D. malerkotliana* and *D. pseudoananassae*, which is in consistent with the phylogenetic relationships among the four species traced out by cytological, biochemical and molecular studies. The genetic basis of inheritance of sex comb patterns, its plausible implication with biogeographical distribution of species and the relationship between degree of hybridization and phylogenetic proximity have been addressed.

DNA microsatellite analysis of naturally occurring colour intermediates between *Bactrocera tryoni* (Froggatt) and *Bactrocera neohumeralis* (Hardy) (Diptera : Tephritidae)

Gilchrist, AS; Ling, AE. 2006

The sympatric tephritid fruit flies *Bactrocera tryoni* (Froggatt) (Queensland fruit fly) and *B. neohumeralis* (Hardy) differ in time of mating and for the colour of the humeral callus ('shoulder pad'), which is typically entirely yellow in *B. tryoni* and typically entirely brown in *B. neohumeralis*. Field collections in sympatric regions usually include at least 1% of individuals whose humeral calli show mixed patches of yellow and brown ('intermediates'). Over 40 years, a number of studies have debated the possibility that these intermediates are interspecific hybrids. In the present study, we have used microsatellites to show that few if any of these intermediates are hybrids. Instead, most variation humeral callus appears to be confined to one species, *B. tryoni*. We discuss these results in the context of gene flow between the two species and suggest directions for future research.

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.

Identification of quantitative trait loci for larval morphological traits in interspecific hybrids of *Ochlerotatus triseriatus* and *Ochlerotatus hendersoni* (Diptera : Culicidae)

Anderson, JR; Schneider, JR; Grimstad, PR; Severson, DW. 2006

Ochlerotatus triseriatus is the natural vector of La Crosse virus, a common cause of pediatric encephalitis in the United States; the closely related *Ochlerotatus hendersoni* transmits this virus at low frequency. Adults of these mosquito species are difficult to distinguish morphologically; however, the larval stages show species-specific differences in several characters. We identified genomic regions contributing to the differences between the larvae of these species through interspecific hybridizations. Quantitative trait loci (QTL) were identified by standard interval mapping techniques and by univariate marker association analyses. We examined 159 F-2 progeny from an *Oc. hendersoni* female by *Oc. triseriatus* male interspecific cross for variation in the number of saddle and siphon hair branches, attachment of the acus, and morphology of the anal papillae. At least one putative QTL was identified for each of the phenotypes examined. QTL most commonly mapped to linkage group (LG) III, although QTL were identified on LGI and LGII for three phenotypes each. Several of these QTL, and particularly those on LGIII, also map to genome regions controlling adult female body size and ability to orally transmit La Crosse virus. Further studies are required to elucidate the relationships among these traits and the impact they may have had on the ecological specialization and speciation of these mosquitoes.

Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera : Culicidae)

Savage, HM; Anderson, M; Gordon, E; McMillen, L; Colton, L; Charnetzky, D; Delorey, M; Aspen, S; Burkhalter, K; Biggerstaff, BJ; Godsey, M. 2006

Oviposition activity and West Nile virus (family Flaviviridae, genus *Flavivirus*, WNV) infection rates were assessed for members of the *Culex pipiens* complex from July through December 2002 by using gravid traps placed at four ecologically different sites in the southern portion of the hybrid zone in Shelby County, TN. Molecular assays identified three members of the *Cx. pipiens* complex: *Cx. pipiens pipiens* L., *Cx. p. quinquefasciatus* Say, and *Cx. p. pipiens*-*Cx. p. quinquefasciatus* hybrids (hybrids). The *Cx. pipiens* complex accounted for 90% of mosquitoes collected in gravid traps. All 285 WNV-positive mosquitoes were *Culex* mosquitoes, and 277 (97%) were *Cx. pipiens* complex mosquitoes. Infection rates among members of the *Cx. pipiens* complex were not significantly different. Infection rates were significantly higher at two urban sites than at a rural site, and WNV was not detected at a forested site. At urban sites, abundances of members of the *Cx. pipiens* complex corresponded to a simple latitude model of the hybrid zone. *Cx. p. quinquefasciatus* was most abundant (46.4%), followed by hybrids (34.1%) and *Cx. p. pipiens* (19.5%). The relative abundances at a rural site were reversed with *Cx. p. pipiens* (48.4%) being most abundant. This demonstrates that spatial habitat variation may profoundly influence the distribution of members of the *Cx. pipiens* complex within the hybrid zone. Members of the *Cx. pipiens* complex did not display different oviposition patterns. However, oviposition patterns assessed hourly at urban and rural sites were significantly different. At urban sites, oviposition activity of *Cx. pipiens* complex mosquitoes was bimodal with an evening peak associated with sunset and a morning peak associated with sunrise. At the rural site, the evening peak was pronounced and the morning peak weak and similar to nighttime activity.

Isolation and characterization of microsatellite loci in the sand fly *Phlebotomus papatasi* (Diptera : Psychodidae)

Hamarsheh, O; Presber, W; Abdeen, Z; Sawalha, S; Al-Lahem, A; Schoenian, G. 2006

Phlebotomus papatasi is a proven vector of *Leishmania major* which is one of the causative agents of cutaneous leishmaniasis in the Old World. Although it has a wide geographical range, its population structure is not yet well understood. In an effort to better understand the population dynamics of this vector, we developed a panel of di- and trinucleotide microsatellite markers, using a magnetic bead hybridization enrichment protocol. These microsatellite loci showed three to seven alleles with an expected heterozygosity range between 0.702 and 0.876. The level of polymorphisms found in this study suggests that these microsatellite loci can be used for population analysis of *P. papatasi*.

Influence of cytoplasmic male sterility on expression of different mechanisms of resistance in sorghum to *Atherigona soccata* (Diptera : Muscidae)

Dhillon, MK; Sharma, HC; Naresh, JS; Singh, R; Pampapathy, G. 2006

Atherigona soccata (Rondani) (Diptera: Muscidae) is one of the most important pests of sorghum, *Sorghum bicolor* (L.) Moench, in Asia, Africa, and the Mediterranean Europe. Exploitation of cytoplasmic male sterility (CMS) for hybrid production has resulted in considerable narrowing of the genetic base and may increase the vulnerability of this crop to insect pests. Therefore, we studied the expression of different mechanisms of resistance in sorghum to *A. soccata* in CMS (A) and maintainer (B) lines of 12 genotypes under field and greenhouse conditions. The CMS lines of *A. soccata*-resistant genotypes were preferred for oviposition (78.5 versus 71.5% plants with eggs) and suffered greater deadheart incidence (47.6 versus 41.6%) than the corresponding maintainer lines, whereas such differences were not apparent in CMS lines belonging to the susceptible genotypes (92.7 versus 92.3% plants with eggs and 75.6 versus 74.6% deadhearts) under multichoice field conditions. Similar differences also were observed under controlled conditions in the greenhouse. The larval period (9.0 versus 8.8 d) and pupal mortality (18.4 versus 13.4%) were greater on maintainer lines than that on the CMS lines in the resistant group. The male and female pupal weights, fecundity, and antibiosis index were greater on the CMS than on the maintainer lines. The maintainer lines showed better recovery resistance than the CMS lines, but no such differences were observed in tiller deadhearts. The differences in susceptibility to *A. soccata* were greater in the *A. soccata* resistant CMS and maintainer lines than in the CMS and maintainer lines belonging to susceptible genotypes. Conversion of *A. soccata*-resistant genotypes into alternate less susceptible cytoplasmic backgrounds may be undertaken for developing sorghum hybrids with stable resistance to *A. soccata*.

Quantitative trait loci for resistance to herbivores in willow: field experiments with varying soils and climates

Ronnberg-Wastljung, AC; Ahman, I; Glynn, C; Widenfalk, O. 2006

As a basis for genetic improvement of willow (*Salix* spp.) for use in wood biomass production, quantitative trait loci (QTLs) responsible for resistance to herbivores have been identified in a tetraploid hybrid F-2 population originating from a cross between *Salix dasyclados* (Wimm.) and *Salix viminalis* (L.) (Salicaceae). Symptoms of herbivory, caused by various insects and game, and, in addition, leaf rust, were assessed in three field locations with varying soils and climates. Eleven damage traits (lost leaf area, leaf discoloration, leaf blisters, leaf-mite symptoms, leaf-margin cuts, and various estimates of shoot-tip damage by a gall midge, game, and lepidopterans) were submitted to QTL analysis. A composite interval mapping approach was used to estimate the number of QTLs, the magnitude of the QTLs, and their position on genetic linkage maps. Most of the identified QTLs were specific for each trait and location, but a few QTLs common across the locations were also detected. Each QTL explained between 8 and 24% of the phenotypic variation, depending on damage trait and field location. Clusters of QTLs for different traits were found at several linkage groups, indicating either a common genetic base or tightly linked QTL. Our results emphasize the need for verification of QTL studies over different environments.

Rapid assay to identify the two genetic forms of *Culex* (*Culex*) *pipiens* L. (Diptera : Culicidae) and hybrid populations

Bahnck, CM; Fonseca, DM. 2006

A previously developed method to identify members of the *Culex pipiens* complex exploiting polymorphisms in a nuclear intron (acetylcholinesterase [ACE] based-assay) cannot differentiate the two forms of *Cx. pipiens*: form *pipiens* and form *molestus*. Notably, the two forms seem to differ extensively in behavior and physiology and likely have very different epidemiologic importance. Because they are morphologically indistinguishable, molecular methods are critical for the evaluation of their relative importance. Although the two forms of *Cx. pipiens* have been distinguished using a panel of microsatellite loci, such a protocol is laborious and expensive. We developed a rapid assay based on polymorphisms in the flanking region of a microsatellite locus. Used in conjunction with the ACE-assay, this new assay allows the identification of pure and hybrid populations of the two *Cx. pipiens* forms as well as those including *Cx. quinquefasciatus*. We discuss the usefulness of the method as well as limitations to its application.

Inheritance of resistance to sorghum shoot fly, *Atherigona soccata*

Dhillon, MK; Sharma, HC; Reddy, BVS; Singh, R; Naresh, JS. 2006

The sorghum shoot fly, *Atherigona soccata* Rond. (Diptera: Muscidae), is one of the most important pests of sorghum [*Sorghum bicolor* (L.) Moench], and host plant resistance is an important component for the management of this pest. Most of the sorghum hybrids currently under cultivation are based on cytoplasmic male-sterility (CMS). To develop a strategy to develop sorghum hybrids with resistance to shoot fly, we studied the nature of gene action for resistance to this pest in F₂ hybrids derived from shoot fly-resistant and -susceptible CMS and restorer lines. The hybrids based on shoot fly-resistant CMS and restorer lines were glossy and trichomed and had lower proportion of plants with eggs (78.5% vs. 88.4 to 93.3%) and deadhearts (40.8% vs. 60.8 to 75.3%) than the hybrids based on other cross combinations, suggesting that resistance is required in both CMS and restorer lines for obtaining shoot fly-resistant hybrids. Proportional contributions of CMS lines for oviposition, deadhearts, leaf glossiness, and recovery resistance were greater than those of the restorer lines. The general (GCA) and specific combining ability (SCA) estimates suggested that inheritance for oviposition nonpreference, deadhearts, recovery resistance, and the morphological traits associated with resistance or susceptibility to *A. soccata* were governed by additive-type of gene action. The SCA effects and heterosis estimates indicated that heterosis breeding would not be rewarding in breeding for resistance to shoot fly.

A comparison of the intraspecific variability of *Phlebotomus sergenti* Parrot, 1917 (Diptera : Psychodidae)

Dvorak, V; Aytekin, AM; Alten, B; Skarupova, S; Votypka, J; Volf, P. 2006

Phlebotomus sergenti populations from different areas of the Mediterranean basin are known to exhibit high intraspecific variability. Previous studies of ITS2 revealed the presence of two branches that may represent sibling species. To corroborate this finding by other tools, two colonies of *P. sergenti* originating from Turkey and Israel, each belonging to a different ITS2 branch, were compared by three different methods: geometric morphometric analysis of wing shape, RAPD

(random amplified polymorphic DNA), and cross-mating study. For geometric morphometric analysis, two-dimensional Cartesian coordinates of 16 landmarks from the wings were digitized and analyzed. Significant shape differences were found between colonies but not between sexes within each colony. RAPD results formed two distinctive clades corresponding to the origin of the colony but also showed heterogeneity among members of both colonies. In cross-mating studies, viable hybrid F1 and F2 progeny were obtained when both Turkish males/Israeli females and Israeli males/Turkish females were crossed. F1 progeny was included in RAPD analysis and these hybrids formed a distinctive clade with an intermediate position between the two parental clades. No significant differences were found in egg production of crossed sand flies. The cross-mating study showed that there is no reproductive barrier between *P. sergenti* from different geographical areas. On the other hand, RAPD and geometric morphometric analysis revealed a significant difference between colonies and confirmed the suitability of previous ITS2 analysis for discrimination among sand fly populations. Further development of molecular markers should resolve a possible existence of sibling species within *Phlebotomus sergenti*.

Effects of different protein concentrations on longevity and feeding behavior of two adult populations of *Ceratitis capitata* Wiedemann (Diptera : Tephritidae)

Placido-Silva, MD; Neto, AMD; Zucoloto, FS; Joachim-Bravo, IS. 2006

The effects of protein intake on two adult male and female populations of *Ceratitis capitata* Wiedemann were assessed. One population consisted of flies reared for twenty years in the laboratory (Lab-pop); the other population consisted both of flies reared in the laboratory for approximately fifteen years and of the periodically introduced wild flies (Hybrid-pop). Three diets were tested: a no-yeast diet and two diets containing yeast (protein source) at the concentrations 6.5 g or 1.5 g per 100 ml diet. The parameters analyzed were: adult longevity, diet intake with and without yeast, and discrimination threshold for yeast. Protein intake increased Lab-pop adult longevity and did not affect longevity of the Hybrid-pop. Longevity in each population was similar for males and females fed on the same diet. Food behavior were similar for male and female adults of both populations; all preferred diets containing protein (yeast). Males and females in both populations ingested similar amounts of each diet. The discrimination threshold for yeast was similar for all males (0.5 g yeast/100 ml diet); Lab-pop females were able to detect the presence of smaller quantities of yeast in their diet, thus having a higher discrimination capacity (0.4 g/100 ml diet) as compared to the Hybrid-pop females (0.6 g/100 ml diet).

Development and localization of microsatellite markers for the sibling species *Chironomus riparius* and *Chironomus piger* (Diptera : Chironomidae)

Nowak, C; Hankeln, T; Schmidt, ER; Schwenk, K. 2006

Five variable microsatellite loci are reported for the nonbiting midge species *Chironomus riparius* and *Chironomus piger*. All loci show considerable intraspecific variation and species-specific alleles, which allow to discriminate among the two closely related species and their interspecific hybrids, and to estimate genetic diversity within and between populations. Additionally, the loci were localized on *C. riparius* polytene chromosomes to verify their single copy status and investigate possible chromosomal linkage. The described markers are used in different studies with regard to population and ecological genetics and evolutionary ecotoxicology of *Chironomus*.

Mixed swarms of the molecular M and S forms of *Anopheles gambiae* (Diptera : Culicidae) in sympatric area from Burkina Faso

Diabate, A; Dabire, RK; Kengne, P; Brengues, C; Baldet, T; Ouari, A; Simard, F; Lehmann, T. 2006

The M and S molecular forms of *Anopheles gambiae* sensu stricto Giles are thought to be reproductively isolated through premating barriers. However, the exact mechanisms of recognition of conspecific partners are unknown. Because mating in *An. gambiae* occurs in swarms, one might expect swarming behavior between the M and S forms to be different and that this probably reduces the risk of contact between males and females of the different forms in areas where they are sympatric. We report the occurrence of four mixed swarms, containing males of M and S forms, out of a total of 26 swarms sampled in Soumouso, a typical savannah village of Burkina Faso, West Africa. However, the frequency of mixed swarms was lower than that expected by chance. This observation suggests partial segregation between the swarms of the molecular forms, which may contribute to their isolation. Because the frequency of mixed swarms seems too high to explain the low frequency of cross-mating and hybrids, we suggest that mate recognition in a swarm is more important than swarm segregation.

Taxonomic problems in the *Drosophila melanica* species group (Diptera : Drosophilidae) from southern China, with special reference to karyotypes and reproductive isolation

Wang, BC; Ohtani, K; Watabe, H; Gao, JJ; Zhang, YP. 2006

Karyotypes and reproductive isolation were studied in two allopatric populations of *Drosophila tsigana*, one from Guizhou Province in southern China and the other from Hokkaido in northern Japan, and in one population of a closely related species, *D. longiserrata*, from Guizhou. In metaphase plates of larval brain cells, both geographic strains of *Drosophila tsigana* showed $2n=10$ chromosomes, with 2 pairs of metacentric (V-shape), 2 pairs of acrocentric (R-shape), and 1 pair of dot-like (D-shape) chromosomes. *Drosophila longiserrata* showed the same number, 10 chromosomes, comprising 2V, 1J (sub-metacentric chromosome), 1 R, and 1 D. X chromosomes of both species were acrocentric, the presumed ancestral form. Premating isolation was complete between *D. tsigana* and *D. longiserrata*, and successful mating was also limited in crosses between the two geographic populations of *D. tsigana*, especially in crosses between Japanese (JP) females and Guizhou (GZ) males. F1 hybrids were obtained only from crosses between GZ females and JP males, and fertilities of both F1 females and males were quite incomplete. The results of morphological observations, karyotypic analyses, and crossing experiments clearly showed that the GZ and JP populations of "*D. tsigana*" were highly divergent from each other and that each population should be recognized as a biologically valid species. The present morphological observations and chromosomal analyses, together with the original descriptions, strongly suggest that "Guizhou *D. tsigana*" might be conspecific with *D. bisetata* Toda, 1988 from Myanmar, and that *D. longiserrata* might be conspecific with *D. afer* Tan, Hsu, and Sheng, 1949 from Meitan, Guizhou.

Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat-types within the hybrid zone, Shelby County, TN, 2002 (Diptera : Culicidae)

Savage, HM. 2006

When ecological isolation breaks down: sexual isolation is an incomplete barrier to hybridization between *Rhagoletis* species

Schwarz, D; McPherson, BA. 2007

Question: Environmental disturbance can disrupt habitat choice as an ecological barrier to hybridization between host-specific parasites that mate on their host. Is environment-independent mate choice a sufficient barrier to prevent hybridization when ecological isolation breaks down? Hypothesis: Males and females will not discriminate between conspecific and heterospecific mating partners in the absence of host cues. Study system: *Rhagoletis mendax* and *R. zephyria* (Diptera: Tephritidae). Hybridization between these two taxa resulted in the *Lonicera* fly, an example of hybrid speciation in animals. The *Lonicera* fly is found only on non-native

honeysuckle. *Rhagoletis mendax* and *R. Zephyria* discriminate against each other's host but not honeysuckle. This suggests the local breakdown of reproductive isolation via host choice following the introduction of an invasive plant. Methods: We combined males and females of both species in a multi-choice experiment in the laboratory and recorded mating events. Conclusion: Without host plant cues, mate choice is an incomplete barrier to hybridization. Reproductive isolation between host-specific parasites can be influenced by environmental disturbance because a non-ecological barrier (mate choice) alone is too weak to maintain reproductive isolation.

Diptera leafminers and their parasitoids in spontaneous vegetation in organic citrus orchard in Montenegro, RS, Brazil

dos Santos, JP; Redaelli, LR; Dal Sogli, FK. 2007

Diptera leafminers and their parasitoids in spontaneous vegetation in organic citrus orchard in Montenegro, RS, Brazil. Leafminers and their parasitoids communities analysis is necessary to supply information about the biotic regulation and to maintenance of the biodiversity in the agroecosystem. This study aimed to register Diptera leafminers and their parasitoids, present in the vegetation spontaneously growing at the citrus orchard from May 2003 to May 2004. The work was conducted in Montenegro, RS, in an organic orchard of the hybrid 'Morcott'. Samplings were taken fortnightly, collecting in each occasion all the plants with mines found in an area delimited by a 0.28 m(2) arc thrown in the lines and between lines of 30 randomly chosen trees. In the lab, the number of larvae and pupae per leaf of Diptera leafminers were recorded. Throughout the study, it was found 15 species of Diptera leafminers, 15 species of leafminer host plants (distributed in six families) and 15 species of micro hymenopterans parasitoids (distributed in three families). It was verified that the Diptera leafminers had presented great specificity to their host plants; therefore, the appropriate management of the spontaneous vegetation of the orchards may favor the establishment and multiplication of natural enemies of these leafminers insects.

Survival and replication of dengue-2 virus in diapausing eggs of *Aedes albopictus* (Diptera : Culicidae)

Guo, XX; Zhao, TY; Dong, YD; Lu, BL. 2007

Survival and replication of dengue-2 virus (family Flaviviridae, genus Flavivirus, DENV-2) was determined in diapausing eggs of *Aedes albopictus* (Skuse) (Diptera: Culicidae) to evaluate the importance of this mechanism for viral maintenance during adverse climatic conditions. Infected and uninfected eggs of *Ae. albopictus* were induced to diapause under low temperature and short photoperiod. Virus was detected by reverse transcription-polymerase chain reaction and isolated in C6/36 cells from both diapausing eggs and nondiapausing eggs. Nucleic acid hybridization was used to monitor viral replication in diapausing eggs. DENV-2 might survive in infected diapausing eggs in a relatively quiescent status based on the absence of a replicative intermediate RNA. In contrast, the viral replicative intermediate RNA along with the replicative form RNA was detected in nondiapausing eggs. It seemed that the virus replicated more actively in nondiapausing eggs than in diapausing eggs. Infected diapausing eggs may play an important role in the maintenance of DENV during adverse climatic conditions in nature.

Unusually short tandem repeats in the chromosome end structure of *Rhynchosciara* (Diptera : Sciaridae)

Rossato, RM; Madalena, CRG; Gorab, E. 2007

The characterisation of chromosome end (terminal and sub-terminal) sequences of *Rhynchosciara americana* chromosomes was continued with the screening of a plasmid library made of amplified DNA fragments from a microdissected chromosome tip. An insert chosen for analysis hybridised to two chromosome ends and contains two microsatellite arrays in close vicinity to a sequence (named M-47), part of which is significantly similar to minisatellites of Salmonidae that are frequently present in the vicinity of microsatellite arrays. PCR results using a single primer representative of M-47 elements suggest that they are also repetitive in *Rhynchosciara* genomes. In addition, total single primer PCR products hybridised to the non-telocentric end subset of *R. americana* chromosomes. Another plasmid microlibrary made of chromosome tips amplified by a single M-47 primer was screened for repeats of *Rhynchosciara* chromosomes. Selected inserts that hybridised strongly to non-telocentric ends of *R. americana* and *R. hollaenderi* have a tandem array of 22 bp repeats (M-22). There is sequence divergence among M-22 repeats but their mean similarity is significantly high in relation to the M-22 consensus sequence derived from the cloned tandem array. M-22 elements lie distal to the 414 bp sub-telomeric satellite array characterised previously as suggested by double labelling for M-22 hybridisation and reverse transcriptase. M-47 elements, formerly identified in Salmonidae, thus contribute to specify unusually short repeats composing the sub-telomeric structure of two *Rhynchosciara* species.

Genetic differentiation between populations of the European rose hip fly *Rhagoletis alternata*

Vaupel, A; Klinge, K; Brandle, M; Wissemann, V; Tscharnkte, T; Brandl, R. 2007

We tested for genetic differentiation between populations of *Rhagoletis alternata* Fall. (Diptera: Tephritidae) on three different host species. We collected larvae from three rose species of the section Caninae (*Rosa canina* L., *Rosa corymbifera* Borkh., and *Rosa rubiginosa* L.) from 15 sites across Germany, where the three roses occurred together. Additionally, we sampled three sites in Switzerland. Roses differ in morphology (e.g. leaf glands) as well as phenology. We were able to score nine allozyme loci (five polymorphic). Populations from the three hosts did not differ in genetic variability. We found significant genetic differentiation between populations from different host species. However, the differentiation was very low (0.9%). Hence, we found no indication for host races. Furthermore, surprisingly little geographical structure of genetic differentiation was found between populations of this fruit fly across central Europe. We offer three mutually non-exclusive explanations for these findings. First, gene flow between populations of *Rh. alternata* is high. Second, the pattern of genetic differentiation is based on a recent expansion of the distributional range. Third, the ongoing gene flow between roses of the section Caninae acts as a hybrid bridge. (c) 2007 The Linnean Society of London.

Colonization of a hybrid strain to restore male *Anastrepha ludens* (Diptera : Tephritidae) mating competitiveness for sterile insect technique programs

Rull, J; Barreda-Landa, A. 2007

To restore male mating competitiveness of Mexican fruit flies, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), reared for sterile insect releases by the Mexican Fruit fly Eradication Campaign, two strain replacement techniques were evaluated. Field cage male competitiveness tests revealed that laboratory males of the Metapa strain mated 3 times less often with wild females than field-collected wild males. A strain developed from the cross of wild males and laboratory females (hybrid strain) was similar to a strain developed from the cross of laboratory males and females (laboratory strain) in that its females produced similar amounts of eggs and the eggs displayed similar levels of hatch and egg-to-pupa transformation in artificial diet. By contrast, a strain developed from the cross of wild males and females (wild strain), forced into artificial rearing, experienced a series of bottlenecks involving reduced egg laying and extremely poor development in diet. The male F1 progeny of the hybrid strain and field-collected wild males outcompeted F1 laboratory males in field cage tests for matings with field-collected wild females. In conclusion, we found that strains developed from the cross of wild males and laboratory females allowed us to restore male mating competitiveness of F1 Mexican fruit flies without compromising mass-rearing production.

Natural infection of *Phlebotomus* (Larrousius) *langeroni* (Diptera : Psychodidae) with *Leishmania infantum* in Tunisia

Guerbouj, S; Chemkhi, J; Kaabi, B; Rahali, A; Ben Ismail, R; Guizani, I. 2007

Phlebotomine sand flies were captured from an active transmission focus of sporadic cutaneous leishmaniasis, caused by *Leishmania infantum*, in El Kef region, northern Tunisia. Both *Phlebotomus perniciosus* and *P. langeroni* were found. *Phlebotomus langeroni* females showed a statistically significant intradomiciliary dominance ($P < 0.01$ for the 2003 and 2004 seasons) when compared to animal shelters. During the 2003 season, dissection of collected female specimens showed the presence of flagellates within the digestive tracts of two *P. perniciosus* among 1086 observed, but none in 232 *P. langeroni*. Amplification of kinetoplast minicircles of *Leishmania* parasites was applied to DNA samples extracted from 298 frozen females including 249 *P. perniciosus*, 36 *P. langeroni*, 5 *P. longicuspis* and 8 *P. perfiliewi* and revealed by radioactive probe hybridization. Two *P. langeroni* females showed a signal of the size expected for *L. infantum* (800 bp) indicating infection with these parasites. However, this PCR-hybridization method failed to identify any positive *P. perniciosus* females in pools of specimens. These results show for the first time the natural infection of *P. langeroni* with *L. infantum* in Tunisia, and support the existence of different *L. infantum* transmission cycles in Tunisia, with a potential, role for *P. langeroni* as a vector. (C) 2006 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.

Morphological adaptation in host races of *Tephritis conura*

Diegisser, T; Seitz, A; Johannesen, J. 2007

The present study investigates morphological differentiation among host races of the fruit fly *Tephritis conura* Loew (Diptera: Tephritidae) for two fitness-related traits and whether these traits are host induced or genetically determined. Flies were analyzed from independent sympatric regions, and from one syntopic site where parental host plants [*Cirsium heterophyllum* (L.) Hill. and *Cirsium oleraceum* (L.) Scop. (Cardueae)] and hybrid plants (*C. heterophyllum* x *C. oleraceum*) co-occur. As both host races may oviposit on hybrid plants and hybrid plants provide an identical environment for larvae of both host races, flies emerging from *C. heterophyllum* x *C. oleraceum* hybrids were used to assess whether host-race morphological differences are genetically determined or due to phenotypic plasticity. No significant size (wing length) differences were found among host races, whereas flies emerging from *C. heterophyllum* had on average 8.4% longer ovipositors than flies emerging from *C. oleraceum*. The mean size-corrected ovipositor length (i.e., the ratio ovipositor/wing length) was 10.3% longer. These proportions were repeated among host races emerging from hybrid plants. Although flies of the *C. heterophyllum* host race from hybrid plants were smaller than on parental host plants, the ratio ovipositor/wing length was constant. Hybrid flies (which emerged only on hybrid plants) were intermediate in relative and absolute ovipositor length. Thus, ovipositor-length differences among *T. conura* host races most likely have a genetic basis. This suggests that host-related differences in ovipositor length reflect adaptations to the respective host-plant species, most likely to the host's flower-head size, whereas both host races experience similar selection regimes on body size.

Geographic origin affects larval competitive ability in European populations of the blow fly, *Lucilia sericata*

Martinez-Sanchez, A; Smith, KE; Rojo, S; Marcos-Garcia, MA; Wall, R. 2007

The blow fly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), is found throughout Europe. In northern areas, in addition to breeding in carrion, it can also commonly act as primary facultative ectoparasite of sheep, resulting in animal suffering and production losses. However, in the south of Europe, *L. sericata* is not usually implicated in myiasis and is found almost entirely in carrion. The causes of this behavioural difference are unclear. To begin to address the possible reasons for this difference, the present study examined the effects of competition on the survival and development of *L. sericata* populations derived from southern Spain, England, and a hybrid derived from a cross between Spanish females and UK males. Larvae were reared at a range of different initial densities and the mortality, development rate, and size of the resultant adults were measured. Mortality increased significantly with density, but the Spanish population had a significantly higher mortality rate than the UK population at all densities. Similarly, adult size declined with increased larval density, but individuals from the Spanish population were consistently larger than those from the UK population at all densities. The mortality and size of the hybrids was consistently intermediate between the Spanish and UK populations. No consistent effects on development rate were observed. This study demonstrates that insects from northwestern and southern European population have qualitatively different life history traits and competitive abilities and may contribute towards explaining the different behaviour of populations of this species in different part of its distribution range.

Analysis of the immune-inducible transcriptome from microbial stress resistant, rat-tailed maggots of the drone fly *Eristalis tenax*

Altincicek, B; Vilcinskas, A. 2007

Background: The saprophagous and coprophagous maggots of the drone fly *Eristalis tenax* (Insecta, Diptera) have evolved the unique ability to survive in aquatic habitats with extreme microbial stress such as drains, sewage pools, and farmyard liquid manure storage pits. Therefore, they represent suitable models for the investigation of trade-offs between the benefits resulting from colonization of habitats lacking predators, parasitoids, or competitors and the investment in immunity against microbial stress. In this study, we screened for genes in *E. tenax* that are induced upon septic injury. Suppression subtractive hybridization was performed to selectively amplify and identify cDNAs that are differentially expressed in response to injected crude bacterial endotoxin (LPS). Results: Untreated *E. tenax* maggots exhibit significant antibacterial activity in the hemolymph which strongly increases upon challenge with LPS. In order to identify effector molecules contributing to this microbial defense we constructed a subtractive cDNA library using RNA samples from untreated and LPS injected maggots. Analysis of 288 cDNAs revealed induced expression of 117 cDNAs corresponding to 30 novel gene clusters in *E. tenax*. Among these immune-inducible transcripts we found homologues of known genes from other Diptera such as *Drosophila* and *Anopheles* that mediate pathogen recognition (e. g. peptidoglycan recognition protein) or immune-related signaling (e. g. relish). As predicted, we determined a high diversity of novel putative antimicrobial peptides including one *E. tenax* defensin. Conclusion: We identified 30 novel genes of *E. tenax* that were induced in response to septic injury including novel putative antimicrobial peptides. Further analysis of these immune-related effector molecules from *Eristalis* may help to elucidate the interdependency of ecological adaptation and molecular evolution of the innate immunity in Diptera.

Using COI barcodes to identify forensically and medically important blowflies

Nelson, LA; Wallman, JF; Dowton, M. 2007

The utility of cytochrome oxidase I (COI) DNA barcodes for the identification of nine species of forensically important blowflies of the genus *Chrysomya* (Diptera: Calliphoridae), from Australia, was tested. A 658-bp fragment of the COI gene was sequenced from 56 specimens, representing all nine *Chrysomya* species and three calliphorid outgroups. Nucleotide sequence divergences were calculated using the Kimura-two-parameter distance model and a neighbour-joining (NJ) analysis was performed to provide a graphic display of the patterns of divergence among the species. All species were resolved as reciprocally monophyletic on the NJ tree. Mean intraspecific and interspecific sequence divergences were 0.097% (range 0-0.612%, standard error [SE] = 0.119%) and 6.499% (range 0.458-9.254%, SE = 1.864%), respectively. In one case, a specimen that was identified morphologically was recovered with its sister species on the NJ tree. The hybrid status of this specimen was established by sequence analysis of the second ribosomal internal transcribed spacer (ITS2). In another instance, this nuclear region was used to verify four cases of specimen misidentification that had been highlighted by the COI analysis. The COI barcode sequence was found to be suitable for the identification of *Chrysomya* species from the east coast of Australia.

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.

The localization of ribosomal DNA in Sciaridae (Diptera : Nematocera) reassessed

Madalena, CRG; Arnabis, JM; Stocker, AJ; Gorab, E. 2007

The chromosomal localization of ribosomal DNA (rDNA) was studied in polytene and diploid tissues of four sciarid species, Trichosia pubescens, Rhynchosciara americana, R. milleri and Schwenkfeldina sp. While hybridization to mitotic chromosomes showed the existence of a single rDNA locus, ribosomal probes hybridized to more than one polytene chromosome region in all the species analyzed as a result of micronucleolar attachment to specific chromosome sites. Micronucleoli are small, round bodies containing transcriptionally active, probably extrachromosomal rDNA. In T. pubescens the rDNA is predominantly localized in chromosome sections X-10 and X-8. In R. americana the rDNA is frequently found associated with centromeric heterochromatin of the chromosomes X, C, B and A, and also with sections X-1 and B-13. Ribosomal probes in R. milleri hybridized with high frequency to pericentric and telomeric regions of its polytene complement. Schwenkfeldina sp. displays a remarkably unusual distribution of rDNA in polytene nuclei, characterized by the attachment of micronucleoli to many chromosome regions. The results showed that micronucleoli preferentially associate with intercalary or terminal heterochromatin of all sciarid flies analyzed and, depending on the species, are attached to a few (Trichosia), moderate (Rhynchosciara) or a large (Schwenkfeldina sp.) number of polytene chromosome sites.

Asymmetry of frontal bristles and postocular setae in species and hybrids of the Anastrepha fraterculus complex (Diptera, Tephritidae)

Souza, JMGA; Gouveia, M; Perondini, ALP; Selivon, D. 2007

Asymmetry of the frontal bristles and postocular setae was studied in samples from natural populations and laboratory colonies of Anastrepha sp. 1 aff fraterculus, of A. sp. 2 aff. fraterculus, and in F1 hybrids obtained from laboratory reciprocal crosses. Natural populations were sampled in a zone of sympatry and in two geographically distant regions with different climatic conditions. Asymmetry was scored as the differences between the number of bristles and of setae on the right and left sides of the head, males and females analyzed independently. The two traits exhibited variability according to the model of fluctuating asymmetry (FA). No significant differences among samples were found in the FA of frontal bristles. A significant FA was observed for the postocular setae of A. sp. 1 males from a southern population (Vacaria, RS) as compared to the asymmetry exhibited by males and females of some other samples. No significant differences in FA were observed among the interspecific hybrids and the laboratory samples of both parental species. The higher FA found in the males from Vacaria was attributed to climatic conditions prevailing in that region. The absence of a higher FA in hybrids may be related to the relatively recent evolutionary history of the two species.

Molecular phylogenetics and the evolution of host plant associations in the nematode genus Fergusobia (Tylenchida : Fergusobiinae)

Ye, W; Gibling-Davis, RM; Davies, KA; Purcell, MF; Scheffer, SJ; Taylor, GS; Center, TD; Morris, K; Thomas, WK. 2007

Fergusobia nematodes (Tylenchida: Fergusobiinae) and Fergusonina flies (Diptera: Fergusoninidae) are putative mutualists that develop together in galls formed in meristematic tissues of many species of the plant family Myrtaceae in Australasia. Fergusobia nematodes were sampled from a variety of myrtaceous hosts and gall types from Australia and one location in New Zealand between 1999 and 2006. Evolutionary relationships of these isolates were inferred from phylogenetic analyses of the DNA sequences of the nuclear ribosomal DNA near-full length small subunit (up to 1689 bp for 21 isolates), partial large subunit D2/D3 domain (up to 889 bp for 87 isolates), partial mitochondrial cytochrome oxidase subunit 1 (618 bp for 82 isolates), and combined D2/D3 and mtCOI (up to 1497 bp for 66 isolates). The SSU data supported a monophyletic Fergusobia genus within a paraphyletic Howardula. A clade of Drosophila-associated Howardula, including Howardula aoronymphium, was the closest sequenced sister group. Phylogenetic analysis of sequences from D2/D3 and mtCOI, separately and combined, revealed many monophyletic clades within Fergusobia. The relationships inferred by D2/D3 and mtCOI were congruent with some exceptions. Well-supported clades were generally consistent with host plant species and gall type. However, phylogenetic analysis suggested host switching or putative hybridization events in many groups, except the lineage of shoot bud gallers on the broad-leaved Melaleuca species complex. Published by Elsevier Inc.

DNA barcoding cannot reliably identify species of the blowfly genus Protocalliphora (Diptera : Calliphoridae)

Whitworth, TL; Dawson, RD; Magalon, H; Baudry, E. 2007

In DNA barcoding, a short standardized DNA sequence is used to assign unknown individuals to species and aid in the discovery of new species. A fragment of the mitochondrial gene cytochrome c oxidase subunit 1 is emerging as the standard barcode region for animals. However, patterns of mitochondrial variability can be confounded by the spread of maternally transmitted bacteria that cosegregate with mitochondria. Here, we investigated the performance of barcoding in a sample comprising 12 species of the blow fly genus Protocalliphora, known to be infected with the endosymbiotic bacteria Wolbachia. We found that the barcoding approach showed very limited success: assignment of unknown individuals to species is impossible for 60% of the species, while using the technique to identify new species would underestimate the species number in the genus by 75%. This very low success of the barcoding approach is due to the non-monophyly of many of the species at the mitochondrial level. We even observed individuals from four different species with identical barcodes, which is, to our knowledge, the most extensive case of mtDNA haplotype sharing yet described. The pattern of Wolbachia infection strongly suggests that the lack of within-species monophyly results from introgressive hybridization associated with Wolbachia infection. Given that Wolbachia is known to infect between 15 and 75% of insect species, we conclude that identification at the species level based on mitochondrial sequence might not be possible for many insects. However, given that Wolbachia-associated mtDNA introgression is probably limited to very closely related species, identification at the genus level should remain possible.

Cytogenetic and molecular evidence for two species in the Anopheles barbirostris complex (Diptera : Culicidae) in Thailand

Saeung, A; Otsuka, Y; Baimai, V; Somboon, P; Pitasawat, B; Tuetun, B; Junkum, A; Takaoka, H; Choochote, W. 2007

Seventeen isolines of Anopheles barbirostris derived from animal-biting female mosquitoes showed three karyotypic forms: Form A (X-2, Y-1) in five isolines from Phetchaburi province; Form B (X-1, X-3, Y-2) in three and eight isolines from Chiang Mai and Ubon Ratchathani provinces, respectively; Form C (X-2, Y-3) in one isolate

from Phetchaburi province. All 17 isolines exhibited an average branch summation of seta 2-VI pupal skins ranging from 12.1-13.0 branches, which was in the limit of *A. barbirostris* (6-18 branches). Of the 12 human-biting isolines from Chiang Mai province, five isolines showed Form B (X-2, Y-2), and seven isolines exhibited a new karyotypic form designated as Form E (X-2, Y-5). All of 12 isolines had an average branch summation of seta 2-VI pupal skins ranging from 22.4-24.5 branches, which was in the limit of *Anopheles campestris* (17-58 branches). Thus, they were tentatively designated as *A. campestris*-like Forms B and E. Hybridization between *A. campestris*-like Forms B and E showed that they were genetically compatible, yielding viable progeny for several generations suggesting conspecific relationships of these two karyotypic forms. Reproductive isolation among crosses between *A. campestris*-like Form B and *A. barbirostris* Forms A, B, and C strongly suggested the existence of these two species. In addition, the very low intraspecific variation (genetic distance < 0.005) of the nucleotide sequence of ITS2 of the rDNA and COI and COII of mitochondrial DNA of the seven isolines of *A. campestris*-like Forms B and E supported their conspecific relationship. The large sequence divergence of ITS2 (0.203-0.268), COI (0.026-0.032), and COII (0.030-0.038) from genomic DNA of *A. campestris*-like Forms B and E and the *A. barbirostris* Forms A, B, and C clearly supported cytogenetic and morphological evidence.

Evaluating the effect of postmating isolation between molecular forms of *Anopheles gambiae* (Diptera : Culicidae)

Diabate, A; Dabire, RK; Millogo, N; Lehmann, T. 2007

Multiple families representing all possible combinations of crosses between the two molecular forms of *Anopheles gambiae* sensu stricto Giles and their hybrids were set up using forced mating between offspring of wild-collected females. The results showed that the reproductive output of hybrids and their backcrosses was similar to that of the pure forms as measured by egg batch size, hatching rate, and larval development success. No sex ratio distortion was found among the offspring. We concluded that postmating developmental barriers do not contribute to the isolation between the molecular forms.

Geographic variation in vector competence for West Nile virus in the *Culex pipiens* (Diptera : Culicidae) complex in California

Vaidyanathan, R; Scott, TW. 2007

We evaluated the susceptibility to infection and transmission of West Nile virus (WNV) in seven populations of *Culex pipiens pipiens* (L.), *Cx. p. quinquefasciatus* Say, and from populations containing *Cx. pipiens/quinquefasciatus* hybrids in a north-south transect of California. Samples were identified to species or as hybrid forms based on morphology of male terminalia. After 7 and 14 days of extrinsic incubation, few females were infected and none transmitted WNV from samples of *Cx. p. pipiens* from northern Shasta County and of *Cx. p. quinquefasciatus* from southern Los Angeles County. Seven days after infective feeding, 13%-36% of mosquitoes from the counties of Merced, Fresno, Kern, and San Bernardino were infected, and 12%-40% of infected mosquitoes expressed WNV in salivary excretate. Fourteen days after infective feeding, 18%-43% of mosquitoes from these counties were infected, and 50%-69% of infected mosquitoes transmitted WNV in salivary excretate. A sample of *Cx. p. quinquefasciatus* from Riverside County did not transmit WNV after 7 days, but 71% transmitted 14 days after infective feeding. These results reveal extensive geographic variation in vector competence for WNV in the *Culex pipiens* complex in California.

Taxonomy and ecology of the pitcher plant mosquito, *Wyeomyia smithii* (Coquillett) (Diptera : Culicidae), in Mississippi

Goddard, J; Waggy, G; Varndo, WC; Harrison, BA. 2007

During 2005, a population of the Gulf Coast race of *Wyeomyia smithii* (Coquillett) was studied in the purple pitcher plant, *Sarracenia purpurea* L. and one hybrid plant in the Grand Bay National Wildlife Refuge located in extreme southeastern Mississippi. Twenty-nine larval specimens of *Wy. smithii* were collected during this study from two clusters of plants approximately 100 m apart. A short review is provided of the changing taxonomic concept for *Wy. smithii* in the United States. Mississippi populations of *Wy. smithii* represent a Gulf Coast race of the species, but current evidence does not support providing a subspecies name for this race. Also provided are ecological observations of the effects of a controlled burn of the pitcher plant field as well as the storm surge caused by Hurricane Katrina on the plants and *Wy. smithii* population densities.

Genetic differentiation between the sibling and sympatric flower-head infesting tephritids: The polyphage, *Trupanea nigricornis* (Coquillett), and the narrowly oligophagous, *T. bisetosa* (Coquillett) (Diptera : Tephritidae)

Knio, KM; Goeden, RD; Headrick, DH. 2007

Trupanea nigricornis and *T. bisetosa* are sibling, cryptic species that occur in sympatry in southern California. *Trupanea nigricornis* is generalist, infesting at least 8 tribes in the Asteraceae while *T. bisetosa* is mainly a specialist on wild sunflowers. Although the two species are very similar in morphology and biology, genetic differentiation between them was possible using isozyme electrophoresis. Among 14 resolved loci, 5 were polymorphic. Significant allele frequency differences were found between the two species for PGI, PGM, ME, and EST-1. Moreover, a fixed allele difference for the locus ACPH was detected, indicating absence of gene flow between *T. nigricornis* and *T. bisetosa* in the sampled sympatric populations. The absence of hybrids could be explained by the results of cross-mating studies, which indicated that the two species did mate in the laboratory, but produced few, if any, viable offspring. Hybrid inviability acted as a post-mating barrier reducing gene flow between sympatric populations of *T. nigricornis* and *T. bisetosa*.

Disruption of the *Wolbachia* surface protein gene *wspB* by a transposable element in mosquitoes of the *Culex pipiens* complex (Diptera, Culicidae)

Sanogo, YO; Dobson, SL; Bordenstein, SR; Novak, RJ. 2007

Culex pipiens quinquefasciatus Say and *Culex pipiens pipiens* Linnaeus are sibling species incriminated as important vectors of emerging and re-emerging infectious diseases worldwide. The two forms differ little morphologically and are differentiated mainly based upon ecological, behavioural, physiological and genetic traits. Within the North American zone of sympatry, populations of *Cx. p. quinquefasciatus* and *Cx. p. pipiens* undergo extensive introgression and hybrid forms have been reported in nature. Both *Cx. p. quinquefasciatus* and *Cx. p. pipiens* are infected with the endosymbiotic bacteria *Wolbachia pipiensis*. Here, we report the presence of a transposable element belonging to the IS256 family (IS256wPip) associated with *Wolbachia* in both *Cx. p. quinquefasciatus* and *Cx. p. pipiens* populations. Using reverse transcriptase PCR and sequence analysis, we show that IS256wPip has disrupted the *wspB* locus, a paralogue of the *Wolbachia* outer membrane protein (*wspA*) gene. The inactivation of the *wspB* appears to be specific to *Cx. p. quinquefasciatus* and to hybrids of the two forms, and was not observed in the surveyed *Cx. p. pipiens* mosquitoes. Our results support the hypothesis of a different origin of North American *Cx. p. quinquefasciatus* and *Cx. p. pipiens* populations. The flux of mobile genetic elements in the *Wolbachia* wPip genome could explain the high level of crossing types observed among different *Culex* populations. The insertion of IS256wPip into *wspB* may comprise a genetic candidate for discriminating *Wolbachia* symbionts in *Culex*.

Host-related life history traits in interspecific hybrids of cactophilic *Drosophila*

Soto, EM; Soto, IM; Carreira, VP; Fanara, JJ; Hasson, E. 2008

In the genus *Drosophila* (Diptera: Drosophilidae), interspecific hybridization is a rare phenomenon. However, recent evidence suggests a certain degree of introgression between the cactophilic siblings *Drosophila buzzatii* Patterson & Wheeler and *Drosophila koepferae* Fontdevila & Wasserman. In this article, we analyzed larval viability and developmental time of hybrids between males of *D. buzzatii* and females of *D. koepferae*, raised in media prepared with fermenting tissues of natural host plants that these species utilize in nature as breeding sites. In all cases, developmental time and larval viability in hybrids was not significantly different from parental lines and, depending on the cross, hybrids developed faster than both parental species or than the slowest species. When data of wing length were included in a discriminant function analysis, we observed that both species can be clearly differentiated, while hybrids fell in two categories, one intermediate between parental species and the other consisting of extreme phenotypes. Thus, our results point out that hybrid fitness, as measured by developmental time and viability, is not lower than in the parental species.

Hybridization between two polyphagous fruit-fly species (Diptera : Tephritidae) causes sex-biased reduction in developmental stability

Erbout, N; De Meyer, M; Lens, L. 2008

When hybridization modifies the genetic constitution of individuals or populations, the stability of phenotypic development may either decrease or increase, depending on the divergence in the gene systems that control the development between the hybridizing taxa, i.e. on the relative effects of outbreeding and heterosis. In genetically closely related species, strong heterotic effects are less likely to occur, and hence hybridization may be expected to cause an overall decrease in developmental stability (DS) resulting from the disruption of coadapted gene complexes. To test this hypothesis, we experimentally crossed two closely related species of *Ceratitis* fruit flies and compared multiple-trait fluctuating asymmetry (FA, a measure of DS) in male and female offspring between parental species and two crossbred types. All traits measured play an important role in the fanning and buzzing behaviour associated with male courtship in *Ceratitis*, or are located on body parts involved in this behaviour. As predicted, hybrid offspring developed more asymmetrically than offspring of either parental species - most notably in meristic traits - and the increase in FA was consistently and significantly stronger in females than in males. The fact that males buffered their development more efficiently than females is in concordance with the presumed between-sex variation in functionality, and hence cost of asymmetry, of the measured traits. Absence of a similar sex difference in DS among parental offspring is believed to result from overall weak association between DS and FA in the absence of genetic stress, resulting from the random nature of the underlying processes that trigger asymmetric development. (c) 2008 The Linnean Society of London.

Laboratory hybridization between the two clades of *Liriomyza huidobrensis* (Diptera: Agromyzidae)

Takano, SI; Iwaizumi, R; Nakanishi, Y; Someya, H. 2008

We conducted mating experiments between two clades of the pea leafminer, *Liriomyza huidobrensis*, i.e., California Clade (CC) and South America Clade (SC). Development times from egg to pupa and egg to adult at 25 C of the two clades and the hybrid progeny were compared. In the interclade mating cross, adult hybrid progenies were obtained only from matings between CC females and SC males. The number of hybrid progeny was quite smaller than those from combinations of the same clade. Furthermore, no F2 progeny was obtained from matings between F1 hybrid progenies. CC progeny required more development times from egg to pupa and egg to adult at 25 C than SC and hybrid progeny. Our biological data indicated that the two clades of *L. huidobrensis* were different species, as indicated by their molecular characters in previous studies.

Identification of male specimens of the *Culex pipiens* complex (Diptera : Culicidae) in the hybrid zone using morphology and molecular techniques

Sanogo, YO; Kim, CH; Lampman, R; Halvorsen, JG; Gad, AM; Novak, RJ. 2008

The identification of the members of the *Culex pipiens* L. complex in arbovirus surveillance programs relies heavily on the use of morphology. In this work, we studied *Cx. pipiens* complex male mosquitoes collected from nine different locations, from northern, southern, and the hybrid zone sites in North America; Cairo, Egypt; and Nairobi, Kenya. Specimens were identified using DV/D ratio and also using amplification of the acetylcholinesterase (ACE.2) gene by both conventional and real-time polymerase chain reaction, and examination of the CQ11 locus. Consistent with previous findings, the morphological and molecular identifications did not always agree, particularly in regions of extensive introgression. There was an increased frequency of hybrid forms in late summer and early fall in Champaign Co., IL, that is north of the previously described *Cx. pipiens* complex hybrid zone. This represents an expansion of the North American hybrid zone. The biological and epidemiological relevance of the high degree of introgression and the late season increase in the proportion of intermediate forms is discussed.

Molecular and Morphometrical Revision of the *Zaprionus tuberculatus* Species Subgroup (Diptera: Drosophilidae), with Descriptions of Two Cryptic Species

Yassin, A. 2008

Zaprionus is an important drosophilid genus in the Afrotropical region. Here, two new species, *Z. burlai* n. sp. and *Z. tsacasi* n. sp., are described from Tanzania and Sao Tome, respectively. The two species show incomplete reproductive isolation with *Z. tuberculatus* Malloch and *Z. sepsoides* Duda, respectively, with intercrosses producing fertile females but sterile males. The latter two have long been considered sibling species and together with three other species (*Z. mascariensis* Tsacas & David, *Z. kolodkinae* Chassagnard & Tsacas, and *Z. cerruca* Chassagnard & McEvey) form the *tuberculatus* subgroup. The phylogenetic relationships of these seven species of the subgroup were revised in light of mitochondrial (COII) gene sequences and wing morphometrics. Mitochondrial DNA firmly distinguished most of the species, except for a triad of *Z. tuberculatus*, *Z. cerruca*, and *Z. burlai*. Wing morphometrics was able to distinguish between closely related species and also indicated the altitudinal origin of each species. Most species can be identified through internal anatomy of the reproductive system (testis and seminal receptacle lengths), and the discovery of the new species with incomplete reproductive isolation may help in understanding the genetic basis of this variation through interspecific hybridization. The molecular phylogeny reconfirmed the Malagasy origin of the subgroup during the Late Pliocene. Colonization of Africa probably involved two independent events during the Pleistocene.

The *Lutzomyia longipalpis* species complex: does population sub-structure matter to *Leishmania* transmission?

Maingon, RDC; Ward, RD; Hamilton, JGC; Bauzer, LGS; Peixoto, AA. 2008

Leishmania chagasi causes visceral leishmaniasis and, to a lesser extent, atypical cutaneous leishmaniasis in Central and South America. Its main sand fly vector, *Lutzomyia longipalpis* s.l. (Diptera: Psychodidae) displays a complex population structure that might contribute to the observed clinical pleomorphism and to recent major urban epidemics. This article summarises our understanding on reproductive barriers and hybridisation among this vector's sibling incipient species. Identifying genes important for sand fly ecological adaptability and sand fly-*Leishmania* genetic covariation could be helpful for interrupting *Leishmania* transmission.

Susceptibility of fruit from diverse apple and crabapple germplasm to attack from apple maggot (Diptera : Tephritidae)

Myers, CT; Reissig, WH; Forsline, PL. 2008

Apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), is a pest of major concern to apple, *Malus x domestica* (Borkh.) production in eastern North America. Host plant resistance to apple maggot among apple germplasm has been previously evaluated among a small number of exotic *Malus* accessions and domestic hybrid selections. However, a large number of exotic accessions housed in USDA collections have never been evaluated for their susceptibility to apple pests. Additionally, previous reports of resistance need to be confirmed under both field conditions and with more rigorous laboratory evaluations. Thus, studies were conducted to evaluate the susceptibility of a number of *Malus* accessions housed at the USDA Plant Genetic Resources Unit "core" collection. Contrary to earlier published reports, these results suggest that some selections previously described as "resistant" are in fact susceptible to both oviposition damage and larval feeding damage by apple maggot. One domestic, disease-resistant apple accession, 'E36-7' is resistant to survival of apple maggot larvae except when the fruit is nearly ripe in late fall. This is the first report of an apple cultivar that is confirmed to be resistant to larval feeding of apple maggot. Although adults can successfully oviposit on all accessions examined, larval survival was zero in a number of small-fruited crabapple accessions classified as resistant in previous studies and also in two accessions, *Malus tschonoskii* (Maxim) C. K. Schneid. and *M. spectabilis* (Aiton) Borkh., that have not been previously evaluated.

Identification of immune-responsive genes in the mosquito *Culex quinquefasciatus* infected with the filarial parasite *Wuchereria bancrofti*

Kumar, BA; Paily, KP. 2008

Several antimicrobial/parasitic peptides are known to be upregulated in mosquitoes upon infection with parasites. The aim of this study was to identify immune-responsive genes in the vector mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae) against the human lymphatic filarial parasite, *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae). Suppression subtractive hybridization was performed using RNA from filarial infected and non-infected mosquitoes to obtain differentially expressed transcripts, and their identities were confirmed through reverse transcriptase polymerase chain reaction (RT-PCR). Out of 23 clones selected from the suppression subtractive library, three corresponded to antimicrobial peptide genes, defensins, and four corresponded to regulatory serpin peptide genes. RT-PCR using defensin-specific primers and sequencing of the product showed a 284-bp defensin cDNA. Sequence alignment with defensins of the mosquitoes *Anopheles gambiae* s.s. Giles and *Aedes aegypti* (L.) showed maximum homology with the former. Similarly, that of serpin-specific primers showed a 406-bp cDNA encoding serpins. Sequence alignment showed maximum homology with that of *An. gambiae*, as in the case of defensins. Hence, this investigation revealed upregulation of defensins and serpins in *Cx. quinquefasciatus* infected with *W. bancrofti*. Antimicrobial peptide genes such as defensins may have limited or no specific role in regulating parasite development. Serpins may prove to be facilitating molecules, by regulating melanization of the parasite. However, the exact functions of these molecules in the immune system of the vector mosquito are yet to be investigated.

Genetic structure of *Cirsium palustre* (Asteraceae) and its role in host diversification of *Tephritis conura* (Diptera: Tephritidae)

Johannesen, J; Tritsch, C; Seitz, A; Diegisser, T. 2008

Whether or not a host plant is incorporated into a phytophagous insect's diet depends on both the insect's ability to colonize the novel host and the host plant's susceptibility to the insect. The latter, again, will be influenced by the genetic structure of the host plant. *Cirsium palustre* (marsh thistle) is heavily infested by the tephritid fly *Tephritis conura* in northern Britain, whereas infestation is not only absent in southern England, but also absent on the European continent where *T. conura* is common on other *Cirsium*. To understand why regional infestation patterns evolve, we studied how genetic structure and phylogeographic ancestry of *C. palustre* are related to the probability of infestation in Britain. The probability of infestation in Britain was related neither to phylogeographic lineage (two lineages were observed in Europe), nor to introgressive hybridization with the original host of *T. conura*, *C. heterophyllum*. However, infested British *C. palustre* were strongly genetically deprived. British *C. palustre* showed continuous loss of genetic variability towards the northern range edge. The loss was explained best by genetic drift during geographic range expansion, rather than by genetic drift in isolated patches or mating system change from predominately outcrossing to selfing. The phylogeographic pattern of *C. palustre* strongly suggests Scotland as the geographic origin of a recent host-plant expansion of *T. conura*. (C) 2008 The Linnean Society of London, Biological Journal of the Linnean Society, 2008, 95, 221-232.

A homolog of the vaccinia virus D13L rifampicin resistance gene is in the entomopoxvirus of the parasitic wasp, *Diachasmimorpha longicaudata*

Lawrence, PO; Dillard, BE. 2008

The parasitic wasp, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), introduces an entomopoxvirus (DIEPV) into its Caribbean fruit fly host, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), during oviposition. DIEPV has a 250-300 kb unipartite dsDNA genome, that replicates in the cytoplasm of the host's hemocytes, and inhibits the host's encapsulation response. The putative proteins encoded by several DIEPV genes are highly homologous with those of poxviruses, while others appear to be DIEPV specific. Here, a 2.34 kb sequence containing a 1.64 kb DIEPV open reading frame within a cloned 4.5 kb EcoRI fragment (designated R1-1) is described from a DIEPV EcoRI genomic library. This open reading frame is a homolog of the vaccinia virus rifampicin resistance (rif) gene, D13L, and encodes a putative 546 amino acid protein. The DIEPV rif contains two EcoRV, two HindIII, one XbaI, and one DraIII restriction sites, and upstream of the open reading frame the fragment also contains EcoRV, HindII, SspEI, and BspI06 sites. Early poxvirus transcription termination signals (TTTTTnT) occur 236 and 315 nucleotides upstream of the consensus poxvirus late translational start codon (TAAATG) and at 169 nucleotides downstream of the translational stop codon of the rif open reading frame. Southern blot hybridization of HindIII-, EcoRI-, and BamHI-restricted DIEPV genomic DNA probed with the labeled 4.5 kb insert confirmed the fidelity of the DNA and the expected number of fragments appropriate to the restriction endonucleases used. Pairwise comparisons between DIEPV amino acids and those of the *Amsacta moorei*, *Heliothis armigera*, and *Melanoplus sanguinipes* entomopoxviruses, revealed 46, 46, and 45 % similarity (identity + substitutions), respectively. Similar values (41-45%) were observed in comparisons with the chordopoxviruses. The mid portion of the DIEPV sequence contained two regions of highest conserved residues similar to those reported for *H. armigera* entomopoxvirus rifampicin resistance protein. Phylogenetic analysis of the amino acid sequences suggested that DIEPV arose from the same ancestral node as other entomopoxviruses but belongs to a separate clade from those of the grasshopper-infecting *M. sanguinipes* entomopoxvirus and from the Lepidoptera-infecting (Genus B or Betaentomopoxvirus) *A. moorei* entomopoxvirus and *H. armigera* entomopoxvirus. Interestingly, the DIEPV putative protein had only 3-26.4 % similarity with RIF-like homologs/orthologs found in other large DNA non-poxviruses, demonstrating its closer relationship to the Poxviridae. DIEPV remains an unassigned member of the Entomopoxvirinae (<http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm>) until its relationship to other diptera-infecting (Gammaentomopoxvirus or Genus C) entomopoxviruses can be verified. The GenBank accession number for the nucleotide sequence data reported in this paper is EF541029.

Crossing experiment of *Anopheles maculatus* form K and *Anopheles willmori* (James) (Diptera : Culicidae)

Somboon, P; Thongwat, D; Morgan, K; Walton, C. 2008

We recently reported crossing experiments between *Anopheles maculatus* form K and five members of the *Maculatus* group to support the specific status of form K. In the present study, we performed further crosses between form K and a sixth species of the *Maculatus* group, *Anopheles willmori* (James). Low viability was observed in hybrid males and females. All hybrid males were sterile with atrophied testes, or partially sterile with abnormal spermatozoa. The hybrid females showed normal ovaries. The ovarian nurse cell polytene chromosomes of the F-1 hybrid females displayed complete asynapsis. Backcrossing showed low viability. All males were sterile with atrophied testes or partially sterile with abnormal spermatozoa, and the females showed varying degrees of atrophied ovaries. The results provide clear evidence that form K is not conspecific to *A. willmori* supporting previous studies that form K represents another species of the *Maculatus* group.

Detection of natural infection in *Lutzomyia cruzi* and *Lutzomyia forattinii* (Diptera : Psychodidae : Phlebotominae) by *Leishmania infantum* chagasi in an endemic area of visceral leishmaniasis in Brazil using a PCR multiplex assay

de Pita-Pereira, D; Cardoso, MAB; Alves, CR; Brazil, RP; Britto, C. 2008

In order to identify *Lutzomyia* spp. naturally infected by *Leishmania* parasites a PCR multiplex assay coupled to non-isotopic hybridization was used for the analysis of insect samples collected by CDC light traps in an endemic area of visceral leishmaniasis (VL) in the municipality of Corumba, Mato Grosso do Sul State, Brazil in May/June 2006. Wild sand flies were identified and grouped into pools of 10 female specimens and 27 groups in total were collected. Positive results were obtained from *Lutzomyia cruzi* (2 out of 13 pools) and *Lutzomyia forattinii* (1 out of 14 pools). The positive pools were confirmed as being infected by *Leishmania infantum* chagasi after hybridizing the PCR products with a species-specific biotinylated probe derived from the kinetoplast minicircle conserved sequence. Given that we detected infection in 3 out of 27 groups and that there was at least 1 infected insect in each, it was possible to infer an infection rate of 1.5% for *Lu. cruzi* and 0.7% for *Lu. forattinii* in the analyzed samples. These results confirm the vectorial role of *Lu. cruzi* in transmitting *L. infantum* chagasi and suggest *Lu. forattinii* as a potential VL vector in the municipality of Corumba, where notifications of the disease in humans and dogs have increased over the last two decades. (C) 2008 Elsevier B.V. All rights reserved.

New Attractants for Males of the Solanaceous Fruit Fly *Bactrocera latifrons*

Ishida, T; Enomoto, H; Nishida, R. 2008

alpha-Ionone, alpha-ionol, and their mixtures with phenolic volatiles act as potential male lures for the solanaceous fruit fly *Bactrocera latifrons* (Hendel). However, the attractiveness of these compounds is not as strong as that of other well-known tephritid male lures, such as methyl eugenol for *Bactrocera dorsalis*. Isophorone and isophorol, which have a partial skeletal structure of alpha-ionone/alpha-ionol (i.e., trimethylcyclohexene), were attractive to *B. latifrons* males, and their mixtures with alpha-ionol exhibited stronger activity than any of the individual compounds. We also tested 3-oxo-alpha-ionone, 3-oxo-alpha-ionol, 3-hydroxy-alpha-ionone, and 3-hydroxy-alpha-ionol, hybrid compounds between isophorone/isophorol and alpha-ionone/alpha-ionol. 3-Oxo-alpha-ionone and 3-oxo-alpha-ionol were active both as attractants and phagostimulants for males. The results suggest that the introduction of an oxygen atom at the 3-position of the alpha-ionone/alpha-ionol molecule optimizes the specific chemosensory responses in *B. latifrons* males.

Molecular and cytogenetic evidence of three sibling species of the *Anopheles barbirostris* Form A (Diptera : Culicidae) in Thailand

Saeung, A; Baimai, V; Otsuka, Y; Rattanarithikul, R; Somboon, P; Junkum, A; Tuetun, B; Takaoka, H; Choochote, W. 2008

Nine isoline colonies of *Anopheles barbirostris* Form A, derived from individual isofemale lines from Chiang Mai, Phetchaburi, and Kanchanaburi, were established in our insectary at Chiang Mai University. All isolines shared the same mitotic karyotype (X-1, X-2, Y-1). Molecular analysis of deoxyribonucleic acid (DNA) sequences and polymerase chain reaction (PCR) products of ITS2, COI, and COII regions revealed three distinct groups: A1 (Chiang Mai), A2 (Phetchaburi), and A3 (Kanchanaburi). Crossing experiments among the three groups exhibited strong reproductive isolation, producing low and/or non-hatched eggs, and inviable and/or abnormal development of the reproductive system of F-1-progenies. Asynaptic regions along the five polytene chromosome arms of F-1-hybrid larvae clearly supported the existence of three sibling species within *A. barbirostris* Form A, provisionally named species A1, A2, and A3.

New Distribution Record and Behavioral Observations for *Pseudacteon curvatus* (Diptera: Phoridae), a Parasitoid of Imported Fire Ants and Their Hybrid (Hymenoptera: Formicidae)

Vogt, JT; Rashid, T; Chen, J. 2008

Host-seeking heights, host-seeking activity patterns, and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera : Culicidae)

Savage, HM; Anderson, M; Gordon, E; Mcmillen, L; Colton, L; Delorey, M; Sutherland, G; Aspen, S; Charnetzky, D; Burkhalter, K; Godsey, M. 2008

Host-seeking heights, host-seeking activity patterns, and West Nile virus (family Flaviviridae, genus *Flavivirus*, WNV) infection rates were assessed for members of the *Culex pipiens* complex from July to December 2002, by using chicken-baited can traps (CT) at four ecologically different sites in Shelby County, TN. Host-seeking height was assessed by CT placed at elevations of 3.1, 4.6, and 7.6 m during one 24-h period per month. Host-seeking activity was assessed by paired CT placed at an elevation of 4.6 m. Can traps were sampled at one 10-h daytime interval and at seven 2-h intervals during the evening, night, and morning. *Cx. pipiens* complex mosquitoes accounted for 87.1% of collected mosquitoes. *Culex (Melanoconion) erraticus* (Dyar & Knab) accounted for 11.9% of specimens. The average number of *Cx. pipiens* complex mosquitoes collected per 24-h CT period from July to September was lowest at a rural middle income site (1.7), intermediate at an urban middle income site (11.3), and highest at an urban low income site (47.4). Can traps at the forested site failed to collect *Cx. pipiens* complex mosquitoes. From July to September at urban sites, *Culex pipiens pipiens* L. was the rarest of the three complex members accounting for 11.1-25.6% of specimens. At the rural site, *Culex pipiens quinquefasciatus* Say was the rarest member of the complex. *Cx. p. pipiens* was not collected after September. Mean abundance of *Cx. pipiens* complex mosquitoes was higher in traps at 7.6 m than in traps at 4.6 m. Abundances at 3.1 m were intermediate and not significantly different from abundances at the other heights. Initiation of host-seeking activity was associated with the end of civil twilight and activity occurred over an extended nighttime period lasting 8-10 h. All 11 WNV-positive mosquitoes were *Cx. pipiens* complex mosquitoes collected from urban sites in traps placed at elevations of 4.6 and 7.6 m. Infection rates were marginally nonsignificant by height. Infection rates, host-seeking heights, and activity patterns were not significantly different among members of the *Cx. pipiens* complex.

Microsatellite Characterization of Subspecies and Their Hybrids in *Culex pipiens* Complex (Diptera: Culicidae) Mosquitoes Along a North-South Transect in the Central United States

Kothera, L; Zimmerman, EM; Richards, CM; Savage, HM. 2009

Mosquitoes in the *Culex pipiens* complex, *Cx. p. pipiens* L. and *Cx. p. quinquefasciatus* Say are morphologically similar and important vectors of West Nile and St. Louis Encephalitis viruses in the United States. The subspecies differ with respect to overwintering strategies, with *Cx. p. pipiens* entering diapause in response to winter conditions and *Cx. p. quinquefasciatus* lacking this ability, yet they hybridize when found in sympatry. Specimens (n = 646) were collected using gravid traps set along a transect from New Orleans, LA, to Chicago, IL. Microsatellite markers were used to: genetically characterize subspecies and hybrids, determine the degree and extent of hybridization to better define the hybrid zone, and examine the efficacy of hybrid detection between microsatellites and a single-gene assay based on the acetylcholinesterase 2 gene (HotAce.2). The results support the presence of two distinct genetic entities, with a broad, stable hybrid zone in between. Admixture analyses classified >40% of individuals as hybrids. Allelic richness was markedly different at the northern and southern ends of the transect, and there was a significant isolation by distance effect. The hybrid zone seems to be wider and extends further to the south than previous work indicated, and as a result, we propose new boundaries compared with those indicated by a previous study. Microsatellites detected more hybrids than the HotAce.2 assay, although the latter assay

remains useful as a screening tool. We suggest that the parental subspecies and the hybrid zone are maintained by heterosis combined with selection for diapause at northern latitudes.

Septic injury-inducible genes in medicinal maggots of the green blow fly *Lucilia sericata*

Altincicek, B; Vilcinskas, A. 2009

Lucilia sericata maggots are used world-wide in biosurgery for the medical treatment of nonhealing wounds because they ingest necrotic tissues and significantly promote healing. To gain further insight into interdependencies between ecological adaptation and molecular evolution of innate immunity in Diptera, we used the suppression subtractive hybridization method to screen for genes that are differentially expressed in response to septic wounding of sterile second instar larvae of *L. sericata*. This approach resulted in the identification of 65 novel *Lucilia* genes including potential signalling proteins (e.g. inhibitor of apoptosis 2 protein) and a number of digestive enzymes including lipases and proteinases. Additionally, we found numerous putative antimicrobial peptides (AMPs), such as a potential *Lucilia* defensin, dipteracin and three novel proline-rich AMPs. The identified genes may facilitate access to both peptides and proteins within the beneficial excretions, secretions and haemolymph of medicinal maggots and provide novel insights into the evolution of innate immunity in Diptera.

Flies in the ointment: a morphological and molecular comparison of *Lucilia cuprina* and *Lucilia sericata* (Diptera: Calliphoridae) in South Africa

Tourle, R; Downie, DA; Villet, MH. 2009

Complementary nuclear (28S rRNA) and mitochondrial (COI) genes were sequenced from blowflies that phenotypically resembled *Lucilia cuprina* (W.), *Lucilia sericata* (Meigen) or exhibited characters of both species. The aim was to test a long-held hypothesis that these species hybridize under natural conditions in South Africa (Ullyett, 1945). Blowflies were obtained predominantly from the Cape Town metropolitan area, but reference samples were acquired for *L. sericata* from Pretoria. Several *L. cuprina*-like flies were shown to possess a conflicting combination of nuclear and mitochondrial genes that has also been seen in Hawaiian specimens. Homoplasmy, sampling of pseudogenes, hybridization and incomplete lineage sorting are discussed as possible hypotheses for the pattern and the latter is concluded to represent the most likely explanation.

Asymmetric reproductive isolation between *Lutzomyia pseudolongipalpis* and *Lutzomyia longipalpis* (species C2), Neotropical vectors of visceral leishmaniasis (Diptera: Phlebotomidae).

Arrivillaga, J; Salerno, P; Rangel, Y. 2009

Asymmetric reproductive isolation between *Lutzomyia pseudolongipalpis* and *Lutzomyia longipalpis* (species C2), Neotropical vectors of visceral leishmaniasis (Diptera: Phlebotomidae). *Lutzomyia pseudolongipalpis* and *Lutzomyia longipalpis* (species C2, *L. sp. n.*) are two endemic species of Phlebotominae sand fly vectors from Venezuela. The two insects are sympatric and monophyletic but have deeply diverging, morphological differences. They belong to the *L. longipalpis* complex. A study of their reproductive isolation is necessary to understand the process of speciation and maintenance of the two sister species as two discrete taxonomic and biological entities. Cross-mating tests were conducted (homo and hetero-specific) and monitored under two criteria: biological (presence of copulation and offspring) and genetic (using two isozymic markers diagnostic for the *L. longipalpis* complex; enzyme diagnosis AK and HK). Results indicate reproductive isolation, with an asymmetrical genetic exchange in a direction of hybridization between the two species under experimental conditions, and production of a low number of heterozygotes. These findings support the existence of negative selection on hybrids, and explain the absence of hybrids under natural conditions, in the sympatric locality. Rev. Biol. Trop. 57 (1-2): 23-31, Epub 2009 June 30.

Incipient speciation revealed in *Anastrepha fraterculus* (Diptera; Tephritidae) by studies on mating compatibility, sex pheromones, hybridization, and cytology

Caceres, C; Segura, DF; Vera, MT; Wornoayporn, V; Cladera, JL; Teal, P; Sapountzis, P; Bourtzis, K; Zacharopoulou, A; Robinson, AS. 2009

It has long been proposed that the nominal species *Anastrepha fraterculus* is a species complex and earlier studies showed high levels of pre-zygotic isolation between two laboratory strains from Argentina and Peru. Further experiments were carried out on the same populations and on their reciprocal hybrids, including pre- and post-zygotic isolation studies, pheromone analysis, and mitotic and polytene chromosome analysis. A high level of pre-zygotic isolation had been maintained between the parental strains despite 3 years of laboratory rearing under identical conditions. The level of pre-zygotic isolation was reduced in matings with hybrids. There were also differences in other components of mating behaviour. There were quantitative and qualitative differences in the sex pheromone of the two strains with the hybrids producing a mixture. The pre-zygotic isolation barriers were complemented by high levels of post-zygotic inviability and sex ratio distortion, most likely not due to Wolbachia, although there was evidence of some cytoplasmic factor involved in sex ratio distortion. Analysis of polytene chromosomes revealed a high level of asynapsis in the hybrids, together with karyotypic differences between the parental strains. The combined results of the present study indicate that these two strains belong to different biological entities within the proposed *A. fraterculus* complex. (C) 2009 The Linnean Society of London, Biological Journal of the Linnean Society, 2009, 97, 152-165.

Alcohol dehydrogenase activities and ethanol tolerance in *Anastrepha* (Diptera, Tephritidae) fruit-fly species and their hybrids

Carvalho, E; Solferini, VN; Matioli, SR. 2009

The ADH (alcohol dehydrogenase) system is one of the earliest known models of molecular evolution, and is still the most studied in *Drosophila*. Herein, we studied this model in the genus *Anastrepha* (Diptera, Tephritidae). Due to the remarkable advantages it presents, it is possible to cross species with different Adh genotypes and with different phenotype traits related to ethanol tolerance. The two species studied here each have a different number of Adh gene copies, whereby crosses generate polymorphisms in gene number and in composition of the genetic background. We measured certain traits related to ethanol metabolism and tolerance. ADH specific enzyme activity presented gene by environment interactions, and the larval protein content showed an additive pattern of inheritance, whilst ADH enzyme activity per larva presented a complex behavior that may be explained by epistatic effects. Regression models suggest that there are heritable factors acting on ethanol tolerance, which may be related to enzymatic activity of the ADHs and to larval mass, although a pronounced environmental effect on ethanol tolerance was also observed. By using these data, we speculated on the mechanisms of ethanol tolerance and its inheritance as well as of associated traits.

Asymmetric introgression between sympatric *molestus* and *pipiens* forms of *Culex pipiens* (Diptera: Culicidae) in the Comporta region, Portugal

Gomes, B; Sousa, CA; Novo, MT; Freitas, FB; Alves, R; Corte-Real, AR; Salgueiro, P; Donnelly, MJ; Almeida, APG; Pinto, J. 2009

Background: *Culex pipiens* L. is the most widespread mosquito vector in temperate regions. This species consists of two forms, denoted *molestus* and *pipiens*, that exhibit important behavioural and physiological differences. The evolutionary relationships and taxonomic status of these forms remain unclear. In northern European

latitudes molestus and pipiens populations occupy different habitats (underground vs. aboveground), a separation that most likely promotes genetic isolation between forms. However, the same does not hold in southern Europe where both forms occur aboveground in sympatry. In these southern habitats, the extent of hybridisation and its impact on the extent of genetic divergence between forms under sympatric conditions has not been clarified. For this purpose, we have used phenotypic and genetic data to characterise *Cx. pipiens* collected aboveground in Portugal. Our aims were to determine levels of genetic differentiation and the degree of hybridisation between forms occurring in sympatry, and to relate these with both evolutionary and epidemiological tenets of this biological group. Results: Autogeny and stenogamy was evaluated in the F1 progeny of 145 individual *Cx. pipiens* females. Bayesian clustering analysis based on the genotypes of 13 microsatellites revealed two distinct genetic clusters that were highly correlated with the alternative traits that define *pipiens* and *molestus*. Admixture analysis yielded hybrid rate estimates of 8-10%. Higher proportions of admixture were observed in *pipiens* individuals suggesting that more *molestus* genes are being introgressed into the *pipiens* form than the opposite. Conclusion: Both physiological/behavioural and genetic data provide evidence for the sympatric occurrence of *molestus* and *pipiens* forms of *Cx. pipiens* in the study area. In spite of the significant genetic differentiation between forms, hybridisation occurs at considerable levels. The observed pattern of asymmetric introgression probably relates to the different mating strategies adopted by each form. Furthermore, the differential introgression of *molestus* genes into the *pipiens* form may induce a more opportunistic biting behaviour in the latter thus potentiating its capacity to act as a bridge-vector for the transmission of arboviral infections.

Natural infection of *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae, Phlebotominae) by *Leishmania* (Viannia) spp. in Brazil

Marcondes, CB; Bittencourt, IA; Stoco, PH; Eger, I; Grisard, EC; Steindel, M. 2009

A study of the natural infection of phlebotomine sand flies by *Leishmania* (Viannia) was conducted in a focus of cutaneous leishmaniasis in Pi arras, on the northeastern coast of the Brazilian state of Santa Catarina. In total, 562 female *Nyssomyia neivai* were collected by miniature light traps near houses, separated in 61 pools and examined by PCR and Southern blot hybridization. Eight pools, four of them from the same light trap/night, were positive. This is the first finding of natural infection by *Le. braziliensis* of adequately identified *Ny. neivai* in Brazil. In this preliminary observation we observed the abundance and predominance of *Ny. neivai* among the captured phlebotomine species (98.5%), indicating that *Ny. neivai* may be the dominant vector of *Leishmania* in the subgenus Viannia in this area. (C) 2008 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.

Evolutionary Differentiation of Fitness Traits Across Multiple Geographic Scales in *Aedes albopictus* (Diptera: Culicidae)

O'Donnell, D; Armbruster, P. 2009

Aedes albopictus (Skuse) (Diptera: Culicidae) is an invasive, container-inhabiting mosquito that was introduced into North America from Japan in 1985. Although previous studies using neutral biochemical markers have found high levels of population differentiation across both the North American and global distribution of *Ae. albopictus*, relatively little is known regarding evolutionary differentiation of life history traits underpinning fitness. We performed common-garden and line-cross experiments to examine the genetic differentiation of fitness (r^2) and underlying performance traits at three spatial scales: 1) local (among populations within Florida), 2) regional (between populations from Florida and New Jersey), and 3) long distance (between populations from Florida and Hawaii, Malaysia, and Japan). We found that North American populations (Florida and New Jersey) had lower fitness (r^2) than populations from outside North America (Hawaii, Malaysia, and Japan). Bivariate means of female, but not male, pupal mass and developmental time differed among regions (Florida, New Jersey, outside North America). Larval survivorship did not differ among regions nor among populations within regions, whereas size-specific fecundity varied among populations within regions but not among regions. Population crosses resulted in significant heterosis and outbreeding depression of F₁ hybrids but the results of crossing did not depend on the geographic distance separating parental populations in a consistent manner. Together, our results imply a role for local genetic drift affecting the life-history differentiation of *Ae. albopictus* populations. These conclusions corroborate previous studies of population structure based on neutral biochemical markers.

The species delimitation problem in the *Simulium damnosum* complex, blackfly vectors of onchocerciasis

Morales-Hojas, R; Krueger, A. 2009

The *Simulium damnosum* Theobald complex (Diptera: Simuliidae) comprises 57 cytoforms grouped into six subcomplexes. Previous phylogenetic studies using gene sequences have not completely resolved the evolutionary relationships of the cytoforms. The present study investigated the systematics of the complex using a phylogeographic approach. The differentiation between eastern and western forms observed in the phylogenetic studies is confirmed in the estimated haplotype networks. However, haplotypes tend to group in geographical clades and not according to cytoforms. Spatial analyses of the molecular variance also resulted in optimal groupings of sequences that did not correspond to cytoform boundaries. Moreover, Mantel tests showed significant correlations, although not strong, between genetic and geographical distances. This suggests an isolation-by-distance model of differentiation. Furthermore, there are instances in which genetic differentiation between cytoforms is low and not significant. These results indicate a lack of clear genetic differentiation between the cytoforms, which may be explained either by a separation of the taxa recent enough to allow the accumulation of few genetic differences or by recombination between the genomes of the cytoforms, which may be the result of hybridization with introgression or of non-independent evolutionary lineages. The results also emphasize the need for further sampling and for the use of more variable markers in order to clarify the evolutionary history of the group.

Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae)

Arthofer, W; Riegler, M; Schneider, D; Krammer, M; Miller, WJ; Stauffer, C. 2009

The European cherry fruit fly *Rhagoletis cerasi* has been a field model for cytoplasmic incompatibility since the mid 1970s. Two *Wolbachia* strains were detected in this tephritid species and wCer2 was described as the CI inducing agent dividing European populations into two unidirectional incompatible groups, i.e. southern females produce viable offspring with northern males, whereas the reciprocal cross results in incompatibility. We detected three new *Wolbachia* strains by sequencing a multitude of plasmids derived from *Wolbachia* surface protein gene (wsp) polymerase chain reaction (PCR) products. Strain-specific primers were developed allowing individual diagnosis without need for cloning. Hybridization of specific PCR products with a wsp oligonucleotide enhanced the detection limit significantly and revealed the presence of low-titre infections in some strains, in different ontogenetic stages and in adults of different age. We then performed a survey of strain prevalence and infection frequency in eight European regions. wCer1 was fixed in all populations, whereas wCer2 was detected only in the South. wCer3 frequency was the lowest without a clear distribution pattern. The abundance of wCer4 was homogenous across Europe. Like wCer2, wCer5 showed significant differences in spatial distribution. Our new findings of previously undetected and recombinant *Wolbachia* strains in *R. cerasi* reveal a major caveat to the research community not to overlook hidden *Wolbachia* diversity in field populations. Low-titres and geographical variability in *Wolbachia* diversity are expected to influence the outcome of *Wolbachia* population dynamics and *Wolbachia*-based insect population control and may create invasion barriers for expanding and artificially introduced *Wolbachia* strains.

Sensitivity of *Aedes aegypti* adults (Diptera: Culicidae) to the vapors of *Eucalyptus* essential oils

Lucia, A; Licastro, S; Zerba, E; Audino, PG; Masuh, H. 2009

Vapors of essential oils extracted from various species of *Eucalyptus* (*E. gunnii*, *E. tereticornis*, *E. grandis*, *E. camaldulensis*, *E. dunni*, *E. cinerea*, *E. saligna*, *E. sideroxylon*, *E. globulus* ssp. *globulus*, *E. globulus* ssp. *maidenii*, *E. viminalis* and the hybrids *E. grandis* x *E. tereticornis* and *E. grandis* x *E. camaldulensis*) and their major components were found to be toxic to *Aedes aegypti* adults, the yellow fever mosquito. An aliquot of each oil was placed in a cylindrical test chamber and the number of knocked-down mosquitoes was recorded as function of time. Knockdown time 50% was then calculated. Results showed that *E. viminalis* had the fastest knockdown time at of 4.2 min, on the same order as dichlorvos, a standard knockdown agent. A correlation was observed between the content of 1,8-cineole in the *Eucalyptus* essential oils and the corresponding toxic effect. The correlation between KT50 values and calculated vapor pressures of the essential oil components showed that the fumigant activity of simple organic compounds in insects is correlated with their volatility. (C) 2009 Elsevier Ltd. All rights reserved.

Cytogenetic and molecular evidence for an additional new species within the taxon *Anopheles barbirostris* (Diptera: Culicidae) in Thailand

Suwannamit, S; Baimai, V; Otsuka, Y; Saeung, A; Thongsahuan, S; Tuetun, B; Apiwathnasorn, C; Jariyapan, N; Somboon, P; Takaoka, H; Choochote, W. 2009

ITS2 DNA sequences of 42 isoline colonies of *Anopheles barbirostris* species A1 and A2 were analyzed and a new genetic species, temporarily designated as species A4 (Chiang Mai), was revealed. The large sequence divergences of the ITS2 (0.116-0.615), COI (0.023-0.048), and COII (0.030-0.040) genes between *A. barbirostris* species A4/A1 (Chiang Mai), A4/A2 (Phetchaburi), A4/A3 (Kanchanaburi), and A4/*Anopheles campestris*-like Form E (Chiang Mai) provided good supporting evidence. Species A1, A2, A3, and A4 share a mitotic karyotype of Form A (X-1, X-2, Y-1). Crossing experiments between species A4 and the other four species yielded strong reproductive isolation producing few and/or non-hatched eggs and inviable and/or abnormal development of the reproductive system of F-1 progenies. Moreover, available F-1 hybrid larvae showed asynaptic polytene chromosome arms. Hence, molecular and cytogenetic evidence strongly support the existence of *A. barbirostris* species A4, which is more closely related to *A. campestris*-like Form E than to species A1, A2, and A3. Additionally, crossing experiments among 12 and seven isolines of different cytological forms of species A1 (A, B, C, D) and A2 (A, B), respectively, yielded fertile and viable F-1 progenies. Thus, different karyotypic forms occurring in natural populations of species A1 and A2 merely represent intraspecies variation of sex chromosomes due to the extra blocks of heterochromatin.

Differential gene expression from midguts of refractory and susceptible lines of the mosquito, *Aedes aegypti*, infected with Dengue-2 virus

Baron, OL; Ursic-Bedoya, RJ; Lowenberger, CA; Ocampo, CB. 2010

Suppressive subtractive hybridization was used to evaluate the differential expression of midgut genes of feral populations of *Aedes aegypti* (Diptera: Culicidae) from Colombia that are naturally refractory or susceptible to Dengue-2 virus infection. A total of 165 differentially expressed sequence tags (ESTs) were identified in the subtracted libraries. The analysis showed a higher number of differentially expressed genes in the susceptible *Ae. aegypti* individuals than the refractory mosquitoes. The functional annotation of ESTs revealed a broad response in the susceptible library that included immune molecules, metabolic molecules and transcription factors. In the refractory strain, there was the presence of a trypsin inhibitor gene, which could play a role in the infection. These results serve as a template for more detailed studies aiming to characterize the genetic components of refractoriness, which in turn can be used to devise new approaches to combat transmission of dengue fever.

Comparative Genomic Hybridization (CGH) Reveals a Neo-X Chromosome and Biased Gene Movement in Stalk-Eyed Flies (Genus *Teleopsis*)

Baker, RH; Wilkinson, GS. 2010

Chromosomal location has a significant effect on the evolutionary dynamics of genes involved in sexual dimorphism, impacting both the pattern of sex-specific gene expression and the rate of duplication and protein evolution for these genes. For nearly all non-model organisms, however, knowledge of chromosomal gene content is minimal and difficult to obtain on a genomic scale. In this study, we utilized Comparative Genomic Hybridization (CGH), using probes designed from EST sequence, to identify genes located on the X chromosome of four species in the stalk-eyed fly genus *Teleopsis*. Analysis of log(2) ratio values of female-to-male hybridization intensities from the CGH microarrays for over 3,400 genes reveals a strongly bimodal distribution that clearly differentiates autosomal from X-linked genes for all four species. Genotyping of 33 and linkage mapping of 28 of these genes in *Teleopsis dalmanni* indicate the CGH results correctly identified chromosomal location in all cases. Syntenic comparison with *Drosophila* indicates that 90% of the X-linked genes in *Teleopsis* are homologous to genes located on chromosome 2L in *Drosophila melanogaster*, suggesting the formation of a nearly complete neo-X chromosome from Muller element B in the dipteran lineage leading to *Teleopsis*. Analysis of gene movement both relative to *Drosophila* and within *Teleopsis* indicates that gene movement is significantly associated with 1) rates of protein evolution, 2) the pattern of gene duplication, and 3) the evolution of eyespan sexual dimorphism. Overall, this study reveals that diopsids are a critical group for understanding the evolution of sex chromosomes within Diptera. In addition, we demonstrate that CGH is a useful technique for identifying chromosomal sex-linkage and should be applicable to other organisms with EST or partial genomic information.

Good species behaving badly: Non-monophyly of black fly sibling species in the *Simulium arcticum* complex (Diptera: Simuliidae)

Conflitti, IM; Kratochvil, MJ; Spironello, M; Shields, GF; Currie, DC. 2010

Mitochondrial based phylogenetic reconstructions often show deviations from species-level monophyly. We used the *Simulium arcticum* species complex (Diptera: Simuliidae) as a model system for interpreting non-monophyly in light of chromosomal data supporting species status of siblings. For cytogenetic identification of morphologically indistinguishable black fly sibling species, larvae must be preserved in Carnoy's solution, a fixative known to degrade DNA. Consequently, we reconstructed phylogenetic relationships based on 12S, COII, cyt b, and ITS-1 gene sequences obtained from larvae sampled from presumed taxon-pure localities. As species composition at 'taxon-pure' sites may have changed at the time of sampling, we performed a second study that aimed to: (1) assess phylogenetic relationships among cytologically verified members of the *S. arcticum* species complex using COI and COII gene sequences; (2) determine whether useable genetic information could be gleaned from Carnoy's fixed specimens; and (3) determine the extent to which Carnoy's fixative degrades DNA over time. We consistently obtained genetic data from material stored in Carnoy's solution for two to three months. Genetic analysis of samples fixed in Carnoy's solution for up to six years indicates that larvae preserved for a maximum of five years can provide useable information for molecular analysis. Our preliminary and cytologically confirmed phylogenetic analyses demonstrate that mitochondrial DNA fails to resolve species-level monophyly of chromosomally distinct *S. arcticum* taxa. As results of analyses based on cytologically verified larvae mirror those of our preliminary study, we rule out imperfect taxonomy as the reason for species-level non-monophyly. Although we cannot confidently reject either inadequate phylogenetic information or incomplete lineage sorting as the cause of non-monophyly, the sharing of alleles between sympatric siblings suggests introgressive hybridization between taxa. We conclude that the patterns present in the *S. arcticum* phylogeny likely represent the initial stages of chromosome based sibling speciation. (C) 2010 Elsevier Inc. All rights reserved.

Host use and developmental instability in the cactophilic sibling species *Drosophila gouveai* and *D. antonietae*

Soto, IM; Carreira, VP; Corio, C; Soto, EM; Hasson, E. 2010

P>The *Drosophila* repleta group encompasses an ensemble of species that inhabit desertic areas that are inhospitable to other drosophilids. These species have a tractable ecology, as they breed and feed on necrotic tissues of a wide diversity of species of Cactaceae, with a certain degree of host specificity, which makes them suitable models to investigate the role of host plant shifts in diversification. Most species have their own primary host plant, which may be shared with a closely

related species. However, the consequences of host plant shifts from primary to secondary hosts have not been thoroughly studied so far. We investigated the effects of the cactus host on developmental instability and performance of *D. gouveai* Tidon-Sklorz & Sene and *D. antonietae* Tidon-Sklorz & Sene (Diptera: Drosophilidae), a pair of closely related sibling species, and of their F1 interspecific hybrids reared in primary and secondary host plants. Fluctuating asymmetry (FA) for wing size was significantly greater in flies of both species reared in their respective secondary cactus host than in those grown in the primary host. Interspecific hybrids also exhibited host-dependent levels of FA. However, hybrids did not show greater FA than the parental species, suggesting that hybridization per se did not affect developmental stability. Even though cactus host shifts affected different measures of fitness (larval viability and adult size) both in *D. antonietae* and *D. gouveai*, we did not detect a consistent relationship between FA and fitness. Our results suggest that FA cannot be used as a sensitive indicator of genetic and phenotypic quality and we discuss its application as a fitness predictor.

mtDNA-based identification of *Lucilia cuprina* (Wiedemann) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) in the continental United States

DeBry, RW; Timm, AE; Dahlem, GA; Stamper, T. 2010

Existing data suggest that the forensically important dipteran species *Lucilia cuprina* (Wiedemann) and *Lucilia sericata* (Meigen) may be particularly difficult to discriminate using DNA sequence data. *L. cuprina* is paraphyletic with respect to *L. sericata* in mtDNA phylogenies, with some *L. cuprina* having mtDNA haplotypes that are very similar to those of *L. sericata*. We examine this problem by providing the first DNA data for *L. cuprina* from North America, including portions of both the mitochondrial COI gene and the nuclear 28S rRNA gene. With the new data, *L. cuprina* remains monophyletic for 28S but paraphyletic with respect to *L. sericata* for COI. However, we find that all flies that are identified as *L. cuprina* by morphology and have *L. sericata*-like mtDNA form a distinctly monophyletic mtDNA clade. This clade may possibly have originated by hybridization between *L. cuprina* and *L. sericata*, but its wide geographic distribution strongly suggests a singular origin as opposed to repeated incidents of hybridization. The phylogenetic results strongly support the hypothesis that *L. cuprina* and *L. sericata* can be discriminated using mtDNA sequence data. We find that a fragment of COI spanning approximately 1200 base pairs is sufficient to discriminate between the two species with greater than 95% bootstrap support. (C) 2010 Elsevier Ireland Ltd. All rights reserved.

Natural *Leishmania infantum* infection in *Migonemyia migonei* (Franca, 1920) (Diptera:Psychodidae:Phlebotominae) the putative vector of visceral leishmaniasis in Pernambuco State, Brazil

de Carvalho, MR; Valenca, HF; da Silva, FJ; de Pita-Pereira, D; Pereira, TD; Britto, C; Brazil, RP; Brandao, SP. 2010

A study of the natural infection of phlebotomine sand flies by *Leishmania* (*Leishmania*) *infantum* was conducted in an area of visceral leishmaniasis in Sao Vicente Ferrer, located in the northern part of the Atlantic rain forest region in the State of Pernambuco, Brazil. In a previous study, *Migonemyia migonei* have been found predominantly in peridomestic houses in this endemic area. The analysis of *M. migonei*, collected by CDC light trap, by multiplex PCR assay coupled to non-isotopic hybridization showed that 2 females out of 50 were infected by *L. infantum*. This is the first finding of natural infection of *M. migonei* by *L. infantum* suggesting that *M. migonei* may be the vector of *L. infantum* in areas of visceral leishmaniasis where *Lutzomyia longipalpis*, the usual vector, is absent. (C) 2010 Elsevier B.V. All rights reserved.

Evolution of intrinsic reproductive isolation among four North American populations of *Rhagoletis pomonella* (Diptera: Tephritidae)

Rull, J; Aluja, M; Feder, JL. 2010

Across its range in North America, four geographically separated, ecologically and genetically diverged populations of hawthorn (*Crataegus*)-infesting *Rhagoletis pomonella* (Diptera: Tephritidae) flies inhabit the Eje Volcanico Trans Mexicano (EVTM), the Sierra Madre Oriental (SMO), the Chiapas Highlands (CHIS) and the USA. Here, we tested whether these four populations are reproductively isolated by any intrinsic, nonhost-related, pre- or postmating barriers to gene flow. Crossing experiments suggested that a low level of host-independent prezygotic isolation may exist between hawthorn flies from EVTM and the three other populations, but only with respect to a slight reduction in copulation duration in EVTM matings. Some evidence for postmating isolation was found, again primarily involving EVTM crossed to SMO, CHIS and US flies. Certain crosses produced no (SMO male x EVTM female) or few (EVTM male x CHIS female; CHIS male x SMO female) F1 hybrid offspring. F2 crosses were generally fertile, except for US male x CHIS female matings. Inherent reproductive isolation therefore appears to be quantitative rather than absolute between populations, as the possibility for gene flow exists through at least some combinations of mating among EVTM, SMO, CHIS and US flies. Our results are consistent with a recently advanced hypothesis that episodic introgression from Mexico into the USA has played a role in providing genetic variation, facilitating sympatric host race formation and the adaptive radiation of the *R. pomonella* sibling species' complex in the USA. (C) 2010 The Linnean Society of London, Biological Journal of the Linnean Society, 2010, 100, 213-223.

Construction and characterisation of a BAC library made from field specimens of the onchocerciasis vector *Simulium squamosum* (Diptera: Simuliidae)

Crainey, JL; Hurst, J; Wilson, MD; Hall, A; Post, RJ. 2010

A Bacterial Artificial Chromosome (BAC) library was made from wild-caught *Simulium squamosum*, which is an important vector of human onchocerciasis. The library is composed of 12,288 BACs, with an average insert size of 128 kb, and is expected to contain similar to 1.54 GB of cloned DNA. Random BAC-end sequencing generated over 95 kb of DNA sequence data from which putative *S. squamosum* gene sequences and novel repetitive DNA families were identified, including DNA transposons, retrotransposons and simple sequence repeats (SSRs). The sequence survey also provided evidence of DNA of microbial origin, and dissection of sample blackflies indicated that some of those used to prepare the library were likely to be parasitized by the mermithid *Isomeris lairdi*. Hybridisations with a set of three independent blackfly single-copy genes and two *Wolbachia* genes suggest that the library provides around 13-fold coverage of the *S. squamosum* genome and about 12-fold coverage of its *Wolbachia* endosymbiont. Crown Copyright (C) 2010 Published by Elsevier Inc. All rights reserved.

Taxonomic boundaries, phylogenetic relationships and biogeography of the *Drosophila willistoni* subgroup (Diptera: Drosophilidae)

Robe, LJ; Cordeiro, J; Loreto, ELS; Valente, VLS. 2010

The *Drosophila willistoni* subgroup represents a complex with varying taxonomic levels. It encompasses *D. willistoni* and its five sibling species: *D. equinoxialis*, *D. insularis*, *D. paulistorum*, *D. pavlovskiana* and *D. tropicalis*. Of these, *D. equinoxialis*, *D. tropicalis* and *D. willistoni* present differentiation at subspecific level, whereas *D. paulistorum* represents a superspecies, formed by six semispecies. Despite this taxonomic and evolutionary complexity, many of these semi and subspecific taxa have not yet had their phylogenetic status tested in an explicitly molecular study. Aiming to contribute to the understanding of the evolution of this challenging group, we analyzed nucleotide sequences from two mitochondrial and four nuclear datasets, both individually and simultaneously, through different phylogenetic methods. High levels of incongruence were detected among partitions, especially concerning the mitochondrial sequences. As this incongruence was found to be statistically significant and robust to the use of different models and approaches, and basically restricted to mitochondrial loci, we suggest that it may stem mainly from hybridization-mediated asymmetrical introgression. Despite this, our nuclear data finally led to a phylogenetic hypothesis which further refines several aspects related to the *willistoni* subgroup phylogeny. In this respect, *D. insularis*, *D. tropicalis*, *D. willistoni* and *D. equinoxialis* successively branched off from the *willistoni* subgroup main stem, which recently subdivided to produce *D. paulistorum* and *D. pavlovskiana*. As regards the semispecies evolution, we found evidence of a recent

diversification, which highly influenced the obtained results due to the associated small levels of genetic differentiation, further worsened by the possibly associated incompletely sorted ancestral polymorphisms and by the possibility of introgression. This study also raises the question of whether these semispecies are monophyletic at all. This reasoning is particularly interesting when one considers that similar levels of reproductive isolation could be attained through infection with different *Wolbachia* strains.

Phlebotomines (Diptera, Psychodidae) in the Speleological Province of the Ribeira Valley: 3. Serra district - area of hostels for tourists who visit the Parque Estadual do Alto Ribeira (PETAR), state of Sao Paulo, Brazil

Galati, EAB; Marassa, AM; Fonseca, MB; Goncalves-Andrade, RM; Consales, CA; Bueno, EFM. 2010

Phlebotomines (Diptera, Psychodidae) in the Speleological Province of the Ribeira Valley: 3. Area of hostels for tourists who visit the Parque Estadual (10 Alto Ribeira (PE-FAR), state of Sao Paulo, Brazil. The study characterizes some ecological aspects of the phlebotomine fauna in an endemic area of cutaneous leishmaniasis (CL) situated in the Serra district, Iporanga municipality where the hostels for tourists visiting the PETAR are located. Captures were undertaken on a smallholding and a small farm situated near the hostels, monthly between January/2001 and December/2003 with automatic light traps (ALT) in pigsty, henhouse and veranda of a domicile at the two sites, and in peridomestic of the small farm also with black/white Shannon traps. With the ALT a total of 87,224 phlebotomines representing 19 species and also two hybrids of *Nyssomyia intermedia* (Lutz & Neiva) and *Nyssomyia neivai* (Pinto) and two anomalous specimens were captured. The standardized index species abundance was for *Ny. intermedia* = 1.0 and *Ny. neivai* = 0.935. The highest frequencies of the smallholding occurred in the pigsty, the Williams' mean/capture for *Ny. intermedia* being 63.7 specimens and for *Ny. neivai* 29.2, and on the small farm, in the hen-house, *Ny. intermedia* 402.6 and *Ny. neivai* 116.2. A total of 863 phlebotomines (*Ny. intermedia*: 75.4%; *Ny. neivai*: 24.3%) were captured with black/white Shannon traps; females of both species being predominant in the white trap. The high frequencies of *Ny. intermedia* and *Ny. neivai*, both implicated in CL transmission, indicate the areas presenting risk of the disease.

Performance of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Fruits: Comparison of Two Laboratory Populations

Joachim-Bravo, IS; Guimaraes, AN; Magalhaes, TC; Nascimento, AS. 2010

This study evaluated the influence of two fruits hosts (orange and papaya) on biological and behavioral parameters of two populations of *Ceratitis capitata* (Wiedemann) reared under laboratory conditions. One of these populations has been reared under laboratory conditions by 25 years without introduction of wild flies (Lab-pop), while the other has been maintained under the same conditions by 15 years but with occasional introduction of wild specimens (Hybrid-pop). The following parameters were analyzed: emergence percentage, life cycle duration (from eclosion to emergence), adult size, longevity, female fecundity and oviposition preference. The best performance of immatures of both populations was obtained on papaya as a host. Larvae reared on orange had longer life cycle, low emergence percentage and smaller adults. The fruit type did not affect fecundity and longevity of the Lab-pop, but in the Hybrid-pop males lived longer when reared on papaya, while females had higher longevity and fecundity when reared on orange. Females of both populations preferred to lay eggs in papaya (better host for larvae) when compared to orange. However, some eggs were deposited on orange only by females of Lab-pop, suggesting a lower ability for host selection of this population. These data are discussed regarding to the effects of continuous laboratory rearing on the biological parameters of this species.

Morphometric and molecular differentiation of *Phlebotomus* (Phlebotomus) sandflies

Khalid, N; Elnaïem, D; Aboud, M; Al Rabba, F; Tripet, F. 2010

The closely related sandfly species of the subgenus *Phlebotomus* namely, *Phlebotomus papatasi* (Scopoli, 1786), *Phlebotomus duboscqi* Neveu-Lemair, 1906 and *Phlebotomus bergeroti* Parrot, 1934 (Diptera: Psychodidae), are major vectors of *Leishmania major* (Kinetoplastida: Trypanosomatidae), the causative agent of cutaneous leishmaniasis in the Old World. Although allopatric in most of their distribution, the three species exist sympatrically in many places in central and eastern Sudan. Males of the three species can be distinguished using morphological characters; however, females are much harder to identify, thus complicating epidemiological studies. We carried out a morphometric and a molecular study to determine reliable morphological features and develop a polymerase chain reaction (PCR) assay for distinguishing females of these species. Males and females from each species were collected from sites in Sudan, East Africa and from one site in Mali, West Africa. Males were analysed morphologically and 20 characters and 10 character ratios were used in a stepwise discriminant analysis. This led to the identification of four characters with high discriminant loading scores sufficient for accurate male species identification. Male DNA was then used for the development of a PCR-based species diagnostic based on the second internal transcribed spacer (ITS2) of the ribosomal DNA. A set of four primers was developed to generate fragment sizes that are specific to each species and can reliably identify females as well as hybrid DNA. Both the morphometric and the molecular findings of this study have important applications for studies of the epidemiology of cutaneous leishmaniasis.

Morphological and Isozyme Analyses of Natural Populations of *Bactrocera* spp. (Diptera: Tephritidae) from Guimaras Island and Laguna, Philippines

Velasco, VMH; Laude, RP; Medina, CD; Velasco, LRI. 2010

Morphological and isozyme analyses were used to determine if there were differences among groups of *Bactrocera* spp. collected from Guimaras Island and Laguna, Philippines. Each population was divided into seven groups based on six morphological criteria. Morphological analysis of aedeagus/aculeus length and wing cell diameter showed that these characteristics did not differ considerably among the groups. Isozyme analysis revealed eight presumptive loci coding for three enzymes. Acid phosphatase (ACP) and esterase (EST) had three loci each, while alkaline phosphatase (ALP) had two. Two alleles, S and F, were found for ACP-1, ACP-3, EST-1, ALP-1 and ALP-2. ACP-2 and EST-3 showed one allele, S, while EST-2 showed three S, M and F. Generally, the groups of *Bactrocera* spp. from Guimaras Island had higher genetic variation than those from Laguna. For both locations, however, intrapopulation variation was generally higher in groups 0, 1 and 2 (presumed to be *B. occipitalis*) than in groups 3 and 4 (presumed to be "hybrids"). Estimates of interpopulation variation showed the presence of considerable variation among the groups. Genetic differences were generally higher between groups from the opposite ends of the classification scheme. In Laguna, groups 0 and 2 with genetic identity (*I*) = 0.8271 and genetic distance *D* = 0.1898 were the most genetically similar while groups 1 and 5 were the most genetically different (*I* = 0.2456; *D* = 1.4040). In Guimaras, groups 2 and 4 were the most genetically similar (*I* = 0.8580, *D* = 0.1532) while groups 2 and 6 were the most genetically different (*I* = 0.3211; *D* = 1.136). The morphological data did not conform with the isozyme data for the eight presumptive loci.

A Comparison of Aboveground and Belowground Populations of *Culex pipiens* (Diptera: Culicidae) Mosquitoes in Chicago, Illinois, and New York City, New York, Using Microsatellites

Kothera, L; Godsey, M; Mutebi, JP; Savage, HM. 2010

Aboveground and belowground populations of the mosquito *Culex pipiens* L. are traditionally classified as form *pipiens* and form *molestus*, respectively, and gene flow between forms is thought to be limited. Relatively few *f. molestus* populations have been found in the United States, which has hindered their study in North America. In this study, we used microsatellites to characterize a newly discovered population off. *molestus* in Chicago, IL, and compared levels of genetic diversity and differentiation in aboveground and belowground populations from Chicago and New York City, NY. Levels of genetic diversity, as measured by expected heterozygosity and allelic richness, were markedly lower in both *f. molestus* populations. Allele frequencies were distinctly different between the two *f. molestus*

populations, and some alleles were present in one belowground population and not the other. Pairwise F-ST, values between populations indicated that *f. molestus* populations were highly divergent from each other, as well as from their associated aboveground populations. Cluster analysis suggested the most likely number of groups was three, with the four *f. pipiens* populations in one cluster, and each of the *f. molestus* populations in its own cluster. Admixture analysis detected a low number of hybrids, 8%, between forms. We also tested the efficacy of two assays purported to distinguish between the forms, the CQ11 assay and a restriction fragment-length polymorphism assay of the COI gene, and found neither assay reliable in this regard. Our findings support the hypothesis that *f. molestus* populations in Chicago and New York City arose from local aboveground populations.

Use of the checkerboard DNA-DNA hybridization technique for bacteria detection in *Aedes aegypti* (Diptera: Culicidae) (L.)

Gaio, AD; Rodrigues, RCC; do Nascimento, C; Secundino, NFC; Lemos, FJA; Pimenta, PFP; Monesi, N. 2011

Background: Bacteria associated with insects can have a substantial impact on the biology and life cycle of their host. The checkerboard DNA-DNA hybridization technique is a semi-quantitative technique that has been previously employed in odontology to detect and quantify a variety of bacterial species in dental samples. Here we tested the applicability of the checkerboard DNA-DNA hybridization technique to detect the presence of *Aedes aegypti*-associated bacterial species in larvae, pupae and adults of *A. aegypti*. Findings: Using the checkerboard DNA-DNA hybridization technique we could detect and estimate the number of four bacterial species in total DNA samples extracted from *A. aegypti* single whole individuals and midguts. *A. aegypti* associated bacterial species were also detected in the midgut of four other insect species, *Lutzomyia longipalpis*, *Drosophila melanogaster*, *Bradysia hygida* and *Apis mellifera*. Conclusions: Our results demonstrate that the checkerboard DNA-DNA hybridization technique can be employed to study the microbiota composition of mosquitoes. The method has the sensitivity to detect bacteria in single individuals, as well as in a single organ, and therefore can be employed to evaluate the differences in bacterial counts amongst individuals in a given mosquito population. We suggest that the checkerboard DNA-DNA hybridization technique is a straightforward technique that can be widely used for the characterization of the microbiota in mosquito populations.

Molecular characterization of *Psytalia lounsburyi*, a candidate biocontrol agent of the olive fruit fly, and its *Wolbachia* symbionts as a pre-requisite for future intraspecific hybridization

Cheyppe-Buchmann, S; Bon, MC; Warot, S; Jones, W; Malausa, T; Fauvergue, X; Ris, N. 2011

Numerous arthropod species are genetically differentiated across their distribution area. Diversifying the geographical origins of a biocontrol agent species can be used to favour their perennial establishment by the sampling of pre-adapted genotypes and/or the production of new genotypes through hybridization. Hybridization can be nevertheless challenged by reproductive isolations induced by some common microbial endosymbionts. In this study, we aimed at characterizing (i) the genetic diversity of six populations of *Psytalia lounsburyi* (Hymenoptera: Braconidae), a candidate biocontrol agent of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) and (ii) the diversity of their *Wolbachia* endosymbionts. Both mitochondrial and microsatellite markers evidence clustering between the South African population and several Kenyan/Namibian populations. The survey of the *Wolbachia* also distinguished two main variants with a spatial heterogeneity in the infection status. All these results are discussed in the context of the use of these *P. lounsburyi* populations for hybridization and further field releases.

Mating frequencies and production of hybrids by *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae) in the laboratory

Yee, WL; Goughnour, RB. 2011

Evidence indicates low levels of hybridization in nature between the apple maggot (AM), *Rhagoletis pomonella* (Walsh), and snowberry maggot (SB), *Rhagoletis zephyria* Snow, sibling species in the *R. pomonella* complex. We determined the effects of AM and SB pairings on mating frequencies and production of hybrid offspring in the laboratory. Mating frequency was lowest in SB female x AM male pairings, higher in AM female x SB male and AM female x AM male pairings, and highest in SB female x SB male pairings. A greater percentage of AM female x AM male pairs produced offspring (puparia) than did AM female x SB male and SB female x AM male pairs, and a greater percentage of AM female x SB male pairs produced puparia than did SB female x AM male pairs. Male or female F(1) hybrids backcrossed with AM males and with other F1 hybrids were fertile. Results suggest most *R. pomonella* x *R. zephyria* hybrids found in nature are the result of *R. zephyria* males mating with *R. pomonella* females, with few from reciprocal matings. If true, this asymmetry could lower the incidence of hybridization in nature.

Assortative mating among *Anastrepha fraterculus* (Diptera: Tephritidae) hybrids as a possible route to radiation of the *fraterculus* cryptic species complex

Segura, DF; Vera, MT; Rull, J; Wornoayporn, V; Islam, A; Robinson, AS. 2011

Reproductive isolation among populations of *Anastrepha fraterculus* has been found acting at the pre- and post-zygotic levels. Differences in timing of sexual activity and male sexual pheromone composition among populations could partially account for prezygotic isolation. Hybrid males were found to produce a novel pheromone, which is a mix of parental pheromones. In the present study, we found that the hybrid females showed a significant preference to mate with hybrid males than with parental males. Male location during pheromone emission is associated with its reproductive success and, thus, differences in the location of males during courtship could also play a role in isolation. We found evidence that reproductive isolation is also related to the location of males during courtship. Hybrid male behaviour regarding location during pheromone release was found to be influenced by the maternal lineage. If these populations hybridized in the field, the hybrid females would tend to mate with hybrid males probably leading to the formation of a new entity within the *A. fraterculus* complex. This simple and fast process could be one reason explaining the high number of taxonomic entities within this complex. Further studies on other members of the *fraterculus* species group may reveal whether this can be considered as an example of homoploid hybrid speciation. (C) 2011 The Linnean Society of London, Biological Journal of the Linnean Society, 2011, 102, 346-354.

The molecular systematics of blowflies and screwworm flies (Diptera: Calliphoridae) using 28S rRNA, COX1 and EF-1 alpha: insights into the evolution of dipteran parasitism

McDonagh, LM; Stevens, JR. 2011

The Calliphoridae include some of the most economically significant myiasis-causing flies in the world-blowflies and screwworm flies-with many being notorious for their parasitism of livestock. However, despite more than 50 years of research, key taxonomic relationships within the family remain unresolved. This study utilizes nucleotide sequence data from the protein-coding genes COX1 (mitochondrial) and EF1 alpha (nuclear), and the 28S rRNA (nuclear) gene, from 57 blowfly taxa to improve resolution of key evolutionary relationships within the family Calliphoridae. Bayesian phylogenetic inference was carried out for each single-gene data set, demonstrating significant topological difference between the three gene trees. Nevertheless, all gene trees supported a Calliphorinae-Luciliinae subfamily sister-lineage, with respect to Chrysomyinae. In addition, this study also elucidates the taxonomic and evolutionary status of several less well-studied groups, including the genus *Bengalia* (either within Calliphoridae or as a separate sister-family), genus *Onesia* (as a sister-genera to, or sub-genera within, Calliphora), genus *Dyscritomyia* and *Lucilia bufonivora*, a specialised parasite of frogs and toads. The occurrence of cross-species hybridisation within Calliphoridae is also further explored, focusing on the two economically significant species *Lucilia cuprina* and *Lucilia sericata*. In summary, this study represents the most comprehensive molecular phylogenetic analysis of family Calliphoridae undertaken to date.

Cytogenetics of a parthenogenetic Arctic species of *Micropsectra* (Diptera, Chironomidae)

Porter, DL; Martin, J. 2011

Micropsectra sedna (Oliver, 1976) is a parthenogenetic midge from the Canadian Arctic. The parthenogenetic mechanism is apomictic thelytoky, with a restitutional division during oogenesis, as found in other parthenogenetic Chironomidae. It is triploid, with two similar chromosome sets, and the third is relatively dissimilar, pairing little with the diploid set. Two karyotypes were observed: a single individual with eight polytene elements in the salivary glands ($3n=12$), considered standard, while the majority of larvae showed only seven polytene chromosomes ($3n=11$). Hybrid speciation is considered likely, although chromosomal recombination following the origin of thelytoky has played some part in karyotype evolution. A single morphologically distinct larva was also found, which might be the donor of the haploid chromosome set. The apomictic restitutional system is compared to that of the other, independently derived, parthenogenetic Chironomids to assess the extent of similarity between species.

Ignatzschineria indica sp nov and *Ignatzschineria ureiclastica* sp nov., isolated from adult flesh flies (Diptera: Sarcophagidae)

Gupta, AK; Dharne, MS; Rangrez, AY; Verma, P; Ghate, HV; Rohde, M; Patole, MS; Shouche, YS. 2011

Two Gram-negative-staining, aerobic, non-motile, rod-shaped bacteria, designated strains FFA1(T) and FFA3(T), and belonging to the class Gammaproteobacteria were isolated from the gastrointestinal tract of adult flesh flies (Diptera: Sarcophagidae). Phylogenetic analysis of 16S rRNA gene sequence data placed these two strains within the genus *Ignatzschineria* with similarities of 98.60/0 (FFA1(T)) and 99.35% (FFA3(T)) to *Ignatzschineria* larvae L1/68(T). The level of gene sequence similarity between strains FFA1(T) and FFA3(T) was 99%, 97.15% and 78.1% based on the 16S rRNA, 23S rRNA and *gyrB* gene sequences, respectively. Strains FFA1(T) and FFA3(T) shared 24% DNA-DNA relatedness. DNA-DNA hybridization revealed a very low level of relatedness between the novel strains (22% for strain FFA1(T) and 44% for strain FFA3(T)) and *I. larvae* L1/68(T) genomic DNA. The respiratory quinone was Q-8 in both novel strains. The DNA G+C contents were 41.1 mol% and 40.1 mol% for strains FFA1(T) and FFA3(T), respectively. The cell membrane of both strains consisted of phosphatidylglycerol, phosphatidylethanolamine, phospholipids and aminophospholipid. The major fatty acids for both strains were C-16:0, summed feature 8 (C-18:1 omega 7c and/or C-18:1 omega 6c), Cyc19:0 omega 8c and C-14:0. The results of DNA-DNA hybridization between the two new strains and *I. larvae* L1/68(T), in combination with phylogenetic, chemotaxonomic, biochemical and electron microscopic data, demonstrated that strains FFA1(T) and FFA3(T) represented two novel species of the genus *Ignatzschineria* for which the names *Ignatzschineria indica* sp. nov. (type strain FFA1(T)=DSM 22309(T)=KCTC 22643(T)=NCIM 5325(T)) and *Ignatzschineria ureiclastica* sp. nov. (type strain FFA3(T)=DSM 22310(T)=KCTC 22644(T)=NCIM 5326(T)) are proposed.

Pollen transport differs among bees and flies in a human-modified landscape

Rader, R; Edwards, W; Westcott, DA; Cunningham, SA; Howlett, BG. 2011

Aim Dispersal distances of insect pollinators are critical in defining their contribution to landscape-wide pollen movement and ultimately gene flow in natural and agricultural systems. We ask whether bee and fly pollinator taxa differ in their dispersal distances and transport of viable pollen in a human-modified system. Location Canterbury and Otago region, South Island, New Zealand. Methods We captured pollen-carrying insects travelling outside of a model mass-flowering agricultural crop, *Brassica rapa*, using insect flight intercept traps at five distances (0, 100, 200, 300 and 400 m) from the pollen source. We examined pollen loads and pollen viability to determine whether pollen transport distance and viability differ among pollinator taxa. Results A total of 5453 insects were collected of which 717 individuals from 26 insect taxa were positively identified as dispersing pollen up to 400 m from the source. These taxa consisted of four species from two bee families (Hymenoptera: Apidae and Halictidae), and eight species from four fly families (Diptera: Bibionidae, Stratiomyidae, Syrphidae and Tachinidae). Apidae generally carried higher pollen loads and more viable pollen than most fly taxa. Taxa in the fly families Stratiomyidae and Syrphidae, however, carried pollen to 400 m, which is further than both bee families. Main conclusions A diverse array of wild and managed flower visitors can transport viable pollen from a pollen source to at least 400 m. Knowledge of the differences in transport distances among generalist pollinators in human-modified environments is crucial to understand the potential extent to which (1) pollen transport can facilitate gene flow and (2) unwanted hybridization may occur between crops and related weeds.

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.

Isolation and Characterization of Microsatellite Loci in the Asian Rice Gall Midge (*Orseolia oryzae*) (Diptera: Cecidomyiidae)

Bentur, JS; Sinha, DK; Padmavathy, C; Revathy, C; Muthulakshmi, M; Nagaraju, J. 2011

Microsatellite loci were isolated from the genomic DNA of the Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) using a hybridization capture approach. A total of 90 non-redundant primer pairs, representing unique loci, were designed. These simple sequence repeat (SSR) markers represented di (72%), tri (15.3%), and complex repeats (12.7%). Three biotypes of gall midge (20 individuals for each biotype) were screened using these SSRs. The results revealed that 15 loci were hyper variable and showed polymorphism among different biotypes of this pest. The number of alleles ranged from two to 11 and expected heterozygosity was above 0.5. Inheritance studies with three markers (observed to be polymorphic between sexes) revealed sex linked inheritance of two SSRs (Oosat55 and Oosat59) and autosomal inheritance of one marker (Oosat43). These markers will prove to be a useful tool to devise strategies for integrated pest management and in the study of biotype evolution in this important rice pest.

The invasive 'Lantana camara L.' hybrid complex (Verbenaceae): a review of research into its identity and biological control in South Africa

Urban, AJ; Simelane, DO; Retief, E; Heystek, F; Williams, HE; Madire, LG. 2011

Recent progress in the nomenclature and genetics of the hybrid-complex 'lantana' is summarized as it pertains to sourcing the best-adapted natural enemies for its

biological control. Reasons are given for viewing the whole array of invasive taxa within *Lantana* L. sect. *Camara* Cham. (Verbenaceae) as a syngameon, and for surveying natural enemies of camara-like *Lantana* entities between Florida and Uruguay. To improve the degree of biological control of *lantana*, additional agents have been selected, evaluated and found suitable for release in South Africa. The quarantine evaluation and current status of 30 candidate biological control agents obtained from the New World is summarized. Of these, seven were found to be suitable for release, according to given criteria, and two new agents, *Aceria lantanae* (Cook) (Acari: Eriophyidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), are improving control of *lantana* in humid, frost-free areas. No significant non-target effects have been detected. Information on the distribution and abundance of 17 agents and *lantana*-associated insects established in South Africa is presented: several are mainly coastal and they are scarce overall. Agent proliferation is constrained by a combination of climatic incompatibility, acquired natural enemies and, probably, the broad spectrum of allelochemicals present in the allopolyploid hybrids within the *L. camara* complex. In the case of *lantana*, biological control plays a subsidiary role in support of essential mechanical-plus-chemical control. Cost benefits justify the continued development of additional agents.

Morphological variability in rupicolous species of the *Acianthera prolifera* complex (Orchidaceae) occurring in southeastern Brazil

de Melo, MC; Borba, EL. 2011

We carried out multivariate morphometric analysis of 23 floral characters in seven populations of a complex of four species of *Acianthera* (Orchidaceae) occurring in Brazilian campo rupestre (rocky field) vegetation (*A. hamosa*, *A. limae*, *A. modestissima*, and *A. prolifera*) that flower synchronously and are partially intercompatible, and one putative hybrid population between *A. limae* and *A. prolifera*. We also carried out cluster analysis involving these eight populations plus 21 populations of a previously published study belonging to another species complex of *Acianthera* occurring in campo rupestre, including 12 floral characters in the analysis. Allopatric species pollinated by the same group of Diptera showed higher floral similarity among themselves than to a sympatric species pollinated by another group of Diptera. Such patterns indicate the existence of floral convergence in allopatric species and/or radiation in sympatric species. The analysis also indicated that there is more floral similarity between species of different complexes but that share the same group of pollinators. Large overlap was observed between *A. limae* and the putative sympatric hybrids, indicating the occurrence of later generations of hybrids and/or individuals of *A. limae* with introgression. The results do not support *A. hamosa* and *A. modestissima* as distinct species. These taxa are geographically isolated, occurring in different environments, are recognized only by vegetative characters that show high phenotypic plasticity, and share the same pollinators, being interfertile.

***Lutzomyia* (*Pintomyia*) *fischeri* (Diptera: Psychodidae: Phlebotominae), a probable vector of American Cutaneous Leishmaniasis: Detection of natural infection by *Leishmania* (*Viannia*) DNA in specimens from the municipality of Porto Alegre (RS), Brazil, using multiplex PCR assay**

de Pita-Pereira, D; Souza, GD; Pereira, TD; Zwetsch, A; Britto, C; Rangel, EF. 2011

In order to determine natural *Leishmania* (*Viannia*) infection in *Lutzomyia* (*Pintomyia*) *fischeri*, a multiplex PCR methodology coupled to non-isotopic hybridization was adopted for the analysis of sand fly samples collected by CDC light traps in an endemic area of American Cutaneous Leishmaniasis (ACL) in the periurban region of the municipality of Porto Alegre, Rio Grande do Sul State, Brazil. We analyzed by PCR methodology 560 specimens of *Lutzomyia* (*Pintomyia*) *fischeri* (520 females and 40 males). The wild sand flies were grouped into 56 pools (52 females and 4 males) of 10 each, and positive results were detected in 2 of the 52 female pools, representing a minimum infection rate of 0.38% based on the presence of at least 1 infected insect in the pool. This result associated with some local evidence such as anthrophily, spatial distribution in accordance with the transmission area and human case incidence, suggests that *L. (P.) fischeri* may be considered as a secondary vector of ACL in the studied locality. (C) 2011 Elsevier B.V. All rights reserved.

Reproductive biology and isolation mechanisms in rupicolous species of the *Acianthera prolifera* complex (Orchidaceae) occurring in southeastern Brazil

de Melo, MC; Taucce, PPG; Borba, EL. 2011

We studied the floral biology and performed experimental intra- and interspecific pollinations in populations of a complex of four *Acianthera* (Orchidaceae) species occurring in Brazilian campo rupestre vegetation (*A. hamosa*, *A. limae*, *A. modestissima*, and *A. prolifera*). All four species flower synchronously, are partially intercompatible, and exhibited some degree of self-sterility. Floral morphology is similar in all the species, with their principal differences associated with size of the floral structures. The four species were visited only by Diptera species of the families Phoridae (*Megaselia* spp.) and Chloropidae, but visits are rare and fruit set is very low. Sympatric species were not pollinated by the same Diptera species. *Acianthera hamosa* and *A. modestissima* have the smallest flowers, and no marked morphological differences between them were observed; they were both pollinated by very similar *Megaselia* species. Both prepollination barriers and postpollination events are important to maintaining the isolation of the species, functioning as overlapping filters that diminish the possibility of gene flow between them. However, putative hybrids between *A. prolifera* and *A. limae* have been found. Conversely, *A. hamosa* and *A. modestissima*, which are recognized only by vegetative characters that show high phenotypic plasticity, seem only to be isolated by geographical barriers, and they may actually constitute a single species or be sister species.

The distribution of potential West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae), in Mexico City

Diaz-Badillo, A; Bolling, BG; Perez-Ramirez, G; Moore, CG; Martinez-Munoz, JP; Padilla-Viveros, AA; Camacho-Nuez, M; Diaz-Perez, A; Beaty, BJ; Munoz, MD. 2011

Background: *Culex* spp. mosquitoes are considered to be the most important vectors of West Nile virus (WNV) detected in at least 34 species of mosquitoes in the United States. In North America, *Culex pipiens pipiens*, *Culex pipiens quinquefasciatus*, and *Culex tarsalis* are all competent vectors of WNV, which is considered to be enzootic in the United States and has also been detected in equines and birds in many states of Mexico and in humans in Nuevo Leon. There is potential for WNV to be introduced into Mexico City by various means including infected mosquitoes on airplanes, migrating birds, ground transportation and infected humans. Little is known of the geographic distribution of *Culex pipiens* complex mosquitoes and hybrids in Mexico City. *Culex pipiens pipiens* preferentially feed on avian hosts; *Culex pipiens quinquefasciatus* have historically been considered to prefer mammalian hosts; and hybrids of these two species could theoretically serve as bridge vectors to transmit WNV from avian hosts to humans and other mammalian hosts. In order to address the potential of WNV being introduced into Mexico City, we have determined the identity and spatial distribution of *Culex pipiens* complex mosquitoes and their hybrids. Results: Mosquito larvae collected from 103 sites throughout Mexico City during 2004-2005 were identified as *Culex*, *Culiseta* or *Ochlerotatus* by morphological analysis. Within the genus *Culex*, specimens were further identified as *Culex tarsalis* or as belonging to the *Culex pipiens* complex. Members of the *Culex pipiens* complex were separated by measuring the ratio of the dorsal and ventral arms (DV/D ratio) of the male genitalia and also by using diagnostic primers designed for the Ace. 2 gene. *Culex pipiens quinquefasciatus* was the most abundant form collected. Conclusions: Important WNV vectors species, *Cx. p. pipiens*, *Cx. p. quinquefasciatus* and *Cx. tarsalis*, are all present in Mexico City. Hybrids of *Cx. p. pipiens* and *Cx. p. quinquefasciatus* were also collected and identified. The presence and abundance of these WNV competent vectors is a cause for concern. Understanding the distribution of these vectors can help improve viral surveillance activities and mosquito control efforts in Mexico City.

Sympatry and Asymmetric Introgression Between the Cactophilic Species *Drosophila serido* and *Drosophila antonietae* (Diptera: Drosophilidae)

Kokudai, CBS; Sene, FM; Manfrin, MH. 2011

The cactophilic species *Drosophila serido* Vilela and Sene 1977 and *Drosophila antonietae* Tidon-Sklorz and Sene 2001 are two closely related species that have partially overlapping distributions in the Florianopolis-SC locality on the southern Brazilian coast. To characterize this contact zone with respect to possible

introgression events and the differential use of cacti hosts, we analyzed male individuals that were collected in their natural environment or from the necrotic cacti in which they were reared, by using aedeagus morphology, isocitrate dehydrogenase isozymes, and the mitochondrial gene cytochrome oxidase I as diagnostic markers. Morphological and molecular markers showed a bimodal distribution, although there was some incongruence, suggesting the presence of asymmetric introgression events. These results suggest that gene flow occurred in the past but then ceased, possibly due to reinforcement of prezygotic reproductive isolation. Analysis of individuals reared in necrotic cacti of two sympatric species in this region showed that *D. antonietae*, but not *D. serido*, preferentially uses *Cereus hildmannianus* Mill. as a cactus host, which may represent an important influence on ecological differentiation by affecting the dynamics of these species in this area of sympatry.

Genetic variation in isolated populations of *Drosophila mercatorum* (Diptera: Drosophilidae) in semi-arid ecosystems

Estrada, AP; Prieto, R. 2011

Variations in external and internal genitalia could constitute into pre-zygote barriers that prevent mating, contributing to the divergence between populations of species and allowing the emergence of new species. Sexual characteristics like mating, were taken into account to study the evolutionary processes involved in the morphology differentiation of the genitalia, for this purpose, there were conducted interpopulation crossings and morphometric analysis of external and internal genitalia of two populations of *Drosophila mercatorum* and its respective hybrids, collected in the Tatacoa Desert (Huila) and the Patia valley (Narino), considering that these are important taxonomic, characters, even to the level of subspecies and races. Morphometric analysis of non-sexual related characteristic such as' wings and abdominal stains were performed to compare the variability between populations, as well as to obtain their correlations, by using 28 individuals per population for geometric morphometrics and 20 for the traditional one, as in the case of the aedeagus. Less variability in these structures and a positive con-elation for the wing size and the aedeagus were found. The number of sows in the clasp, the shape of the wing and abdominal stains did not show significant differences between populations; however, the decrease in the offspring produced through the inter-population crossings and back-crossings, and the morphological variation in the aedeagus, allow differentiating them as geographic races.

Fire ant decapitating fly cooperative release programs (1994-2008): Two *Pseudacteon* species, *P. tricusps* and *P. curvatus*, rapidly expand across imported fire ant populations in the southeastern United States

Callcott, AMA; Porter, SD; Weeks, RD; Graham, LC; Johnson, SJ; Gilbert, LE. 2011

Natural enemies of the imported fire ants, *Solenopsis invicta* Buren, *S. richteri* Forel (Hymenoptera: Formicidae), and their hybrid, include a suite of more than 20 fire ant decapitating phorid flies from South America in the genus *Pseudacteon*. Over the past 12 years, many researchers and associates have cooperated in introducing several species as classical or self-sustaining biological control agents in the United States. As a result, two species of flies, *Pseudacteon tricusps* Borgmeier and *P. curvatus* Borgmeier (Diptera: Phoridae), are well established across large areas of the southeastern United States. Whereas many researchers have published local and state information about the establishment and spread of these flies, here distribution data from both published and unpublished sources has been compiled for the entire United States with the goal of presenting confirmed and probable distributions as of the fall of 2008. Documented rates of expansion were also used to predict the distribution of these flies three years later in the fall of 2011. In the fall of 2008, eleven years after the first successful release, we estimate that *P. tricusps* covered about 50% of the fire ant quarantined area and that it will occur in almost 65% of the quarantine area by 2011. Complete coverage of the fire ant quarantined area will be delayed or limited by this species' slow rate of spread and frequent failure to establish in more northerly portions of the fire ant range and also, perhaps, by its preference for red imported fire ants (*S. invicta*). Eight years after the first successful release of *P. curvatus*, two biotypes of this species (one biotype occurring predominantly in the black and hybrid imported fire ants and the other occurring in red imported fire ants) covered almost 60% of the fire ant quarantined area. We estimate these two biotypes will cover almost 90% of the quarantine area by 2011 and 100% by 2012 or 2013. Strategic selection of several distributional gaps for future releases will accelerate complete coverage of quarantine areas. However, some gaps may be best used for the release of additional species of decapitating flies because establishment rates may be higher in areas without competing species.

Susceptibility of Low-Chill Blueberry Cultivars to Mediterranean Fruit Fly, Oriental Fruit Fly, and Melon Fly (Diptera: Tephritidae)

Follett, PA; Zee, FT; Hamasaki, RT; Hummer, K; Nakamoto, ST. 2011

No-choice tests were conducted to determine whether fruit of southern highbush blueberry, *Vaccinium corymbosum* L., hybrids are hosts for three invasive tephritid fruit flies in Hawaii. Fruit of various blueberry cultivars was exposed to gravid female flies of *Bactrocera dorsalis* Hendel (oriental fruit fly), *Ceratitidis capitata* (Wiedemann) (Mediterranean fruit fly), or *Bactrocera cucurbitae* Coquillett (melon fly) in screen cages outdoors for 6 h and then held on sand in the laboratory for 2 wk for pupal development and adult emergence. Each of the 15 blueberry cultivars tested were infested by oriental fruit fly and Mediterranean fruit fly, confirming that these fruit flies will oviposit on blueberry fruit and that blueberry is a suitable host for fly development. However, there was significant cultivar variation in susceptibility to fruit fly infestation. For oriental fruit fly, 'Sapphire' fruit produced an average of 1.42 puparia per g, twice as high as that of the next most susceptible cultivar 'Emerald' (0.70 puparia per g). 'Legacy', 'Biloxi', and 'Spring High' were least susceptible to infestation, producing only 0.20-0.25 oriental fruit fly puparia per g of fruit. For Mediterranean fruit fly, 'Blue Crisp' produced 0.50 puparia per g of fruit, whereas 'Sharpblue' produced only 0.03 puparia per g of fruit. Blueberry was a marginal host for melon fly. This information will aid in development of pest management recommendations for blueberry cultivars as planting of low-chill cultivars expands to areas with subtropical and tropical fruit flies. Planting of fruit fly resistant cultivars may result in lower infestation levels and less crop loss.

Genomic introgression in laboratory evolved hybrid races, *Cytorace* 1 and *Fissioncytorace*-1 of *Nasuta-albomicans* complex (NAC) of *Drosophila* (Insecta, Diptera) as revealed by RAPD and ISSR markers

Thongatabam, B; Ramachandra, NB. 2012

Nasuta-albomicans complex (NAC) of *Drosophila* is an artificial hybrid zone comprising of *Drosophila nasuta nasuta*, *Drosophila nasuta albomicans* and 16 *Cytoraces*, which are the evolutionary products of a long range hybridization experiment conducted in the laboratory environment. Occurrence of centric fission in the X3 chromosome of *Cytorace* 1 led to the derivation of *Fissioncytorace*-1. Molecular techniques have emerged as powerful and valuable tools for detection and exploitation of genetic polymorphism. In the present study, *Cytorace* 1 and *Fissioncytorace*-1 were subjected to Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) analyses to determine the introgression of *D. n. nasuta* and *D. n. albomicans* genomes. It was found that *Cytorace* 1 and *Fissioncytorace*-1 exhibit similarities in RAPD and ISSR profiles although different combinations of genomic regions could have favoured *Fissioncytorace*-1, for better morphophenotypes and fitness, when compared to *Cytorace* 1, which has existed for over 15 years from the time of its evolution in the laboratory environment.

Sequence, Secondary Structure, and Phylogenetic Analyses of the Ribosomal Internal Transcribed Spacer 2 (ITS2) in Members of the North American Signifera Group of *Orthopodomyia* (Diptera: Culicidae)

Byrd, BD; Harrison, BA; Zavortink, TJ; Wesson, DM. 2012

Mosquitoes of the genus *Orthopodomyia* (Diptera: Culicidae) are little known and of uncertain epidemiological importance. In the United States, there are three *Orthopodomyia* species (i.e., *Or. signifera* (Coquillett), *Or. alba* Baker, and *Or. kummi* Edwards); they are all members of the Signifera Group based on the current

morphological taxonomy. In the course of identifying recently collected specimens, a problem was found with the current key morphological characters for separating the fourth instar larvae of *Or. signifera* and *Or. kummi*. Internal transcribed spacer two sequences of the rDNA were obtained to resolve the identities. The *Orthopodomyia* internal transcribed spacer two ranged in size from 193 (*Or. kummi*) to 244 bp (*Or. signifera*) (mean = 218 bp) and were slightly Adenine/Thymine enriched (44.7% Guanine/Cytosine on average). Putative secondary structures reveal structural homologies (four domains) consistent between species that also feature conserved sequences specific to mosquitoes (e. g., a conserved motif on the 3' aspect of the longest helix: GARTACATCC). Sequence analyses suggest that in certain areas of southwestern North America, hybridization may occur between *Or. kummi* and *Or. signifera*. Furthermore, our analyses confirm that *Or. californica* (a junior synonym of *Or. signifera*) is indeed *Or. signifera*. To our knowledge, this is the first sequence-based phylogenetic and molecular analysis of the *Orthopodomyia*.

A new tool for the molecular identification of *Culicoides* species of the *Obsoletus* group: the glass slide microarray approach

DeBlauwe, I; De Witte, JC; De Deken, G; Deken, R; Madder, M; Van Erk, S; Hoza, FA; Lathouwers, D; Geysen, D. 2012

Culicoides species of the *Obsoletus* group (Diptera: Ceratopogonidae) are potential vectors of bluetongue virus serotype 8 (BTV 8), which was introduced into central Western Europe in 2006. Correct morphological species identification of *Obsoletus* group females is especially difficult and molecular identification is the method of choice. In this study we present a new molecular tool based on probe hybridization using a DNA microarray format to identify *Culicoides* species of the *Obsoletus* group. The internal transcribed spacer 1 (ITS1) gene sequences of 55 *Culicoides* belonging to 13 different species were determined and used, together with 19 *Culicoides* ITS1 sequences sourced from GenBank, to design species-specific probes for the microarray test. This test was evaluated using the amplified ITS1 sequences of another 85 *Culicoides* specimens, belonging to 11 species. The microarray test successfully identified all samples (100%) of the *Obsoletus* group, identifying each specimen to species level within the group. This test has several advantages over existing polymerase chain reaction (PCR)-based molecular tools, including possible capability for parallel analysis of many species, high sensitivity and specificity, and low background signal noise. Hand-spotting of the microarray slide and the use of detection chemistry make this alternative technique affordable and feasible for any diagnostic laboratory with PCR facilities.

Hybrid carrot seed crop pollination by the fly *Calliphora vicina* (Diptera: Calliphoridae)

Howlett, BG. 2012

Many insect species can contribute to crop pollination; however, most growers remain highly dependent on the managed honey bee (*Apis mellifera* L.) for this service. The European Blue Blow Fly *Calliphora vicina* Robineau-Desvoidy, 1830 is one species with potential use as a pollinator. It occurs worldwide and is easy to rear. Caged trials conducted within a hybrid carrot (*Daucus carota* L.) seed crop found *C. vicina* to be an effective pollinator. Seed yield (number and weight) from field-grown carrot plants caged with *C. vicina*, but excluding all other large flower visitors (body width > 3 mm), was similar to seed yield from uncaged plants in the presence of honey bees (*Apis mellifera* L.) and other insects. In contrast, caged plants without *C. vicina* produced 10-fold less seed. Under open field conditions, *C. vicina* spent an average of 71.0 s per umbel compared to 54.4 s for honey bees; however, under caged conditions, *C. vicina* spent more time on average per umbel (128.9 s). Counts of *C. vicina* and honey bees on umbels outside of cages and *C. vicina* inside cages found that honey bees were most abundant on days with maximum temperature > 25 degrees C, while *C. vicina* was more abundant on cooler days around 20 degrees C. *C. vicina* may therefore be a useful pollinator of crops grown in isolation cages for plant breeding purposes as well as in open fields when climatic conditions are less favourable for optimal honey bee activity.

Natural infection of *cortelezzii* complex (Diptera: Psychodidae: Phlebotominae) with *Leishmania braziliensis* in Chaco, Argentina

Rosa, J; Pereira, DP; Brazil, RP; Andrade, JD; Salomon, O; Szelag, E. 2012

In Argentina, American Cutaneous Leishmaniasis (ACL) extends up to 29 degrees S in the phytogeographic regions of the Yungas (west), Chaco (center) and Paranaense (east). Since the Phlebotominae vectors of this disease in the western Chaco (dry Chaco) are unknown, in the present work, we studied the natural infection in Phlebotominae by PCR-ERFLP and Dot blot in order to incriminate these organisms as potential vectors. Captures with CDC-type traps were performed monthly in the domicile, the peridomicile and the forest in the Municipio Mision Nueva Pompeya, Chaco, Argentina, in two sites with human cases of ACL: Los Pozos (24 degrees 54'S, 61 degrees 22'W) and Fortin Arenales (24 degrees 58'S, 61 degrees 21'W), from November 2006 to December 2007. A total of 1702 Phlebotominae were captured: *Mygonomyia migonei* (83.8%), *cortelezzii* complex (11.1%), *Mycropigomyia peresi* (3.3%), *Mycropigomyia quinquefer* (1.2%), *Pintomyia torresi* (0.2%) and *Nyssomyia neivai* (0.2%). Although no significant differences were found in species diversity, there were significant differences in abundance between both sites studied. A total of 80 phlebotomine females were analyzed: 50 of the *cortelezzii* complex and 30 *My. migonei*. No intestinal flagellates were observed by light microscopy. Two pools of 10 individuals of the *cortelezzii* complex of the peridomicile and forest of Fortin Arenales were reactive by PCR and Dot blot for *Leishmania* (*Viannia*) *braziliensis*. In Argentina, *Evandromyia cortelezzii* has been incriminated as a likely vector of ACL because of its abundance in areas of sporadic outbreaks. In the present work, *Ev. cortelezzii* females were found naturally infected, thus reinforcing the hypothesis that the members of the *cortelezzii* complex act as vectors of the disease. (C) 2012 Elsevier B.V. All rights reserved.

Beyond barcoding: A mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae)

Nelson, LA; Lambkin, CL; Batterham, P; Wallman, JF; Dowton, M; Whiting, MF; Yeates, DK; Cameron, SL. 2012

Members of the Calliphoridae (blowflies) are significant for medical and veterinary management, due to the ability of some species to consume living flesh as larvae, and for forensic investigations due to the ability of others to develop in corpses. Due to the difficulty of accurately identifying larval blowflies to species there is a need for DNA-based diagnostics for this family, however the widely used DNA-barcoding marker, *cox1*, has been shown to fail for several groups within this family. Additionally, many phylogenetic relationships within the Calliphoridae are still unresolved, particularly deeper level relationships. Sequencing whole mt genomes has been demonstrated both as an effective method for identifying the most informative diagnostic markers and for resolving phylogenetic relationships. Twenty-seven complete, or nearly so, mt genomes were sequenced representing 13 species, seven genera and four calliphorid subfamilies and a member of the related family Tachinidae. PCR and sequencing primers developed for sequencing one calliphorid species could be reused to sequence related species within the same superfamily with success rates ranging from 61% to 100%, demonstrating the speed and efficiency with which an mt genome dataset can be assembled. Comparison of molecular divergences for each of the 13 protein-coding genes and 2 ribosomal RNA genes, at a range of taxonomic scales identified novel targets for developing as diagnostic markers which were 117-200% more variable than the markers which have been used previously in calliphorids. Phylogenetic analysis of whole mt genome sequences resulted in much stronger support for family and subfamily-level relationships. The Calliphoridae are polyphyletic, with the Polleninae more closely related to the Tachinidae, and the Sarcophagidae are the sister group of the remaining calliphorids. Within the Calliphoridae, there was strong support for the monophyly of the Chrysomyinae and Luciliinae and for the sister-grouping of Luciliinae with Calliphorinae. Relationships within Chrysomya were not well resolved. Whole mt genome data, supported the previously demonstrated paraphyly of *Lucilia cuprina* with respect to *L. sericata* and allowed us to conclude that it is due to hybrid introgression prior to the last common ancestor of modern *sericata* populations, rather than due to recent hybridisation, nuclear pseudogenes or incomplete lineage sorting. (C) 2012 Elsevier B.V. All rights reserved.

Aedeagal Divergence in Sympatric Populations of Two Sibling Species of Cactophilic *Drosophila* (Diptera: Drosophilidae): Evidence of Character Displacement?

Soto, IM. 2012

Aedeagal morphology of two sibling cactophilic species, *Drosophila buzzatii* Patterson & Wheeler and *Drosophila koepferae* Fontdevila & Wasserman, was analyzed in nine allopatric and three sympatric locations throughout South America. Morphological differences were detected for both aedeagus size and shape between sympatric and allopatric populations of *D. buzzatii*, despite the significant variability within both groups. Populations of *D. buzzatii* sympatric with *D. koepferae* displayed smaller aedeagus than the allopatric ones as well as more differentiated aedeagus shape. The shape differences were non-allometric and mainly consisted in a change of curvature of the dorsal margin of the aedeagus being more pronounced in males from populations sympatric with *D. koepferae*. It is concluded that aedeagal morphology presented some degree of character displacement in both size and shape in populations of *D. buzzatii* in sympatry with *D. koepferae*. These results might suggest the existence of mechanisms of interspecific recognition and hybridization prevention between these species that include the morphology of the male genitalia.

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.

LOW GENETIC DIVERSITY IN *Wolbachia*-INFECTED *Culex quinquefasciatus* (DIPTERA: CULICIDAE) FROM BRAZIL AND ARGENTINA

Morais, SA; de Almeida, F; Suesdek, L; Marrelli, MT. 2012

Culex quinquefasciatus is a vector of human pathogens, including filarial nematodes and several viruses. Although its epidemiological relevance is known to vary across geographical regions, an understanding of its population genetic structure is still incipient. In light of this, we evaluated the genetic diversity of *Cx. quinquefasciatus* and *Cx. pipiens* x *Cx. quinquefasciatus* hybrids collected from nine localities in Brazil and one site in Argentina. We used mitochondrial genes *cox1* and *nd4*, along with the *coxA* and *wsp* genes of the maternally-inherited *Wolbachia* endosymbiont. The *nd4* fragment was invariant between samples, whilst *cox1* exhibited four haplotypes that separated two types of *Cx. quinquefasciatus*, one clustered in southern Brazil. Low sequence diversity was generally observed, being discussed. Both Brazilian and Argentinian mosquitoes were infected with a single *Wolbachia* strain. As reported in previous studies with these populations, *cox1* and *nd4* diversity is not congruent with the population structure revealed by nuclear markers or alar morphology. Future *Cx. quinquefasciatus* research should, if possible, evaluate mtDNA diversity in light of other markers.

Random mating and reproductive compatibility among Argentinean and southern Brazilian populations of *Anastrepha fraterculus* (Diptera: Tephritidae)

Rull, J; Abraham, S; Kovaleski, A; Segura, DF; Islam, A; Wornoayporn, V; Dammalage, T; Tomas, US; Vera, MT. 2012

As a prerequisite for area-wide application of the sterile insect technique in an area encompassing northern Argentina and southern Brazil, prezygotic and postzygotic reproductive compatibility among three geographically distant populations in the area was tested. In field cages, sexually mature adults of each population were found to be sexually compatible, mating duration was not affected by fly origin and there was no clear evidence of spatial partition of mating location. In the laboratory, homotypic and heterotypic crosses for all possible combinations displayed similar levels of fertility and yielded F1 adults without distortion of the sex ratio. Finally, F1 hybrid and parental adults produced equally viable F2 eggs. Put together, our results and those from earlier studies suggest that a large area, ranging from Buenos Aires to the surroundings of Sao Paulo, could be managed using a single *A. fraterculus* mass-reared strain. At the northern margin of this area, two *A. fraterculus* morphotypes appear to coexist in sympatry. We delineate future research to further delimit the distribution of the aff1 morphotype (Argentina-southern Brazil) and to gain insight into evolutionary patterns producing divergence and radiation of tropical fruit fly species.

Ancient and modern hybridization between *Lucilia sericata* and *L. cuprina* (Diptera: Calliphoridae)

Williams, K; Villet, MH. 2013

There are important but inconsistent differences in breeding site preference between the blow flies *Lucilia sericata* (Meigen, 1826) and *L. cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae) that have significance for medical and veterinary science. These inconsistencies might arise from hybridisation. The species are difficult to distinguish using external morphology, although the male genitalia are distinctive and there are reliable molecular markers. Molecular evidence of modern hybridisation, derived from a newly developed nuclear marker, the period (*per*) gene, is presented here. This has implications for identifications of these species based on mtDNA, and may lead to an explanation of the medical and veterinary anomalies noted in these species.

Invasion genetics of American cherry fruit fly in Europe and signals of hybridization with the European cherry fruit fly

Johannesen, J; Keyghobadi, N; Schuler, H; Stauffer, C; Vogt, H. 2013

The American cherry fruit fly is an invasive pest species in Europe, of serious concern in tart cherry production as well as for the potential to hybridize with the European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae), which might induce new pest dynamics. In the first European reports, the question arose whether only the eastern American cherry fruit fly, *Rhagoletis cingulata* (Loew) (Diptera: Tephritidae), is present, or also the closely related western American cherry fruit fly, *Rhagoletis indifferens* Curran. In this study, we investigate the species status of European populations by comparing these with populations of both American species from their native ranges, the invasion dynamics in German (first report in 1993) and Hungarian (first report in 2006) populations, and we test for signals of hybridization with the European cherry fruit fly. Although mtDNA sequence genealogy could not separate the two American species, cross-species amplification of 14 microsatellite loci separated them with high probabilities (0.991.0) and provided evidence for *R. cingulata* in Europe. German and Hungarian *R. cingulata* populations differed significantly in microsatellite allele frequencies, mtDNA haplotype and wing pattern distributions, and both were genetically depauperate relative to North American populations. The diversity suggests independent founding events in Germany and Hungary. Within each country, *R. cingulata* displayed little or no structure in any trait, which agrees with rapid local range expansions. In cross-species amplifications, signals of hybridization between *R. cerasi* and *R. cingulata* were found in 2% of *R. cingulata* individuals and in 3% of *R. cerasi*. All putative hybrids had *R. cerasi* mtDNA indicating that the original between-species mating involved *R. cerasi* females and *R. cingulata* males.

Comparative Body Size and Shape Analyses of F-1 Hybrid *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae)

Yee, WL; Chapman, PS; Sheets, HD. 2013

Hybridization between apple maggot, *Rhagoletis pomonella* (Walsh), and *Rhagoletis zephyria* Snow (Diptera: Tephritidae) occurs in Washington State, complicating fly identifications. Here, experimentally generated F1 hybrids of *R. pomonella* and *R. zephyria* were classified using morphometric methods. Five of nine mean body size measurements of hybrids from crossing female *R. pomonella* x male *R. zephyria* were intermediate between those of nonhybrids. Aculeus lengths of hybrid females were intermediate or were more similar to those of *R. pomonella* than *R. zephyria*. Based on six or seven body size measures, 25.0% of female and 44.4% of male hybrids were correctly classified. Misclassified female and male hybrids resembled *R. pomonella* and one or the other parental species, respectively. Mean wing shapes of hybrid females based on landmark measurements were novel, whereas those of males were intermediate between those of nonhybrids. Based on wing shapes + natural log (ln) centroid size, 90.9% of female and 66.7% of male hybrids were correctly classified. Hybrid females were misclassified as *R. pomonella* and more hybrid males as *R. zephyria*. Based on surstylus shape, 27.7% of male hybrids were correctly classified, with more being misclassified as *R. pomonella*. Results show wing shape + ln centroid size is the most useful measure for identifying female and male hybrids and that *R. pomonella* traits for aculeus length and/or wing shape appear dominant to those of *R. zephyria* in hybrid females.

Molecular Species Identification of Cryptic Apple and Snowberry Maggots (Diptera: Tephritidae) in Western and Central Washington

Green, E; Almskaar, K; Sim, SB; Arcella, T; Yee, WL; Feder, JL; Schwarz, D. 2013

In Washington state, identification of the quarantine apple pest *Rhagoletis pomonella* (Walsh) is complicated by the presence of the cryptic species *Rhagoletis zephyria* Snow (Diptera: Tephritidae). Distinguishing the two flies is important because there is a zero tolerance policy for *R. pomonella* in apple production for export. Here, we attempt to distinguish the two species by scoring *R. pomonella* and *R. zephyria* populations from western and south Dcentral Washington for a set of 11 nuclear markers, including four single nucleotide polymorphisms (SNPs) developed for rapid and inexpensive genotyping using Taqman real-time quantitativeDpolymerase chain reaction. We show that the four SNPs maybe adequate in most cases for distinguishing whether a fly originated from apple or black hawthorn (the two major host plants for *R. pomonella* representing an economic risk) versus snowberry (the major host for *R. zephyria*, and not a commercial threat). However, directional introgression of *R. zephyria* alleles into *R. pomonella* can complicate the identification of flies of mixed ancestry based only on the four SNPs. Moreover, this problem is more acute in the sensitive apple-growing regions of central Washington where our results imply hybridization is common. Consequently, application of the four SNP quantitativeDpolymerase chain reaction assay can immediately assist ongoing apple maggot monitoring, while the development of additional genetic markers through next-generation sequencing would be valuable for increasing confidence in species identification and for assessing the threat posed by hybridization as *R. pomonella* further spreads into the more arid apple-growing regions of central Washington.

Suppressive subtraction hybridization reveals that rice gall midge attack elicits plant-pathogen-like responses in rice

Rawat, N; Himabindu, K; Neeraja, CN; Nair, S; Bentur, JS. 2013

The Asian rice gall midge, *Orseolia oryzae* (Diptera: Cecidomyiidae), is the third most destructive insect pest of rice (*Oryza sativa* L.). Till date, 11 gall midge resistance gene loci have been characterized in different rice varieties. To elucidate molecular basis of incompatible (hypersensitive response plus [HR+] type) and compatible rice-gall midge interactions, two suppressive subtraction hybridization cDNA libraries were constructed. These were enriched for differentially expressed transcripts after gall midge infestation in two rice varieties (resistant Suraksha and susceptible TN1). In total, 2784 ESTs were generated and sequenced from the two libraries, of which 1536 were from the resistant Suraksha and 1248 were from the susceptible TN1. Majority (80%) of the ESTs was non-redundant sequences with known functions and was classified into three principal gene ontology (GO) categories and 12 groups. Upregulation of NBS-LRR, Cytochrome P450, heat shock proteins, phenylalanine ammonia lyase and OsPR10 alpha genes from the Suraksha library, as revealed by real-time PCR, indicated that R gene mediated, salicylic acid related defense pathway is likely to be involved in gall midge resistance. Present study suggested that resistance in Suraksha against gall midge is similar in nature to the resistance observed in plants against pathogens. However, in TN1, genes related to primary metabolism and redox were induced abundantly. Results suggested that genes encoding translationally controlled tumor protein and NAC domain proteins are likely to be involved in the gall midge susceptibility. (c) 2012 Elsevier Masson SAS. All rights reserved.

Identification and Transcriptional Profiling of Differentially Expressed Genes Associated With Response to UVA Radiation in *Drosophila melanogaster* (Diptera: Drosophilidae)

Zhou, LJ; Zhu, ZH; Liu, ZX; Ma, WH; Desneux, N; Lei, CL. 2013

UltravioletA (UVA) radiation, the major component of solar ultraviolet (UV) radiation reaching the earth's surface, leads to negative effects in insects, such as oxidative stress, photoreceptor damage, and cell death. To better understand the molecular mechanisms of insect response to UVA radiation, suppression subtractive hybridization (SSH) and real-time quantitative polymerase chain reaction approaches were combined to reveal differential transcript expression in *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae). In this study, two subtractive cDNA libraries were constructed and sequenced, obtaining 131 high-quality unique expressed sequence tags (ESTs) that were up- or downregulated in *D. melanogaster* exposed to UVA radiation for 0.5 h. Of the 131 ESTs, 102 unique ESTs were differentially expressed and classified into 10 functional categories. The results showed that UVA radiation induces expression of genes related to stress and defense response and metabolism. Potential transcription factor binding motifs upstream of these genes are associated with multiple signaling pathways that may help the insect cope with the stress of UVA radiation. To our knowledge, this is the first analysis of insect response to UVA radiation at the transcriptional level. Our results reveal that UVA radiation influences the expression profiles of stress-responsive genes and provide further insights into the mechanisms of adaptive response to UVA radiation stress.

Feeding patterns of molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization

Gomes, B; Sousa, CA; Vicente, JL; Pinho, L; Calderon, I; Arez, E; Almeida, APG; Donnelly, MJ; Pinto, J. 2013

Background: Two biological forms of the mosquito *Culex pipiens* s.s., denoted pipiens and molestus, display behavioural differences that may affect their role as vectors of arboviruses. In this study, the feeding patterns of molestus and pipiens forms were investigated in Comporta (Portugal), where high levels of inter-form admixture have been recorded. Methods: Indoor and outdoor mosquito collections were performed in the summer of 2010. Collected *Cx. pipiens* s.l. females were molecularly identified to species and form by PCR and genotyped for six microsatellites. The source of the blood meal in post-fed females was determined by ELISA and mitochondrial DNA sequencing. Results: The distribution of the forms differed according to the collection method. The molestus form was present only in indoor collections, whereas pipiens and admixed individuals were sampled both indoors and outdoors. In both forms, over 90% of blood meals were made on avian hosts. These included blood meals taken from Passeriformes (*Passer domesticus* and *Turdus merula*) by females caught resting inside domestic shelters. Conclusion: Genetic structure and blood meal analyses suggest the presence of a bird biting molestus population in the study area. Both forms were found to rest indoors, mainly in avian shelters, but at least a proportion of females of the pipiens form may bite outdoors in sylvan habitats and then search for anthropogenic resting sites to complete their gonotrophic cycle. This behaviour may potentiate the accidental transmission of arboviruses to humans in the region.

Application of a reverse dot blot DNA-DNA hybridization method to quantify host-feeding tendencies of two sibling species in the *Anopheles gambiae* complex

Fritz, ML; Miller, JR; Bayoh, MN; Vulule, JM; Landgraf, JR; Walker, ED. 2013

A DNA-DNA hybridization method, reverse dot blot analysis (RDBA), was used to identify *Anopheles gambiae* s.s. and *Anopheles arabiensis* (Diptera: Culicidae) hosts. Of 299 blood-fed and semi-gravid *An. gambiae* s.l. collected from Kisian, Kenya, 244 individuals were identifiable to species; of these, 69.5% were *An. arabiensis* and 29.5% were *An. gambiae* s.s. Host identifications with RDBA were comparable with those of conventional polymerase chain reaction (PCR) followed by direct sequencing of amplicons of the vertebrate mitochondrial cytochrome b gene. Of the 174 amplicon-producing samples used to compare these two methods, 147 were identifiable by direct sequencing and 139 of these were identifiable by RDBA. *Anopheles arabiensis* bloodmeals were mostly (94.6%) bovine in origin, whereas *An. gambiae* s.s. fed upon humans more than 91.8% of the time. Tests by RDBA detected that two of 112 *An. arabiensis* contained blood from more than one host species, whereas PCR and direct sequencing did not. Recent use of insecticide-treated bednets in Kisian is likely to have caused the shift in the dominant vector species from *An. gambiae* s.s. to *An. arabiensis*. Reverse dot blot analysis provides an opportunity to study changes in host-feeding by members of the *An. gambiae* complex in response to the broadening distribution of vector control measures targeting host-selection behaviours.

Feeding Relationships Among *Tilapia zillii* (Gervais, 1848), *Tilapia guineensis* (Bleeker, 1862) and Their Hybrid in Ayame Man-Made Lake, Cote d'Ivoire

Shep, H; Konan, MK; Doumbia, L; Ouattara, M; Boussou, CK; Ouattara, A; Gourene, G. 2013

The stomach contents of 122 specimens of *Tilapia zillii*, 121 of *Tilapia guineensis* and 227 of their hybrid were studied in Ayame man-made lake during two years between August 1995 and September 1997 in order to analyze their diet composition and interspecific diet overlap. The samples were taken using two gill-nets batteries with mesh size 10, 12, 15, 20, 25, 30, 35, 40 and 50 mm. Food items identified from stomach contents were analysed using the Index of Food Preponderance (IFP). The two parental species and their hybrids consumed a great variety of items preys from animal and plant origin. The insect Diptera and the macrophytes were the most important item in the diet of these species. In the food of parental species, Molluscs, Coleoptera and Zooplankton also consisted some important items. The food overlap analysis showed that *T. zillii guineensis* and their hybrids share a wide range of prey types. The three species had a generalized feeding behaviour and the feeding strategy of the hybrid individuals is closer to this of *Tilapia zillii*. Competition for food is probably minimal, since the three fish species eat a wide range of foods and the abundance of main preys.

Occurrence of Phorid Fly (Diptera: Phoridae) Parasitoids of Imported Fire Ants (Hymenoptera: Formicidae) in Georgia

Gardner, WA; Peeler, HB; Diffie, SK. 2013

Ten releases of *Pseudacteon* decapitating flies (Diptera: Phoridae), a classical biological control agent of imported fire ants (Hymenoptera: Formicidae) in the southern United States, were made at 8 different sites in Georgia (USA) over a period spanning 2000 - 2012. Intensive monitoring and survey activities were initiated in 2008 to delineate the dispersal and occurrence of the *Pseudacteon* spp. in the state. Results show that all 159 counties in Georgia have at least 1 species of *Pseudacteon*; *P. curvatus* Borgmeier is established 129 of those counties, *P. tricuspidis* Borgmeier is established in 70 counties, and *P. obtusus* Borgmeier is established in the county in which it was released. Both *P. curvatus* and *P. tricuspidis* occur together in 39 counties, and all 3 of the *Pseudacteon* species occur in the 1 county in which all 3 species were released. These results help target supplemental or additional releases of *P. tricuspidis*, *P. curvatus*, and *P. obtusus* as well as releases of other candidate *Pseudacteon* species.

Transcriptome analysis in *Ceratitis capitata* to unveil genes involved in ageing-maturation process

San Andres, V; Castanera, P; Sabater-Munoz, B. 2013

The sterile insect technique (SIT) is widely used in integrated programmes against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Information on the age distribution of insects, and more particularly, the knowledge of wild female reproductive status (mature or not) at the time of the sterile male release is one of the key factors for the success of the SIT. In recent years, sequencing analysis has become an important tool in molecular biology. In this work we present a genome-wide expression analysis based on SSH (subtractive sequence hybridization) and EST (expressed sequence tag) sequencing and macroarray expression analysis to identify signature genes related to the ageing-maturing process in *C. capitata*, leading to the successful identification of new putative candidate genes of reproductive status in medfly that would serve as molecular markers for ageing. We have sorted out 94 unigenes from 873 single-pass ESTs, of which 57% have homology with known genes. Ageing-maturing process in *C. capitata* presents a marked expression pattern accompanied by the increase of transcription level of genes involved in reproduction (vitellogenins, chorion proteins and male-specific serum proteins). Other identified cDNAs (43%) with a differential expression pattern would be also candidates but deserve further studies, as they belong to the unknown function class.

Metaphase karyotypes of *Anopheles paraliae* (Diptera: Culicidae) in Thailand and evidence to support five cytological races

Taai, K; Baimai, V; Thongsahuan, S; Saeung, A; Otsuka, Y; Srisuka, W; Sriwichai, P; Somboon, P; Jariyapan, N; Choochote, W. 2013

Sixteen isoline colonies of *Anopheles paraliae* were established from wild-caught females collected from cow-baited traps at 4 locations in Thailand. They showed 3 types of X (X-1, X-2, X-3) and 5 types of Y (Y-1, Y-2, Y-3, Y-4, Y-5) chromosomes based on the number and amount of major block(s) of heterochromatin present in the heterochromatic arm, and were designated as Forms A (X-3, Y-1), B (X-1, X-2, X-3, Y-2), C (X-3, Y-3), D (X-1, X-2, X-3, Y-4) and E (X-3, Y-5). Form A was found in Songkhla Province, Form B was obtained in Ratchaburi, Nakhon Si Thammarat and Songkhla Provinces, Form C was acquired in Chanthaburi Province, Form D was recovered in Ratchaburi and Songkhla Provinces, and Form E was encountered in Ratchaburi Province. Hybridization experiments among the 7 isoline colonies, which represented the 5 karyotypic forms of *An. paraliae*, revealed genetic compatibility in providing viable progenies and synaptic salivary gland polytene chromosomes through F-2-generations, and thus suggest the conspecific nature of these karyotypic forms. These results were supported by the very low intraspecific sequence divergence (mean genetic distance = 0.000-0.002) of the nucleotide sequences in ribosomal DNA (ITS2) and mitochondrial DNA (COI and COII) of the 5 forms.

Paenalcaligenes hermetiae* sp nov., isolated from the larval gut of *Hermetia illucens* (Diptera: Stratiomyidae), and emended description of the genus *Paenalcaligenes

Lee, YY; Lee, JK; Park, KH; Kim, SY; Roh, SW; Lee, SB; Choi, Y; Lee, SJ. 2013

A novel Gram-stain-negative, facultatively anaerobic, non-motile and short rod-shaped bacterium, strain KBLO09(T), was isolated from the larval gut of *Hermetia illucens*. Strain KBLO09(T) grew optimally at 37 degrees C, at pH 6.0 and with 1-2% (w/v) NaCl. The 16S rRNA gene sequence of strain KBLO09(T) showed 97.6% similarity to that of *Paenalcaligenes hominis* CCUG 53761A(T) indicating its classification with the genus *Paenalcaligenes*. The major fatty acids were cyclo-C-17:0, C-16:0 and summed feature 2 (comprising C-14:0 3-OH/iso-C-16:1). The respiratory quinones were ubiquinone-8 (Q-8), predominating, and a minor amount of Q-7. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unknown aminolipid and five unknown polar lipids. The polyamine pattern contained predominantly putrescine and relatively high amounts of spermidine. The betaproteobacterial-specific 2-hydroxyputrescine could only be detected in trace amounts. The G+C content of genomic DNA was 56.1 mol%. Results from DNA DNA hybridization with *P. hominis* KCTC 23583(T) unambiguously

demonstrated that strain KBLO09(T) represents a novel species in the genus *Paenalcaligenes*. Based on phenotypic, genotypic and phylogenetic characterization, the novel species *Paenalcaligenes hermetiae* sp. nov. is proposed. The type strain is KBLO09(T) (=KACC 16840(T)=JCM 18423(T)). An emended description of the genus *Paenalcaligenes* is also provided.

Genetic Structure of *Lutzomyia longipalpis* Populations in Mato Grosso Do Sul, Brazil, Based on Microsatellite Markers

Santos, MFC; Ribolla, PEM; Alonso, DP; Andrade, JD; Casaril, AE; Ferreira, AMT; Fernandes, CES; Brazil, RP; Oliveira, AG. 2013

Background: *Lutzomyia longipalpis* (Diptera: Psychodidae) is the major vector of *Leishmania* (Leishmania) *infantum* and thus plays a crucial role in the epidemiology of American visceral leishmaniasis (AVL). This vector is the best studied species of sand fly in the Neotropical region. Many studies claim that this vector is in fact a species complex; however there is still no consensus regarding the number of species that belong into this complex or the geographical distribution of sibling species. The aim of the present study was to analyze the genetic relationships within *Lu. longipalpis* populations in the state of Mato Grosso do Sul (MS), Brazil. Methodology/Principal Findings: We collected 30 *Lu. longipalpis* (15 females and 15 males) from five localities (Campo Grande, Tres Lagoas, Aquidauana, Miranda and Bonito) and 30 *Lu. cruzi* from Corumba totaling 180 sandflies from MS, and 30 *Lu. longipalpis* from Estrela de Alagoas, state of Alagoas (AL), Northeast Brazil. We show that eight previously described microsatellite loci were sufficient in distinguishing *Lu. longipalpis* from *Lu. cruzi*, which is a closely related species, and in differentiating between *Lu. longipalpis* collected in MS versus Estrela de Alagoas. Analyses of the genotypes revealed introgression between sympatric *Lu. longipalpis* and *Lu. cruzi*. Conclusions/Significance: Our findings support the hypothesis of cryptic species within the *Lu. longipalpis* complex. Furthermore, our data revealed introgression between *Lu. longipalpis* and *Lu. cruzi*. This phenomenon should be further investigated to determine the level and incidence of hybridization between these two species. We also demonstrated that microsatellite markers are a powerful tool for differentiating sand fly populations and species. The present study has elucidated the population structure of *Lu. longipalpis* in MS and, by extension, the Neotropical *Lu. longipalpis* complex itself.

Analysis of ovary-specific genes in relation to egg maturation and female nutritional condition in the mosquitoes *Georgacraigius atropalpus* and *Aedes aegypti* (Diptera: Culicidae)

Telang, A; Rechel, JA; Brandt, JR; Donnell, DM. 2013

Analysis of the reproductive physiology of anautogenous mosquitoes at the molecular level is complicated by the simultaneity of ovarian maturation and the digestion of a blood meal. In contrast to anautogenous mosquitoes, autogenous female mosquitoes can acquire greater nutrient stores as larvae and exhibit higher ovarian production of ecdysteroids at adult eclosion. These features essentially replace the role of a blood meal in provisioning the first batch of eggs and initiating egg development. To gain insight into the process of ovary maturation we first performed a transcript analysis of the obligatory autogenous mosquito *Georgacraigius atropalpus* (formerly *Ochlerotatus atropalpus*). We identified ESTs using suppressive subtractive hybridization (SSH) of transcripts from ovaries at critical times during oogenesis in the absence of blood digestion. Preliminary expression studies of genes such as apolipoprotein III (APO) and oxysterol binding protein (OSBP) suggested these genes might be cued to female nutritional status. We then applied our findings to the medically important anautogenous mosquito *Aedes aegypti*. RNAi-based analyses of these genes in *Ae. aegypti* revealed a reduction in APO transcripts leads to reduced lipid levels in carcass and ovaries and that OSBP may play a role in overall lipid and sterol homeostasis. In addition to expanding our understanding of mosquito ovarian development, the continued use of a comparative approach between autogenous and anautogenous species may provide novel intervention points for the regulation of mosquito egg production. (C) 2012 Elsevier Ltd. All rights reserved.

Vector Competence of Argentine Mosquitoes (Diptera: Culicidae) for West Nile virus (Flaviviridae: Flavivirus)

Miceli, MV; Matarachiero, AC; Muttis, E; Fonseca, DM; Aliota, MT; Kramer, LD. 2013

We examined the ability of *Culex pipiens* L. complex mosquitoes from Argentina to vector West Nile virus (WNV) to assess their role in the transmission of WNV in South America. Several egg rafts of *Culex* spp. were collected from different breeding sites in the suburbs of the city of La Plata, Argentina, and a subset of each progeny was scored with morphological and genetic species indicators. Surprisingly, we did not find *Cx. pipiens* form *pipiens*, but found evidence of genetic hybrids of *Culex quinquefasciatus* and *Cx. pipiens* f. *molestus*. We then used morphological traits to create two colonies predominantly composed of one of these two taxa, although some hybrids are likely to have been included in both. These colonies were used in vector competence studies using NY99 and WN02 genotype strains of WNV obtained in New York State. As controls, we also tested colonies of U. S. *Cx. quinquefasciatus* and *Cx. pipiens* f. *molestus*. Additional *Culex* larvae from three drainage ditches near the cities of La Plata and Berisso, Argentina, were identified by morphological and high-resolution molecular markers (microsatellites) as *Cx. quinquefasciatus* Say, *Cx. pipiens* form *molestus*, and hybrids. Results indicate that Argentinian *Culex* are competent but only moderately efficient vectors of WNV and are less susceptible to this virus than comparable U. S. mosquito strains. Studies of vertical transmission of NY99 virus by *Cx. pipiens* f. *molestus* hybrids from Argentina yielded a minimal filial infection rate of 1.19 from females feeding during their second and later bloodmeals.

RESISTANCE OF MELON ACCESSIONS TO LEAFMINER *Liriomyza* spp. (DIPTERA: AGROMYZIDAE)

Nunes, GHD; Medeiros, AC; Araujo, EL; Nogueira, CHF; Sombra, KDD. 2013

The leafminer is the major pest of melon in the Brazilian Northeast. Within a program of integrated pest management using genetic resistance is a method of easy adoption, ecological and provides cost reduction with application of pesticides. The objective of this study was to evaluate the resistance of melon accessions to leafminer. Twenty-two accessions and three hybrids were evaluated in greenhouse and in two experiments under field conditions. The accessions were evaluated with multiple-choice in a design lattice with five replications for the following traits: number of larvae, number of puparium, and number of flies. Under field conditions, the accessions were evaluated in two experiments carried out during 2009 and 2010 in randomized complete block design with three replications. The mean number of mines per leaf was evaluated. There is variability among accessions of melon for resistance to leafminer in the accessions evaluated. Considering the experiments carried out in the greenhouse and field, the accession AC-22 is the most promising as a source of resistance to leafminer evaluations in the field and greenhouse, followed by accession AC-10. The other accessions are susceptible.

Distribution, host plant affiliation, phenology, and phylogeny of walnut-infesting *Rhagoletis* flies (Diptera: Tephritidae) in Mexico

Rull, J; Aluja, M; Tadeo, E; Guillen, L; Egan, S; Glover, M; Feder, JL. 2013

Many taxa of Nearctic origin have diversified in the subtropical highlands of Mexico. In particular, flies in the genus *Rhagoletis* have undergone episodes of isolation and gene flow during Pleistocene glaciations and post-glacial times that have produced lineage differentiation and reproductive isolation. To reach a better understanding of the phylogeography of the genus *Rhagoletis*, a host plant survey of the walnut-infesting *Rhagoletis suavis* species group was conducted across sixteen states comprising 34 different collecting sites in Mexico over a 9-year period. Five species of Juglans were found to be infested by three species of walnut-attacking *Rhagoletis* flies. Several species of parasitoids were also recovered from collections, but in contrast to their walnut fly hosts, they revealed little evidence for host or geographic subdivision. There was no consistent difference in mean eclosion time between walnut fly species or populations associated with different host walnuts in Mexico, unlike the case for other *Rhagoletis* species, in which allochronic isolation arising from variation in diapause timing is a major ecological

adaptation, reproductively isolating flies. We compare the distribution of *R. suavis* flies in Mexico with those of other *Rhagoletis* species attacking hawthorns and cherries, and discuss its implications for population divergence and speciation.(c) 2013 The Linnean Society of London.

Evolution of pre-zygotic and post-zygotic barriers to gene flow among three cryptic species within the *Anastrepha fraterculus* complex

Rull, J; Abraham, S; Kovaleski, A; Segura, DF; Mendoza, M; Liendo, MC; Vera, MT. 2013

Tropical tephritids are ideally suited for studies on population divergence and speciation because they include species groups undergoing rapid radiation, in which morphologically cryptic species and sister species are abundant. The *fraterculus* species group in the Neotropical genus *Anastrepha* is a case in point, as it is composed of a complex of up to seven *A. fraterculus* morphotypes proposed to be cryptic species. Here, we document pre- and post-zygotic barriers to gene flow among adults of the Mexican *A. fraterculus* morphotype and three populations (Argentina, Brazil, and Peru) belonging to two separate morphotypes (Brazilian 1 and Peruvian). We unveiled three forms of pre-zygotic reproductive isolation resulting in strong assortative mating. In field cages, free-ranging male and female *A. fraterculus* displayed a strong tendency to form couples with members of the opposite sex belonging to their own morphotype, suggesting that male pheromone emission, courtship displays, or both intervene in shaping female choice before actual contact and coupling. In addition, males and females of the Peruvian morphotype became receptive and mated significantly later than adults of the Mexican and Brazilian 1 morphotypes. After contact, Mexican females exhibited greater mating discrimination than males when facing adults of the opposite sex belonging to either the Peruvian or the Brazilian 1 morphotype as evidenced by vigorous resistance to penetration once they had been forcefully mounted by heterotypic males. Forced copulations resulted in production of F1 hybrids that were either less viable (and partially fertile) than parental crosses or even sterile. Our results suggest that the Mexican morphotype is a distinct biological entity and that pre-zygotic reproductive isolation through divergence in courtship or male-produced pheromone and other mechanisms appear to evolve faster than post-zygotic isolation in the *fraterculus* species group.

Population Genetic and Admixture Analyses of *Culex pipiens* Complex (Diptera: Culicidae) Populations in California, United States

Kothera, L; Nelms, BM; Reisen, WK; Savage, HM. 2013

Microsatellite markers were used to genetically characterize 19 *Culex pipiens* complex populations from California. Two populations showed characteristics of earlier genetic bottlenecks. The overall *F_{ST}* value and a neighbor-joining tree suggested moderate amounts of genetic differentiation. Analyses using Structure indicated *K* = 4 genetic clusters: *Cx. pipiens* form *pipiens* L., *Cx. quinquefasciatus* Say, *Cx. pipiens* form *molestus* Forskal, and a group of genetically similar individuals of hybrid origin. A Discriminant Analysis of Principal Components indicated that the latter group is a mixture of the other three taxa, with form *pipiens* and form *molestus* contributing somewhat more ancestry than *Cx. quinquefasciatus*. Characterization of 56 morphologically autogenous individuals classified most as *Cx. pipiens* form *molestus*, and none as *Cx. pipiens* form *pipiens* or *Cx. quinquefasciatus*. Comparison of California microsatellite data with those of *Cx. pipiens pallens* Coquillett from Japan indicated the latter does not contribute significantly to genotypes in California.

A Bioinformatics Approach for Integrated Transcriptomic and Proteomic Comparative Analyses of Model and Non-sequenced Anopheline Vectors of Human Malaria Parasites

Mohien, CU; Colquhoun, DR; Mathias, DK; Gibbons, JG; Armistead, JS; Rodriguez, MC; Rodriguez, MH; Edwards, NJ; Hartler, J; Thallinger, GG; Graham, DR; Martinez-Barnette, J; Rokas, A; Dinglasan, RR. 2013

Malaria morbidity and mortality caused by both *Plasmodium falciparum* and *Plasmodium vivax* extend well beyond the African continent, and although *P. vivax* causes between 80 and 300 million severe cases each year, *vivax* transmission remains poorly understood. *Plasmodium* parasites are transmitted by *Anopheles* mosquitoes, and the critical site of interaction between parasite and host is at the mosquito's luminal midgut brush border. Although the genome of the "model" African *P. falciparum* vector, *Anopheles gambiae*, has been sequenced, evolutionary divergence limits its utility as a reference across anophelines, especially non-sequenced *P. vivax* vectors such as *Anopheles albimanus*. Clearly, technologies and platforms that bridge this substantial scientific gap are required in order to provide public health scientists with key transcriptomic and proteomic information that could spur the development of novel interventions to combat this disease. To our knowledge, no approaches have been published that address this issue. To bolster our understanding of *P. vivax*-*An. albimanus* midgut interactions, we developed an integrated bioinformatic-hybrid RNA-Seq-LC-MS/MS approach involving *An. albimanus* transcriptome (15,764 contigs) and luminal midgut subproteome (9,445 proteins) assembly, which, when used with our custom Diptera protein database (685,078 sequences), facilitated a comparative proteomic analysis of the midgut brush borders of two important malaria vectors, *An. gambiae* and *An. albimanus*. *Molecular & Cellular Proteomics* 12: 10.1074/mcp.M112.019596, 120-131, 2012.

Survivorship of adult *Aedes albopictus* (Diptera: Culicidae) feeding on indoor ornamental plants with no inflorescence

Qualls, WA; Xue, RD; Beier, JC; Muller, GC. 2013

The international trade of lucky bamboo (*Dracaena sanderiana* [Asparagaceae]) is responsible for certain introductions of the exotic species *Aedes albopictus* (Skuse) in California and the Netherlands. Understanding the association of this species with lucky bamboo and other ornamental plants is important from a public health standpoint. The aim of this study was to investigate the importance of indoor ornamental plants as sugar sources for adult *A. albopictus*. If exposed to *D. sanderiana*, bromeliad (*Guzmania* spp. hybrid [Bromeliaceae]), Moses-in-the-cradle (*Rhoeo spathacea* [Commelinaceae]), 10 % sucrose solution, and a negative water control as the only nutrient source, adult female *A. albopictus* mean survival time was 12, 7, 6, 15, and 4 days, respectively. Mean survival times for adult males were not significantly different (*P* > 0.05) from the females and were 10, 7, 6, 14, and 3 days, respectively. Combined male and female survival times were not significantly different on lucky bamboo compared to survival times on a 10 % sucrose control. Based on our findings, *A. albopictus* can readily survive long enough to complete a gonotrophic cycle and potentially complete the extrinsic incubation period for many arboviruses when only provided access to lucky bamboo plants or possibly other common ornamentals. Vector control professionals should be aware of potential in-home infestations and public health concerns associated with mosquito breeding and plant tissue feeding on ornamental plants.

Phlebotomus orientalis Sand Flies from Two Geographically Distant Ethiopian Localities: Biology, Genetic Analyses and Susceptibility to *Leishmania donovani*

Seblova, V; Volfova, V; Dvorak, V; Pruzinova, K; Votypka, J; Kassahun, A; Gebre-Michael, T; Hailu, A; Warburg, A; Volf, P. 2013

Background: *Phlebotomus orientalis* Parrot (Diptera: Psychodidae) is the main vector of visceral leishmaniasis (VL) caused by *Leishmania donovani* in East Africa. Here we report on life cycle parameters and susceptibility to *L. donovani* of two *P. orientalis* colonies originating from different sites in Ethiopia: a non-endemic site in the lowlands - Melka Werer (MW), and an endemic focus of human VL in the highlands - Addis Zemen (AZ). Methodology/Principal Findings: Marked differences in life-cycle parameters between the two colonies included distinct requirements for larval food and humidity during pupation. However, analyses using Random Amplified Polymorphic DNA (RAPD) PCR and DNA sequencing of cytb and COI mitochondrial genes did not reveal any genetic differences. F1 hybrids developed successfully with higher fecundity than the parental colonies. Susceptibility of *P. orientalis* to *L. donovani* was studied by experimental infections. Even the lowest infective dose tested (2 x 10³ per ml) was sufficient for successful establishment of *L. donovani* infections in about 50% of the *P. orientalis* females. Using higher infective doses, the infection rates were around 90% for both colonies. *Leishmania* development in *P. orientalis* was fast, the presence of metacyclic promastigotes in

the thoracic midgut and the colonization of the stomodeal valve by haptomonads were recorded in most *P. orientalis* females by day five post-blood feeding. Conclusions: Both MW and AZ colonies of *P. orientalis* were highly susceptible to Ethiopian *L. donovani* strains. As the average volume of blood-meals taken by *P. orientalis* females are about 0.7 μ l, the infective dose at the lowest concentration was one or two *L. donovani* promastigotes per sand fly blood-meal. The development of *L. donovani* was similar in both *P. orientalis* colonies; hence, the absence of visceral leishmaniasis in non-endemic area Melka Werer cannot be attributed to different susceptibility of local *P. orientalis* populations to *L. donovani*.

Phenotypic Variation among *Culex pipiens* Complex (Diptera: Culicidae) Populations from the Sacramento Valley, California: Horizontal and Vertical Transmission of West Nile Virus, Diapause Potential, Autogeny, and Host Selection

Nelms, BM; Kothera, L; Thiemann, T; Macedo, PA; Savage, HM; Reisen, WK. 2013

The vector competence and bionomics of *Culex pipiens* form *pipiens* L. and *Cx. pipiens* f. *molestus* Forskal were evaluated for populations from the Sacramento Valley. Both f. *pipiens* and f. *molestus* females became infected, produced disseminated infections, and were able to transmit West Nile virus. Form *molestus* females also transmitted West Nile virus vertically to egg rafts and F₁ progeny, whereas f. *pipiens* females only transmitted to egg rafts. *Culex pipiens* complex from urban Sacramento blood-fed on seven different avian species and two mammalian species. Structure analysis of blood-fed mosquitoes identified K = 4 genetic clusters: f. *molestus*, f. *pipiens*, a group of genetically similar hybrids (Cluster X), and admixed individuals. When females were exposed as larvae to midwinter conditions in bioenvironmental chambers, 85% (N = 79) of aboveground *Cx. pipiens* complex females and 100% (N = 34) of underground f. *molestus* females did not enter reproductive diapause.

SOIL FAUNA CHARACTERIZATION IN *Eucalyptus* spp. PLANTATIONS

Garlet, J; Costa, EC; Boscardin, J. 2013

Forest soils provide good conditions for the development and the establishment of soil fauna, mainly by the deposition of litter. However, monoculture systems conducted in a single food providing substrate, can promote the development of certain fauna groups over others, causing outbreaks of pest species. The aim of this study was to characterize the soil fauna and its relationship with meteorological variables, in plantations of *Eucalyptus* spp. This study was conducted in six stands of three species of *Eucalyptus*: *Eucalyptus dunni* Maiden, *Eucalyptus grandis* Maiden and *Eucalyptus grandis* x *Eucalyptus urophylla* S. T. Blake (clone hybrid) and two ages (planted in 2006 and 2007). The stands were located at Cabanha da Prata and Chica Barbosa farms in Alegrete, and at Taquari farm in São Francisco de Assis, Rio Grande do Sul state. For sampling soil insects, pitfall traps were used. Eight traps were installed ten meters apart from each other, distributed in two rows in the center of each stand, totaling 48 traps. Samples were collected fortnightly from September 2008 to August 2009. After counting and identifying the taxonomic groups, the parameters of richness (total number of taxonomic groups), abundance and Shannon diversity index were determined. The results were analyzed using 3x2 factorial model (three species and two ages) in a randomized design with Test F. During this study, 8,811 specimens representing 12 taxonomic groups were collected. The groups Coleoptera, Hymenoptera, and Diptera had the highest percentages of individuals collected: 31.1, 23.2, and 19.6%, respectively. The plantation with three years of age had a higher number of individuals compared to the stands of two years of age. The species *Eucalyptus grandis* and *Eucalyptus grandis* x *Eucalyptus urophylla* had a higher number of individuals collected when compared with the species *Eucalyptus dunni* at both ages. Thus, the species and age of the planting exerted influence on the organisms collected.

Evidence to support natural hybridization between *Anopheles sinensis* and *Anopheles kleini* (Diptera: Culicidae): possibly a significant mechanism for gene introgression in sympatric populations

Choochote, W; Min, GS; Intapan, PM; Tantrawatpan, C; Saeung, A; Lulitanond, V. 2014

Background: Malaria caused by *Plasmodium vivax* is still a public health problem in the Republic of Korea (ROK), particularly regarding the recent re-emergence of this malarial species near the demilitarized zone in northwestern Paju City, Gyeonggi-do Province. Currently, at least 4 species (*An. kleini*, *An. pullus*, *An. belenrae* and *An. lesteri*) of the Hyrcanus Group are reported as possible natural vectors of *vivax* malaria in the ROK, and *An. sinensis*, which is the most dominant species, has long been incriminated as an important natural vector of this *P. vivax*. However, *An. sinensis* was ranked recently as a low potential vector. According to the discovery of natural hybrids between *An. sinensis* (a low potential vector for *P. vivax*) and *An. kleini* (a high potential vector for *P. vivax*) in Paju City, intensive investigation of this phenomenon is warranted under laboratory conditions. Methods: Mosquitoes were collected during 2010-2012 from Paju City, ROK. Hybridization experiments used iso-female line colonies of these anophelines together with DNA analysis of ribosomal DNA [second internal transcribed spacer (ITS2)] and mitochondrial DNA [cytochrome c oxidase subunit I (COI)] of the parental colonies, F₁-hybrids and repeated backcross progenies were performed intensively by using a PCR-based assay and pyrosequencing technology. Results: The results from hybridization experiments and molecular investigations revealed that the mitochondrial COI gene was introgressed from *An. sinensis* into *An. kleini*. The *An. sinensis* progenies obtained from consecutive repeated backcrosses in both directions, i.e., F₂-(11) progeny [(*An. sinensis* x *An. kleini*) x *An. sinensis*] and F₃-(5) progeny [(*An. kleini* x *An. sinensis*) x *An. kleini*] provided good supportive evidence. Conclusions: This study revealed introgression of the mitochondrial COI gene between *An. sinensis* and *An. kleini* through consecutive repeated backcrosses under laboratory conditions. This new body of knowledge will be emphasized in reliable promising strategies in order to replace the population of *An. kleini* as a high potential vector for *P. vivax*, with that of a low potential vector, *An. sinensis*, through the mechanism of gene introgression in nature.

Hybridization studies to modify the host preference of *Anopheles gambiae*

Pates, HV; Curtis, CF; Takken, W. 2014

A strategy to decrease the vector competence of *Anopheles gambiae* sensu stricto (Diptera: Culicidae), the most efficient malaria vector in Africa, may consist of exploiting the genes involved in zoophily. Crossing and backcrossing experiments were performed between *An. gambiae* s.s. and the zoophilic sibling species *Anopheles quadriannulatus*. Mosquito strains were tested in a dual-choice olfactometer to investigate their responses to cow odour. Totals of 12% of *An. gambiae* s.s. and 59% of *An. quadriannulatus* selected the port with the cow odour. Crosses and backcrosses did not show a significant preference for the cow-baited port. The results indicated that anthropophilic behaviour in *An. gambiae* s.s. is a dominant or partially dominant trait, which, in conjunction with the unstable zoophilic behaviour observed in *An. quadriannulatus*, poses a serious obstacle to plans to decrease vector competence by modifying the anthropophilic trait.

Cloning, Expression, and Purification of a New Antibacterial Substance Gene From Larvae of *Musca domestica* (Diptera: Muscidae)

Pei, ZH; Bian, L; Zhang, H; Gao, YH; Ma, HX. 2014

Musca domestica L. (Diptera: Muscidae), the housefly, exhibits unique immune defenses and can produce antibacterial substances upon stimulation with bacteria. On the basis of the cDNA library constructed using the suppression subtractive hybridization method, a 1188-bp antibacterial substance gene, which we named AS566, was amplified by rapid amplification of cDNA ends from *M. domestica* larva stimulated with *Salmonella pullorum* (Enterobacteriaceae: *Salmonella*). In this study, the full-length AS566 gene was cloned and inserted into a His-tagged *Escherichia coli* (Enterobacteriaceae: *Escherichia*) prokaryotic expression system to enable production of the recombinant protein. The recombinant AS566 protein was purified in denatured form from inclusion bodies and renatured to obtain functionally

active AS566 protein. The bacteriostatic activity of the recombinant purified AS566 protein was assessed using the Oxford plate assay system and the results indicated that AS566 had antibacterial activity against six bacteria, including an *E. coli* clinical isolate, *S. pullorum*, *Streptococcus bovis* (Streptococcaceae: Streptococcus), *Streptococcus suis*, and *Staphylococcus aureus* (Staphylococcaceae: Staphylococcus) in vitro. The antibacterial activity of AS566 toward Gram - bacteria was two times greater than that against Gram + bacteria. The sequencing results and BLAST analysis showed that the antibacterial substance gene AS566 was not homologous to any other antibacterial substance genes in GenBank. The antibacterial mechanisms of the newly discovered AS566 protein warrant further study.

Ongoing speciation within the *Anastrepha fraterculus* cryptic species complex: the case of the Andean morphotype

Devescovi, F; Abraham, S; Roriz, AKP; Nolzco, N; Castaneda, R; Tadeo, E; Caceres, C; Segura, DF; Vera, MT; Joachim-Bravo, I; Canal, N; Rull, J. 2014

The *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) cryptic species complex is currently composed of seven taxonomically recognized morphotypes. Both, pre- and post-zygotic isolation has been documented among four of these morphotypes, revealing that in fact they appear to be distinct biological entities. In order to progress in the full delimitation of species within the complex, we examined reproductive isolation between a Colombian population of the Andean morphotype and populations belonging to four other morphotypes spanning from Mexico to Argentina. Flies from the Andean morphotype exhibited strong pre-zygotic mating isolation through temporal partitioning of mating activity. Post-zygotic isolation was observed for crosses of males of all morphotypes and Andean morphotype females, yet most of the F1 hybrid male F1 hybrid female self-crosses showed normal levels of fertility, a finding suggesting a nuclear-cytoplasmic interaction according to previous studies. Overall, the Andean morphotype within the complex also appears to be a distinct biological entity. We discuss the implications of these findings for the understanding of speciation mechanisms in the Neotropical genus *Anastrepha*.

Towards the PCR-based identification of Palaearctic *Culicoides* biting midges (Diptera: Ceratopogonidae): results from an international ring trial targeting four species of the subgenus *Avaritia*

Garros, C; Balenghien, T; Carpenter, S; Delecolle, JC; Meiswinkel, R; Pedarrieu, A; Rakotoarivony, I; Gardes, L; Golding, N; Barber, J; Miranda, M; Borrás, DB; Goffredo, M; Monaco, F; Pages, N; Sghaier, S; Hammami, S; Calvo, JH; Lucientes, J; Geysen, D; De Deken, G; Monteys, VSI; Schwenkenbecher, J; Kampen, H; Hoffmann, B; Lehmann, K; Werner, D; Baldet, T; Lancelot, R; Cetre-Sossah, C. 2014

Background: Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are biological vectors of internationally important arboviruses. To understand the role of *Culicoides* in the transmission of these viruses, it is essential to correctly identify the species involved. Within the western Palaearctic region, the main suspected vector species, *C. obsoletus*, *C. scoticus*, *C. dewulfi* and *C. chiopterus*, have similar wing patterns, which makes it difficult to separate and identify them correctly. Methods: In this study, designed as an inter-laboratory ring trial with twelve partners from Europe and North Africa, we assess four PCR-based assays which are used routinely to differentiate the four species of *Culicoides* listed above. The assays based on mitochondrial or ribosomal DNA or microarray hybridisation were tested using aliquots of *Culicoides* DNA (extracted using commercial kits), crude lysates of ground specimens and whole *Culicoides* (265 individuals), and non-*Culicoides* Ceratopogonidae (13 individuals) collected from across Europe. Results: A total of 800 molecular assays were implemented. The in-house assays functioned effectively, although specificity and sensitivity varied according to the molecular marker and DNA extraction method used. The *Obsoletus* group specificity was overall high (95-99%) while the sensitivity varied greatly (59.6-100%). DNA extraction methods impacted the sensitivity of the assays as well as the type of sample used as template for the DNA extraction. Conclusions: The results are discussed in terms of current use of species diagnostic assays and the future development of molecular tools for the rapid differentiation of cryptic *Culicoides* species.

Morphological identification of *Lucilia sericata*, *Lucilia cuprina* and their hybrids (Diptera, Calliphoridae)

Williams, KA; Villet, MH. 2014

Hybrids of *Lucilia sericata* and *Lucilia cuprina* have been shown to exist in previous studies using molecular methods, but no study has shown explicitly that these hybrids can be identified morphologically. Published morphological characters used to identify *L. sericata* and *L. cuprina* were reviewed, and then scored and tested using specimens of both species and known hybrids. Ordination by multi-dimensional scaling indicated that the species were separable, and that hybrids resembled *L. cuprina*, whatever their origin. Discriminant function analysis of the characters successfully separated the specimens into three unambiguous groups-*L. sericata*, *L. cuprina* and hybrids. The hybrids were morphologically similar irrespective of whether they were from an ancient introgressed lineage or more modern. This is the first evidence that hybrids of these two species can be identified from their morphology. The usefulness of the morphological characters is also discussed and photographs of several characters are included to facilitate their assessment.

Phylogenetic analysis of the GST family in *Anopheles (Nyssorhynchus) darlingi*

de Azevedo, GM; Guimaraes-Marques, GM; Bridi, LC; Ohse, KC; Vicentini, R; Tadei, W; Rafael, MS. 2014

Anopheles darlingi Root, 1926 and *Anopheles gambiae* (Diptera: Culicidae) are the most important human malaria vectors in South America and Africa, respectively. The two species are estimated to have diverged 100 million years ago. Studies on the phylogenetics and evolution of gene sequences, such as glutathione S-transferase (GST) in disease-transmitting mosquitoes are scarce. The sigma class GST (KC890767) from the transcriptome of *An. darlingi* captured in the Brazilian Amazon was studied by in silico hybridization, and mapped to chromosome 3 of *An. gambiae*. The sigma class GST of *An. darlingi* was used for phylogenetic analyses to understand the GST base composition of the most recent common ancestor between *An. darlingi*, *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*. The GST (KC890767) of *An. darlingi* was studied to generate the main divergence branches using a Neighbor-Joining and bootstrapping approaches to confirm confidence levels on the tree nodes that separate the *An. darlingi* and other mosquito species. The results showed divergence between *An. gambiae*, *Ae. Aegypti*, *Cx. quinquefasciatus*, and *Phlebotomus papatasi* as outgroup, and the homology relationship between sigma class GST of *An. darlingi* and GSTS1_1 gene of *An. gambiae* was valuable for phylogenetic and evolutionary studies. (C) 2014 Elsevier B.V. All rights reserved.

Cloning, expression, and purification of a new antimicrobial peptide gene from *Musca domestica* larva

Pei, ZH; Sun, XN; Tang, Y; Wang, K; Gao, YH; Ma, HX. 2014

Musca domestica (Diptera: Muscidae), the housefly, exhibits unique immune defences and can produce antimicrobial peptides upon stimulation with bacteria. Based on the cDNA library constructed using the suppression subtractive hybridization (SSH) method, a 198-bp antimicrobial peptide gene, which we named MDAP-2, was amplified by rapid amplification of cDNA ends (RACE) from *M. domestica* larvae stimulated with *Salmonella pullorum* (Enterobacteriaceae: Salmonella). In the present study, the full-length MDAP-2 gene was cloned and inserted into a His-tagged *Escherichia coli* prokaryotic expression system to enable production of the recombinant peptide. The recombinant MDAP-2 peptide was purified using Ni-NTA HisTrap FF crude column chromatography. The bacteriostatic activity of the recombinant purified MDAP-2 protein was assessed. The results indicated that MDAP-2 had in vitro antibacterial activity against all of the tested Gram-bacteria from clinical isolates, including *E. coli* (Enterobacteriaceae: Escherichia), one strain of *S. pullorum* (Enterobacteriaceae: Salmonella), and one strain of *Pasteurella multocida*. DNA sequencing and BLAST analysis showed that the MDAP-2 antimicrobial peptide gene was not homologous to any other antimicrobial peptide genes in GenBank. The

Distribution of *Pseudacteon* spp. (Diptera: Phoridae), biological control agents of *Solenopsis* spp. (Hymenoptera: Formicidae), in Louisiana and associated prevalence of *Kneallhazia solenopsae* (Microsporidia: Thelohaniidae)

Meszaros, A; Oi, DH; Valles, SM; Beuzelin, JM; Reay-Jones, FPF; Johnson, SJ. 2014

Phorid flies, *Pseudacteon* spp. (Diptera: Phoridae), have been released in the United States since 1996 as biological control agents for imported fire ants, *Solenopsis invicta* Buren, *Solenopsis richteri* Forel, and their hybrid (Hymenoptera: Formicidae). A statewide survey was conducted in Louisiana during 2009 and 2010 to determine the distribution of *Pseudacteon tricuspidis* Borgmeier and *Pseudacteon curvatus* Borgmeier, and to quantify the potential spatial association between the two species. Additionally, collected phorid flies were tested for the microsporidium *Kneallhazia* (=Thelohania) *solenopsae* (Knell, Allen, and Hazard), another natural enemy of fire ants, to establish an account of the pathogen's distribution and prevalence in phorid fly populations. *P. tricuspidis* and *P. curvatus* have expanded their range from four and three separate release sites, respectively, in Louisiana. By 2010, *P. tricuspidis* and *P. curvatus* occupied approximately 86,600 and 111,900 km², respectively. Overall, *P. curvatus* was more abundant than *P. tricuspidis*, with 41.9 +/- 7.3 (SE) and 9.8 +/- 1.5 (SE) females, respectively, collected on average per sampling site in 2009. Spatial Analysis by Distance IndicEs (SADIE) suggested aggregation for the two phorid fly species. *P. tricuspidis* collections were positively associated with *P. curvatus* collections, indicating that gaps and clusters of both species generally overlapped. *K. solenopsae* was detected in 22 of 64 parishes, and among 124 collection sites tested, 19% yielded positive responses for *K. solenopsae*. *P. tricuspidis* males and females and *P. curvatus* females were found to harbor *K. solenopsae*. This study is the first to detect *K. solenopsae* in *P. tricuspidis*. (C) 2014 Elsevier Inc. All rights reserved.

Mating Compatibility Between *Bactrocera invadens* and *Bactrocera dorsalis* (Diptera: Tephritidae)

Bo, W; Ahmad, S; Dammalage, T; Tomas, US; Wornoaiporn, V; Ul Haq, I; Caceres, C; Vreysen, MJB; Hendrichs, J; Schutze, MK. 2014

The invasive fruit fly, *Bactrocera invadens* Drew, Tsuruta & White, is a highly polyphagous fruit pest that occurs predominantly in Africa yet has its origins in the Indian subcontinent. It is extremely morphologically and genetically similar to the Oriental fruit fly, *Bactrocera dorsalis* (Hendel); as such the specific relationship between these two species is unresolved. We assessed prezygotic compatibility between *B. dorsalis* and *B. invadens* using standardized field cage mating tests, which have proven effectiveness in tephritid cryptic species studies. These tests were followed by an assessment of postzygotic compatibility by examining egg viability, larval and pupal survival, and sex ratios of offspring produced from parental and subsequent F1 crosses to examine for hybrid breakdown as predicted under a two-species hypothesis. *B. dorsalis* was sourced from two countries (Pakistan and China), and each population was compared with *B. invadens* from its type locality of Kenya. *B. invadens* mated randomly with *B. dorsalis* from both localities, and there were generally high levels of hybrid viability and survival resulting from parental and F1 crosses. Furthermore, all but one hybrid cross resulted in equal sex ratios, with the single deviation in favor of males and contrary to expectations under Haldane's rule. These data support the hypothesis that *B. dorsalis* and *B. invadens* represent the same biological species, an outcome that poses significant implications for pest management and international trade for sub-Saharan Africa.

Development of a genetic sexing strain in *Bactrocera carambolae* (Diptera: Tephritidae) by introgression of sex sorting components from *B. dorsalis*, Salaya1 strain

Isasawin, S; Aketarawong, N; Lertsiri, S; Thanaphum, S. 2014

Background: The carambola fruit fly, *Bactrocera carambolae* Drew & Hancock is a high profile key pest that is widely distributed in the southwestern ASEAN region. In addition, it has trans-continently invaded Suriname, where it has been expanding east and southward since 1975. This fruit fly belongs to *Bactrocera dorsalis* species complex. The development and application of a genetic sexing strain (Salaya1) of *B. dorsalis* sensu stricto (s.s.) (Hendel) for the sterile insect technique (SIT) has improved the fruit fly control. However, matings between *B. dorsalis* s.s. and *B. carambolae* are incompatible, which hinder the application of the Salaya1 strain to control the carambola fruit fly. To solve this problem, we introduced genetic sexing components from the Salaya1 strain into the *B. carambolae* genome by interspecific hybridization. Results: Morphological characteristics, mating competitiveness, male pheromone profiles, and genetic relationships revealed consistencies that helped to distinguish Salaya1 and *B. carambolae* strains. A Y-autosome translocation linking the dominant wild-type allele of white pupae gene and a free autosome carrying a recessive white pupae homologue from the Salaya1 strain were introgressed into the gene pool of *B. carambolae*. A panel of Y-pseudo-linked microsatellite loci of the Salaya1 strain served as markers for the introgression experiments. This resulted in a newly derived genetic sexing strain called Salaya5, with morphological characteristics corresponding to *B. carambolae*. The rectal gland pheromone profile of Salaya5 males also contained a distinctive component of *B. carambolae*. Microsatellite DNA analyses confirmed the close genetic relationships between the Salaya5 strain and wild *B. carambolae* populations. Further experiments showed that the sterile males of Salaya5 can compete with wild males for mating with wild females in field cage conditions. Conclusions: Introgression of sex sorting components from the Salaya1 strain to a closely related *B. carambolae* strain generated a new genetic sexing strain, Salaya5. Morphology-based taxonomic characteristics, distinctive pheromone components, microsatellite DNA markers, genetic relationships, and mating competitiveness provided parental baseline data and validation tools for the new strain. The Salaya5 strain shows a close similarity with those features in the wild *B. carambolae* strain. In addition, mating competitiveness tests suggested that Salaya5 has a potential to be used in *B. carambolae* SIT programs based on male-only releases.

Population genetics of neotropical *Culex quinquefasciatus* (Diptera: Culicidae)

Wilke, ABB; Vidal, PO; Suesdek, L; Marrelli, MT. 2014

Background: *Culex quinquefasciatus* mosquitoes can be found in almost every major city of Brazil and are vectors of filariasis and several arboviruses. Microsatellite markers have been widely used to uncover the genetic structure of various groups of insect populations. The aim of this study was to glimpse the genetic structure of *Cx. quinquefasciatus* in Brazil. Methods: Nine populations were sampled across Brazil (one of them from a laboratory colony - COL) and another one from Argentina and process regarding the variability of six microsatellite loci. Results: The analyzed loci revealed moderate population genetic structure (mean F_{st} = 0.12). Dendrograms of genetic distances evidenced two major population clusters, respectively corresponding to the northern and southern populations. The hybrid population *Cx. pipiens/quinquefasciatus* (from La Plata, Argentina) and the colony population fell outside the major clusters. Those clusters were substructured and there was a significant correlation between genetic and geographic distances and environmental variables ($r = 0.51$; $p > 0.001$ and $r = 0.46$; $p > 0.004$). Conclusions: Multilocus cluster Bayesian analysis confirmed that populations are mutually distinct, and the set of results point to genetic differences among populations. The presumable low gene flow among them may be due to the large geographic distances (> 1000 km) and to the environmental heterogeneity of the sampled areas. The genetic structure observed in this study may lead to the best understanding of *Cx. quinquefasciatus* demographical diversity as well as their genetic variations patterns in Brazil so far unknown.

Physiological Integration Plays Key Role in Cranberry (Ericales: Ericaceae) for Tolerance of Damage by *Dasineura oxycoccana* (Diptera: Cecidomyiidae)

Tewari, S; Buonaccorsi, JP; Averill, AL. 2014

Understanding the mechanisms by which plants tolerate herbivory is important in the study of insect-plant interactions. In cranberry, current season growth has been identified as the main source of photosynthate for the developing fruits. Feeding injury by larvae of cranberry tipworm, *Dasineura oxycoccana* Johnson, disrupts the

apical growth of cranberry shoots or uprights, but does not impact fruit output. To study the effects of experimentally depleting photosynthate available from sources other than the current season growth on fruit output, we girdled tipworm-injured uprights. This technique enabled us to estimate the contribution of current season growth in supplying photosynthate to developing fruits in tipworm-injured uprights. The mean fruit weight declined by >55% in those tipworm-injured uprights that were limited to photosynthate from only the current season growth (girdled uprights). The result was consistent between two phenologically different cultivars of cranberry, one a native selection from wild cranberry stands ('Howes') and the other a hybrid ('Stevens'). In addition, fruit weight was positively correlated to current season leaf area in the girdled uprights only. These results strongly suggest that physiological integration among the different sources of photosynthate plays a key role in the tolerance of tipworm feeding injury for fruit output in cranberry.

Sympatric occurrence of *Culex pipiens* (Diptera, Culicidae) biotypes *pipiens*, *molestus* and their hybrids in Portugal, Western Europe: feeding patterns and habitat determinants

Osorio, HC; Ze-Ze, L; Amaro, F; Nunes, A; Alves, MJ. 2014

Hybridization and the spread of the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae), in the northwestern United States

Arcella, T; Hood, GR; Powell, THQ; Sim, SB; Yee, WL; Schwarz, D; Egan, SP; Goughnour, RB; Smith, JJ; Feder, JL. 2015

Hybridization may be an important process interjecting variation into insect populations enabling host plant shifts and the origin of new economic pests. Here, we examine whether hybridization between the native snowberry-infesting fruit fly *Rhagoletis zephyria* (Snow) and the introduced quarantine pest *R.pomonella* (Walsh) is occurring and may aid the spread of the latter into more arid commercial apple-growing regions of central Washington state, USA. Results for 19 microsatellites implied hybridization occurring at a rate of 1.44% per generation between the species. However, there was no evidence for increased hybridization in central Washington. Allele frequencies for seven microsatellites in *R.pomonella* were more *R.zephyria*-like in central Washington, suggesting that genes conferring resistance to desiccation may be adaptively introgressing from *R.zephyria*. However, in only one case was the putatively introgressing allele from *R.zephyria* not found in *R.pomonella* in the eastern USA. Thus, many of the alleles changing in frequency may have been prestanding in the introduced *R.pomonella* population. The dynamics of hybridization are therefore complex and nuanced for *R.pomonella*, with various causes and factors, including introgression for a portion, but not all of the genome, potentially contributing to the pest insect's spread.

Population Genetic Structure of the Malaria Vector *Anopheles sinensis* (Diptera: Culicidae) Sensu Stricto and Evidence for Possible Introgression in the Republic of Korea

Kang, S; Jung, J; Kim, W. 2015

Anopheles sinensis Wiedemann sensu stricto (s.s.) is a dominant mosquito and considered a secondary malaria vector in the Republic of Korea (ROK). Despite the potential significance for malaria control, population genetics studies have been conducted using only mitochondrial DNA (mtDNA), and studies of the genetics of hybridization have never been attempted. In this study, 346 specimens from 23 localities were subject to experiments. Among them, 305 *An. sinensis* s.s. specimens from 20 localities were used for mtDNA analysis, and 346 specimens comprising 341 *An. sinensis* s.s. from 22 localities and five *Anopheles kleini* Rueda from one locality were examined in the microsatellite study. Neighbor-joining analysis of pairwise F-ST and R-ST based on microsatellite results showed that the populations are divided into two groups, as did the mtDNA results. However, the Bayesian analysis and factorial correspondence analysis plots showed three distinct clusters. Among the mtDNA and microsatellite results, only microsatellites represented small but positive and significant isolation-by-distance patterns. Both molecular markers show the Taebaek and Sobaek Mountain ranges as barriers between the northern and southern parts of the ROK. The newly recognized third group suggests possible introgressive hybridization of *An. sinensis* s.s. with closely related species. The slightly different composition of populations in each group based on different markers is probably because of different population dynamics in each group. These results imply that there is restricted gene flow of epidemiologically important malaria-related genes between the northern and southern parts of the ROK.

Divergent host preferences of above- and below-ground *Culex pipiens* mosquitoes and their hybrid offspring

Fritz, ML; Walker, ED; Miller, JR; Severson, DW; Dworkin, I. 2015

Culex pipiens form *pipiens* and *Cx.pipiens* form *molestus* (Diptera: Culicidae) belong to a cosmopolitan taxonomic group known as the *Pipiens* Assemblage. Hybridization between these forms is thought to contribute to human transmission of West Nile virus (WNV) in North America. Complementary choice and no-choice landing assays were developed to examine host acceptance by North American *Cx.pipiens* in the laboratory. Populations collected from above- and below-ground sites in suburban Chicago were identified as forms *pipiens* and *molestus* using a polymerase chain reaction-based assay. Avian and human host acceptance was then quantified for the two populations, as well as for their hybrid and backcross offspring. No-choice tests were used to demonstrate that both the *pipiens* and *molestus* forms were capable of feeding on human and avian hosts. Choice tests were used to demonstrate that form *pipiens* females were strongly avian-seeking; an individual's probability of accepting the chick host was 85%. Form *molestus* females were more likely to accept the human host (87%). Rates of host acceptance by F-1 and backcross progeny were intermediate to those of their parents. The results suggest that host preferences in *Cx.pipiens* are genetically determined, and that ongoing hybridization between above- and below-ground populations is an important contributor to epizootic transmission of WNV in North America.

Biology of *Anastrepha grandis* (Diptera: Tephritidae) in Different Cucurbits

Bolzan, A; Nava, DE; Garcia, FRM; Valgas, RA; Smaniotto, G. 2015

Anastrepha grandis (Macquart) (Diptera: Tephritidae) is one of the main pests of cucurbits in Brazil. Losses occur due to the damage caused to the fruits and the embargo on exports, as *A. grandis* is considered a quarantine pest in countries that import Brazilian cucurbits. This study aimed to evaluate the development of *A. grandis* in hosts of the Cucurbitaceae family. The hosts used were stem squash (*Cucurbita pepo* L.), squash (*Cucurbita moschata* Duchesne), chayote [*Sechium edule* (Jacq.) Swartz], mini watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai], Spanish melon (*Cucumis melo* L.), hybrid squash "Tetsukabuto" (*C. moschata* x *Cucurbita maxima* Duchesne), and salad cucumber (*Cucumis sativus* L.). We evaluated the viability and duration of egg-to-pupa period, pupal weight, sex ratio, and average number of pupae per fruit under controlled conditions of temperature, relative humidity, and photophase. The preoviposition and oviposition periods, fecundity, fertility, and longevity of females were determined for adults. Hosts of the genus *Cucurbita* provided a better development of *A. grandis* in comparison with other hosts, and presented a greater number of insects on fruit as well as higher infestation rate. Fecundity and longevity were also higher for females that developed in hosts of the genus *Cucurbita*, although values of these biological parameters varied between stem squash, squash, hybrid squash "Tetsukabuto".

Mom Matters: Diapause Characteristics of *Culex pipiens*-*Culex quinquefasciatus* (Diptera: Culicidae) Hybrid Mosquitoes

Meuti, ME; Short, CA; Denlinger, DL. 2015

Females of the northern house mosquito, *Culex pipiens* L., are capable of entering an adult overwintering diapause characterized by arrested ovarian development, enhanced stress tolerance, and elevated lipid stores. In contrast, the southern house mosquito, *Culex quinquefasciatus* Say, lacks this capacity and is therefore unable to survive the harsh winters found in northern regions of North America. These two species are capable of forming fertile hybrids in the United States, yet the diapause characteristics of these hybrids have not been extensively investigated. We crossed *Cx. pipiens* from Columbus, OH, with *Cx. quinquefasciatus* from Vero Beach, FL, and reared F-1 hybrids from all mothers separately under diapause-inducing, short-day conditions (a photoperiod of 8: 16 [L: D] h) at 18 degrees C. Egg follicle length and lipid content were used to assess the diapause status of hybrids. Diapause incidence of hybrids varied widely for progeny from different mothers of the same species, but hybrids with *Cx. pipiens* mothers were consistently more prone to enter diapause than hybrids that had *Cx. quinquefasciatus* mothers. Our results suggest a strong maternal influence on the diapause phenotype and that a high percentage (45-75%) of *Cx. pipiens*-*Cx. quinquefasciatus* hybrids are capable of entering diapause. This implies that many hybrids can successfully overwinter, leading to a possible widening of the hybrid zone of these two species in North America.

Experimental host preference of diapause and non-diapause induced *Culex pipiens pipiens* (Diptera: Culicidae)

Faraji, A; Gaugler, R. 2015

Background: *Culex pipiens pipiens* plays an important role in the transmission of several vector-borne pathogens such as West Nile virus (WNV) in North America. Laboratory and field studies suggest that this species is ornithophilic but because of genetic hybridization with sibling species during the active mosquito season, it may occasionally feed on mammals. Adult female *Cx. p. pipiens* undergo a facultative diapause and may serve as an overwintering mechanism for WNV. To determine the effect of diapause on the innate host preference of *Cx. p. pipiens* emerging from winter hibernation, we conducted host-choice experiments using bird and mammal hosts. Methods: Mosquitoes were reared under non-diapause induced (NDI), diapause induced (DI), and field collected from overwintering (OW) hibernaculae. They were released into a large mesh enclosure housing two lard can traps, and given a choice between feeding on a dove or a rat. Results: Host seeking *Cx. p. pipiens* were four times more likely to feed on the dove than the rat, regardless of experimental conditions. Under NDI conditions, *Cx. p. pipiens* were ($p < 0.001$) more attracted to the bird (79.9 % [75.6-84.1]) than the rat (20.1 [15.9-24.4]). Overwintering mosquitoes and those exposed to DI conditions were also significantly ($p < 0.001$) more attracted to birds (81.6 % [75.9-87.3]) than to rats (18.5 [12.7-24.2])." Conclusions: We provide new information about the innate host preference of *Cx. p. pipiens* emerging from diapause in temperate habitats where winter survival is crucial for disease transmission cycles. Although we showed that *Cx. p. pipiens* prefers an avian to a mammalian host, nearly 20 % of emerging mosquitoes in the spring could feed on mammals. Changes in host preferences may also contain valuable clues about transmission dynamics and subsequent timely interventions by vector control and public health practitioners.

Developmental ecdysteroid titers and DNA puffs in larvae of two sciarid species, *Rhynchosciara americana* and *Rhynchosciara milleri* (Diptera: Sciaridae)

Soares, MAM; Hartfelder, K; de Souza, JMT; Stocker, AJ. 2015

Ecdysteroid titers, developmental landmarks and the presence of prominent amplifying regions (DNA puffs) have been compared during late larval to pupal development in four groups of *Rhynchosciara americana* larvae and in *R. americana* and *Rhynchosciara milleri*. Three prominent DNA puffs (B2, C3 and C8) expand and regress sequentially on the rising phase of the 20-hydroxyecdysone (20E) titer in *R. americana* as a firm, cellular cocoon is being constructed. A sharp rise in 20E coincides with the regression of these puffs. The shape of the 20E curve is similar in *R. milleri*, a species that does not construct a massive cocoon, but the behavior of certain DNA puffs and their temporal relationship to the curve differs. Regions corresponding to B2 and C3 can be identified in *R. milleri* by banding pattern similarity with *R. americana* chromosomes and, in the case of B2, by hybridization to an *R. americana* probe. A B2 puff appears in *R. milleri* as the 20E titer rises but remains small in all gland regions. A puff similar to the *R. americana* C3 puff occurs in posterior gland cells of *R. milleri* (C3(Rm)) after the B2 puff, but this site did not hybridize to *R. americana* C3 probes. C3(Rm) incorporated H-3-thymidine above background, but showed less post-puff DNA accumulation than C3 of *R. americana*. *R. americana* C8 probes hybridized to a more distal region of the *R. milleri* C chromosome that did not appear to amplify or form a large puff. These differences can be related to developmental differences, in particular differences in cocoon construction between the two species.

New evidence for the potential role of *Culex pipiens* mosquitoes in the transmission cycle of West Nile virus in Tunisia

Krida, G; Rhim, A; Daaboub, J; Failloux, AB; Bouattour, A. 2015

Physiological and molecular characteristics of natural populations of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) were investigated to elucidate how this species is potentially involved in the transmission of West Nile virus in Tunisia. A total of 215 *Cx. pipiens* females from 11 breeding habitats were analysed in the laboratory to estimate autogeny and stenogamy rates. They were tested individually for the locus CQ11 to distinguish between the two *Cx. pipiens* forms, *pipiens* and *molestus*. All tested *Cx. pipiens* populations were stenogamous. Females from underground breeding sites were all autogeneious, whereas females from above-ground habitats were mostly anautogeneious. Of all the females tested, 59.7% were identified as *pipiens*, 22.4% as *molestus*, and 17.9% as hybrid *pipiens/molestus*. Furthermore, both *Cx. pipiens* forms and their hybrids were found to co-occur in sympatry in all sites. The results of this study represent the first evidence that both *Cx. pipiens* forms and their hybrids are present in Tunisia. Because hybrids able to act as bridge vectors are present in all studied habitats, Tunisia can be considered to have a high degree of receptivity for the establishment of West Nile virus zoonotic cycles.

Defining Genetic, Taxonomic, and Geographic Boundaries Among Species of the *Psorophora confinnis* (Diptera: Culicidae) Complex in North and South America

Lanzaro, GC; Collier, TC; Lee, Y. 2015

The *Psorophora confinnis* complex is currently composed of three species-*Psorophora confinnis* sensu stricto (Lynch Arribalzaga) in South America, *Psorophora columbiae* (Dyar and Knab) in North America, and *Psorophora jamaicensis* (Theobald) in the Caribbean. Members of the complex are of considerable importance as vectors of arboviruses, for example, Venezuelan equine encephalitis virus, and are significant biting pests throughout their range. The biological and geographic boundaries of *Ps. confinnis* and *Ps. columbiae* are unclear. In fact, the name *Ps. columbiae* is presently designated as "provisional." In this article, we aim to clarify the taxonomy and geographic distributions of species within the *Ps. confinnis* complex. A population genetics approach was employed using gene and genotypic frequency data at 26 isozyme loci. The results suggest that the *Ps. confinnis* complex in North and South America is composed of four species. *Ps. confinnis* s.s. and *Ps. columbiae* are distinct species in South and North America, respectively. Populations in Colombia, South America, formally designated as *Ps. funiculus* (Dyar) and populations in the southwestern United States and western Mexico, formally designated *Ps. toltecum* (Dyar and Knab), are distinct species. *Psorophora toltecum* and *Psorophora funiculus* species names should be resurrected from synonymy. In addition we identified a *Ps. columbiae* and *Ps. toltecum* hybrid zone in central Texas in a region described as being one of 13 North American suture zones, being geographical areas in which closely related species occur in sympatry and frequently hybridize.

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.

Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* mosquitoes in Ibadan, Southwest Nigeria

Okorie, PN; Ademowo, OG; Irving, H; Kelly-Hope, LA; Wondji, CS. 2015

The emergence of insecticide resistance in *Anopheles* (Diptera: Culicidae) mosquitoes has great implications for malaria control in Nigeria. This study aimed to determine the dynamics of insecticide susceptibility levels and the frequency of knock-down resistance (*kdr*) mutations (L1014F) in wild *Anopheles coluzzii* Coetzee & Wilkerson sp. n. and *Anopheles gambiae* Giles from the Ojoo and Bodija areas of Ibadan, in southwest Nigeria. Insecticide susceptibility to pyrethroids, organophosphates, carbamates and organochlorines was assessed using World Health Organization (WHO) bioassays. A subset of the mosquitoes exposed to pyrethroids and DDT was used for species and molecular form identification; *kdr* genotyping was determined using the TaqMan real-time polymerase chain reaction assay. The mosquitoes were resistant to pyrethroids and DDT but completely susceptible to organophosphates and carbamates. Bodija samples (*n*=186) consisted of *An. gambiae* (91.4%) and *An. coluzzii* (8.1%) and included one *An. coluzzii*/*An. gambiae* hybrid specimen. All mosquitoes screened in Ojoo (*n*=26) were *An. gambiae*. The L1014F *kdr* mutation was detected at frequencies of 24.5 and 5.8% in Bodija and Ojoo, respectively. No correlation was observed between *kdr* genotypes and resistance phenotypes. The results indicate that metabolic resistance probably plays an important role in the development of resistance and highlight the need to implement insecticide resistance management strategies.

Phylogeny of anopheline (Diptera: Culicidae) species in southern Africa, based on nuclear and mitochondrial genes

Norris, LC; Norris, DE. 2015

A phylogeny of anthropophilic and zoophilic anopheline mosquito species was constructed, using the nuclear internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome oxidase subunit I (COI) genes. The ITS2 alignment, typically difficult due to its noncoding nature and large size variations, was aided by using predicted secondary structure, making this phylogenetically useful gene more amenable to investigation. This phylogeny is unique in explicitly including zoophilic, non-vector anopheline species in order to illustrate their relationships to malaria vectors. Two new, cryptic species, *Anopheles funestus*-like and *Anopheles rivulorum*-like, were found to be present in Zambia for the first time. Sequences from the D3 region of the 28S rDNA suggest that the Zambian *An. funestus*-like may be a hybrid or geographical variant of *An. funestus*-like, previously reported in Malawi. This is the first report of *An. rivulorum*-like sympatric with *An. rivulorum* (Leeson), suggesting that these are separate species rather than geographic variants.

Inter-regional mating compatibility among *Bactrocera dorsalis* populations in Thailand (Diptera, Tephritidae)

Chinvinijkul, S; Srikachar, S; Kumjing, P; Kimjong, W; Sukamnouyorn, W; Polchaimat, N. 2015

Mating compatibility among recently colonized (wildish) populations of *Bactrocera dorsalis* (Hendel) from different geographic origins in Thailand was assessed through inter-regional mating tests. Outdoor octagonal nylon screen field cages containing single potted mango trees (*Mangifera indica* L.) were used. Sexual compatibility was determined using the index of sexual isolation (ISI), the male relative performance index (MRPI), and the female relative performance index (FRPI). The ISI values indicated that the northern population of *B. dorsalis* from Chiang Mai province was sexually compatible with the southern population of *B. dorsalis* (previously *B. papayae*) from Nakhon Si Thammarat province. The MRPI values showed that the northern males had a slightly higher tendency to mate than southern males, while the FRPI data reflected that females of both origins participated equally in matings. In all combinations there were no differences between homotypic and heterotypic couples in mating latency. Southern males tended to mate first with southern females, followed by northern males mating with northern females, while the latest matings involved heterotypic couples, in particular northern males mating with southern females. Overall, more couples were collected from higher parts of the field cage and the upper tree canopy, while there were no differences between the origins of flies in terms of elevation of couples within the cage. Laboratory assessments of fecundity showed no differences in the average number of eggs resulting from inter-regional crosses. Development of immature stages was also equal in the two hybrid crosses, with no differences found in the number of pupae produced, percentage pupal recovery, and percent adult emergence. The practical implication of this study is that colony of *B. dorsalis* derived from any northern or southern region of Thailand can potentially be used in sterile insect technique programs against this pest.

Assortative mating and maintenance of intrapopulation polymorphism in wild populations and laboratory cultures of insects

Benkovskaya, GV; Nikonorov, YM. 2015

Speciation as a micro-evolutionary process begins with emerging of intraspecies differentiation, which is associated with establishment of reproductive isolation. One of intrinsic isolation factors ensuring physiological isolation is assortative mating. In course of long-term field observations in the Southern Urals (Republic of Bashkortostan) and prolonged laboratory experiments, we have detected assortative mating in populations of potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Three morphotypes were singled out by the extent of integument melanization, namely achromists (A), melanists (M), and intermediate type (I), and frequency of occurrence of these morphotypes imago pairs significantly deviated from random distribution, thus manifesting the assortative mating. Under in copulo conditions, mating between achromist males (A) and melanist females (M) where active choice belonged to males was not registered. In laboratory experiments, in the sample of 40 artificially formed pairs, there were detected significant differences in longevity and fecundity of different morphotype imagoes. Achromists and melanists had significantly (almost two times) higher longevity as compared with intermediate type. Females of intermediate type were significantly more fecund in homonomous crossings than achromists and melanists. It is shown that pairs offspring also differs significantly in viability, with highest viability being characteristic for offspring from females of A- and I-type. The differences revealed are indicative of different reproductive strategies that exist in populations. In laboratory line S of house fly *Musca domestica* L. (Diptera: Muscidae) the presence of individuals with different reproductive strategies related to longevity is detected. Maximum longevity in inbred lines Sh28 (short living) and L2 (long living), isolated from the line S, significantly differed almost twofold. In the line of short living flies, mass reproduction occurs on the first second week of imago stage, while in long living flies it is put off to the third fourth week. In reciprocal crossings between the lines, drastic reduction in females fecundity is observed as well as reduction in viability of females from hybrid offspring. The results obtained are indicative of substantial role of assortative mating and frequency-dependent selection in maintenance of intrapopulation diversity.

Genome sequence of *Phormia regina* Meigen (Diptera: Calliphoridae): implications for medical, veterinary and forensic research

Andere, AA; Li, RNP; Ray, DA; Picard, CJ. 2016

Background: Blow flies (Diptera: Calliphoridae) are important medical, veterinary and forensic insects encompassing 8 % of the species diversity observed in the

calyptate insects. Few genomic resources exist to understand the diversity and evolution of this group. Results: We present the hybrid (short and long reads) draft assemblies of the male and female genomes of the common North American blow fly, *Phormia regina* (Diptera: Calliphoridae). The 550 and 534 Mb draft assemblies contained 8312 and 9490 predicted genes in the female and male genomes, respectively; including > 93 % conserved eukaryotic genes. Putative X and Y chromosomes (21 and 14 Mb, respectively) were assembled and annotated. The *P. regina* genomes appear to contain few mobile genetic elements, an almost complete absence of SINEs, and most of the repetitive landscape consists of simple repetitive sequences. Candidate gene approaches were undertaken to annotate insecticide resistance, sex-determining, chemoreceptors, and antimicrobial peptides. Conclusions: This work yielded a robust, reliable reference calliphorid genome from a species located in the middle of a calliphorid phylogeny. By adding an additional blow fly genome, the ability to tease apart what might be true of general calliphorids vs. what is specific of two distinct lineages now exists. This resource will provide a strong foundation for future studies into the evolution, population structure, behavior, and physiology of all blow flies.

Dominant fitness costs of resistance to fipronil in *Musca domestica* Linnaeus (Diptera: Muscidae)

Abbas, N; Shah, RM; Shad, SA; Azher, F. 2016

House fly, *Musca domestica* L. (Diptera: Muscidae) a common pest of poultry, has developed resistance to the commonly used insecticide fipronil. The life history traits were examined in the fipronil-selected (Fipro-SEL), susceptible counterpart (UNSEL), and their hybrid progeny strains in order to design an effective resistant management strategy. Compared to the UNSEL strain, the Fipro-SEL was 181.94-fold resistant to fipronil. This resistance was unstable after five generations without selection. The Fipro-SEL had a significantly longer larval duration, lower pupal weight, lower fecundity, lower hatchability, lower number of next generation larvae, lower intrinsic rate of population increase and lower biotic potential than the UNSEL strain. Most fitness parameters of the hybrid progeny were similar and significantly lower than that in the UNSEL strain, suggesting autosomal and dominant fitness costs. Compared to the UNSEL strain, relative the fitness of the Fipro-SEL, Hybrid1 and Hybrid2 was 0.13, 0.33 and 0.30, respectively. Fipronil resistance resulted in high fitness costs and these fitness costs were dominant and autosomal in the Fipro-SEL strain of *M. domestica*. Rotation of fipronil with other insecticides having no cross resistance should be useful for delaying the development of resistance in *M. domestica*. (C) 2016 Elsevier B.V. All rights reserved.

Rearing of *Anopheles beklemishevi* (Diptera: Culicidae) and the possibility of its hybridization with *An. atroparvus* under laboratory conditions

Novikov, YM. 2016

Oviposition of *Culex tarsalis* (Diptera: Culicidae) Differs on Water Conditioned by Potential Fish and Insect Predators

Why, AM; Lara, JR; Walton, WE. 2016

The response of egg-laying *Culex tarsalis* Coquillett (Diptera: Culicidae) to water conditioned by three fish species used for mosquito control and three predatory aquatic insect species was examined in laboratory binary choice experiments. Oviposition by *Cx. tarsalis* was 72% less on water conditioned with the arroyo chub, *Gila orcutti* (Eigenmann & Eigenmann) (Cypriniformes: Cyprinidae) relative to control cups containing aged tap water, but no significant difference was found in the numbers of egg rafts laid on water conditioned with the fathead minnow (*Pimephales promelas* (Rafinesque), Cypriniformes: Cyprinidae) and the control treatment (water aged 24 h). Mosquito oviposition on water conditioned with the predominantly herbivorous/algivorous California Mozambique tilapia hybrid (*Oreochromis mossambicus* (Peters) x *Oreochromis urolepis hornorum* L. (Perciformes: Cichlidae)) or predatory insects (nymphs: *Sympetrum corruptum* (Hagen) (Odonata: Libellulidae); adults: *Thermonectus basillaris* (Harris) or *Cybister fimbriolatus* (Say) (Coleoptera: Dytiscidae)) did not differ significantly relative to that onto water aged for 24 h. As compared with water aged 24 h and water conditioned with diving beetles, oviposition by *Cx. tarsalis* was significantly lower ($\geq 53\%$) when live predatory diving beetles were present in oviposition cups. Gravid *Cx. tarsalis* females do not respond equally to putative semiochemicals in water conditioned with the piscine or aquatic insect predators of immature mosquitoes tested here.

Anchored enrichment dataset for true flies (order Diptera) reveals insights into the phylogeny of flower flies (family Syrphidae)

Young, AD; Lemmon, AR; Skevington, JH; Mengual, X; Stahls, G; Reemer, M; Jordaens, K; Kelso, S; Lemmon, EM; Hauser, M; De Meyer, M; Misof, B; Wiegmann, BM. 2016

Background: Anchored hybrid enrichment is a form of next-generation sequencing that uses oligonucleotide probes to target conserved regions of the genome flanked by less conserved regions in order to acquire data useful for phylogenetic inference from a broad range of taxa. Once a probe kit is developed, anchored hybrid enrichment is superior to traditional PCR-based Sanger sequencing in terms of both the amount of genomic data that can be recovered and effective cost. Due to their incredibly diverse nature, importance as pollinators, and historical instability with regard to subfamilial and tribal classification, Syrphidae (flower flies or hoverflies) are an ideal candidate for anchored hybrid enrichment-based phylogenetics, especially since recent molecular phylogenies of the syrphids using only a few markers have resulted in highly unresolved topologies. Over 6200 syrphids are currently known and uncovering their phylogeny will help us to understand how these species have diversified, providing insight into an array of ecological processes, from the development of adult mimicry, the origin of adult migration, to pollination patterns and the evolution of larval resource utilization. Results: We present the first use of anchored hybrid enrichment in insect phylogenetics on a dataset containing 30 flower fly species from across all four subfamilies and 11 tribes out of 15. To produce a phylogenetic hypothesis, 559 loci were sampled to produce a final dataset containing 217,702 sites. We recovered a well resolved topology with bootstrap support values that were almost universally >95 %. The subfamily Eristalinae is recovered as paraphyletic, with the strongest support for this hypothesis to date. The ant predators in the Microdontinae are sister to all other syrphids. Syrphinae and Pipizinae are monophyletic and sister to each other. Larval predation on soft-bodied hemipterans evolved only once in this family. Conclusions: Anchored hybrid enrichment was successful in producing a robustly supported phylogenetic hypothesis for the syrphids. Subfamilial reconstruction is concordant with recent phylogenetic hypotheses, but with much higher support values. With the newly designed probe kit this analysis could be rapidly expanded with further sampling, opening the door to more comprehensive analyses targeting problem areas in syrphid phylogenetics and ecology.

Phylogenetic radiation of the greenbottle flies (Diptera, Calliphoridae, Luciliinae)

Williams, KA; Lamb, J; Villet, MH. 2016

The subfamily Luciliinae is diverse and geographically widespread. Its four currently recognised genera (*Dyscritomyia* Grimshaw, 1901, *Hemipyrellia* Townsend, 1918, *Hypopygiopsis* Townsend 1916 and *Lucilia* Robineau-Desvoidy, 1830) contain species that range from saprophages to obligate parasites, but their pattern of phylogenetic diversification is unclear. The 28S rRNA, COI and Period genes of 14 species of *Lucilia* and *Hemipyrellia* were partially sequenced and analysed together with sequences of 11 further species from public databases. The molecular data confirmed molecular paraphyly in three species-pairs in *Lucilia* that hamper barcode identifications of those six species. *Lucilia sericata* and *L. cuprina* were confirmed as mutual sister species. The placements of *Dyscritomyia* and *Hypopygiopsis* were ambiguous, since both made *Lucilia* paraphyletic in some analyses. Recognising *Hemipyrellia* as a genus consistently left *Lucilia* s.l. paraphyletic, and the occasionally-recognised (sub) genus *Phaenicia* was consistently paraphyletic, so these taxa should be synonymised with *Lucilia* to maintain monophyly. Analysis of a matrix of 14 morphological characters scored for adults of all genera and for most of the species included in the molecular analysis confirmed several of these findings. The different degrees of parasitism were phylogenetically clustered within this genus but did not form a graded series of evolutionary stages, and there was

Chemical Composition and Biological Activity of Essential Oils from Four *Nepeta* Species and Hybrids against *Aedes aegypti* (L.) (Diptera: Culicidae)[illegible]

The species composition of Chironomus in the Curonian Lagoon has been investigated. It was shown that three sibling species of the plumosus group, namely, Ch. plumosus, Ch. balatonicus, and Ch. muratensis, and a first-generation interspecific hybrid Ch. muratensis x Ch. plumosus lived here. The occurrence frequency in the lagoon was 87, 19, 6, and 3% for Ch. plumosus, Ch. balatonicus, Ch. muratensis x Ch. plumosus, and Ch. muratensis, respectively. The species Ch. plumosus was recorded in all the areas (southern, central, and northern), while Ch. balatonicus was found only in the northern area in the village of Juodkrante near the city of Klaipėda. Presumably, the distribution of these sibling species is due to the presence of a salinity gradient in the lagoon.

Millions of people die each year as a result of pathogens transmitted by mosquitoes. However, the morphological identification of mosquito species can be difficult even for experts. The identification of morphologically indistinguishable species, such as members of the *Anopheles maculipennis* complex (Diptera: Culicidae), and possible hybrids, such as *Culex pipiens pipiens*/*Culex pipiens molestus* (Diptera: Culicidae), presents a major problem. In addition, the detection and discrimination of newly introduced species can be challenging, particularly to researchers without previous experience. Because of their medical importance, the clear identification of all relevant mosquito species is essential. Using the direct polymerase chain reaction (PCR) method described here, DNA amplification without prior DNA extraction is possible and thus species identification after sequencing can be achieved. Different amounts of tissue (leg, head; larvae or adult) as well as different storage conditions (dry, ethanol, -20 and -80 degrees C) and storage times were successfully applied and showed positive results after amplification and gel electrophoresis. Overall, 28 different indigenous and non-indigenous mosquito species were analysed using a gene fragment of the COX1 gene for species differentiation and identification by sequencing this 658-bp fragment. Compared with standard PCR, this method is time- and cost-effective and could thus improve existing surveillance and control programmes.

Gall midges are insects specialized in maneuvering plant growth, metabolic and defense pathways for their benefit. The Asian rice gall midge and rice share such an intimate relationship that there is a constant battle for survival by either partner. Diverse responses by the rice host against the midge include necrotic hypersensitive resistance reaction, non-hypersensitive resistance reaction and gall-forming compatible interaction. Genetic studies have revealed that major R (resistance) genes confer resistance to gall midge in rice. Eleven gall midge R genes have been characterized so far in different rice varieties in India. In addition, no single R gene confers resistance against all the seven biotypes of the Asian rice gall midge, and none of the biotypes is virulent against all the resistance genes. Further, the interaction of the plant resistance gene with the insect avirulence gene is on a gene-for-gene basis. Our recent investigations involving suppressive subtraction hybridization cDNA libraries, microarray analyses, gene expression assays and metabolic profiling have revealed several molecular mechanisms, metabolite markers and pathways that are induced, down-regulated or altered in the rice host during incompatible or compatible interactions with the pest. This is also true for some of the pathways studied in the gall midge. Next generation sequencing technology, gene expression studies and conventional screening of gall midge cDNA libraries highlighted molecular approaches adopted by the insect to feed, survive and reproduce. This constant struggle by the midge to overcome the host defenses and the host to resist the pest has provided us with an opportunity to observe this battle for survival at the molecular level. (C) 2015 Elsevier Ltd. All rights reserved.

Phlebotomine sand flies are vectors of several important etiological agents. Sand fly cibaria and genitalia exhibit morphological characters that are essential for species identification. A morphologically anomalous female sand fly of the subgenus *Psychodopygus* Mangabeira was found in a faunistic survey of a forested area near Manaus, Amazonas State, Brazil. This specimen possesses two pairs of spermathecae and a cibarium with supernumerary rows of teeth. Most morphological anomalies in sand flies occur in the number and arrangement of spines in structures associated with the male genitalia. In females, the number of cibarial teeth is the most common form of anomaly. The specimen described here constitutes a rare anomalous record.

Di Luca, M; Toma, L; Boccolini, D; Severini, F; La Rosa, G; Minelli, G; Bongiorno, G; Montarsi, F; Arnoldi, D; Capelli, G; Rizzoli, A; Romi, R. 2016

Mosquitoes in the *Culex pipiens* complex are considered to be involved in the transmission of a range of pathogens, including West Nile virus (WNV). Although its taxonomic status is still debated, the complex includes species, both globally distributed or with a more limited distribution, morphologically similar and characterised by different physiological and behavioural traits, which affect their ability as vectors. In many European countries, *Cx. pipiens* and its sibling species *Culex torrentium* occur in sympatry, exhibiting similar bionomic and morphological characters, but only *Cx. pipiens* appears to play a vector role in WNV transmission. This species consists of two biotypes, *pipiens* and *molestus*, which can interbreed when in sympatry, and their hybrids can act as WNV-bridge vectors, due to intermediate ecological features. Considering the yearly WNV outbreaks since 2008 and given the morphological difficulties in recognising species and biotypes, our aim was to molecularly identify and characterise *Cx. pipiens* and *Cx. torrentium* in Italy, using recently developed molecular assays. *Culex torrentium* was not detected; as in other European countries, the *pipiens* and *molestus* biotypes were widely found in sympatry with hybrids in most environments. The UPGMA cluster analysis applied to CQ11 genotypic frequencies mainly revealed two groups of *Cx. pipiens* populations that differed in ecological features. The high propensity of the *molestus* biotype to exist in hypogean environments, where the habitat's physical characteristics hinder and preclude the gene flow, was shown. These results confirmed the CQ11 assay as a reliable diagnostic method, consistent with the ecological and physiological aspects of the populations analysed. Since the assessment of the actual role of three biotypes in the WNV circulation remains a crucial point to be elucidated, this extensive molecular screening of *Cx. pipiens* populations can provide new insights into the ecology of the species and may give useful indications to plan and implement WNV surveillance activities in Italy.

Chromosomal Translocations in Black Flies (Diptera: Simuliidae)-Facilitators of Adaptive Radiation?

Adler, PH; Yadamsuren, O; Procnier, WS. 2016

A macrogenomic investigation of a Holarctic clade of black flies-the *Simulium choldkovskii* lineage-provided a platform to explore the implications of a unique, synapomorphic whole-arm interchange in the evolution of black flies. Nearly 60 structural rearrangements were discovered in the polytene complement of the lineage, including 15 common to all 138 analyzed individuals, relative to the central sequence for the entire subgenus *Simulium*. Three species were represented, of which two Palearctic entities (*Simulium choldkovskii* and *S. decimatum*) were sympatric; an absence of hybrids confirmed their reproductive isolation. A third (Nearctic) entity had nonhomologous sex chromosomes, relative to the other species, and is considered a separate species, for which the name *Simulium nigricoxum* is revalidated. A cytophylogeny is inferred and indicates that the two Palearctic taxa are sister species and these, in turn, are the sister group of the Nearctic species. The rise of the *S. choldkovskii* lineage encompassed complex chromosomal and genomic restructuring phenomena associated with speciation in black flies, viz. expression of one and the same rearrangement as polymorphic, fixed, or sex linked in different species; taxon-specific differentiation of sex chromosomes; and reciprocal translocation of chromosome arms. The translocation is hypothesized to have occurred early in male spermatogonia, with the translocated chromosomal complement being transmitted to the X- and Y-bearing sperm during spermatogenesis, resulting in alternate disjunction of viable F1 translocation heterozygotes and the eventual formation of more viable and selectable F2 translocation homozygous progeny. Of 11 or 12 independently derived whole-arm interchanges known in the family Simuliidae, at least six are associated with subsequent speciation events, suggesting a facilitating role of translocations in adaptive radiations. The findings are discussed in the context of potential structural and functional interactions for future genomic research.

Sexual behavior and male volatile compounds in wild and mass-reared strains of the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae) held under different colony management regimes

Bosa, CF; Cruz-Lopez, L; Zepeda-Cisneros, CS; Valle-Mora, J; Guillen-Navarro, K; Liedo, P. 2016

We compared the calling and mating behavior and volatile release of wild males *Anastrepha ludens* (Loew) with males from 4 mass-reared strains: (i) a standard mass-reared colony (control), (ii) a genetic sexing strain (Tap-7), (iii) a colony started from males selected on their survival and mating competitiveness abilities (selected), and (iv) a hybrid colony started by crossing wild males with control females. Selected and wild males were more competitive, achieving more matings under field cage conditions. Mass-reared strains showed higher percentages of pheromone calling males under field conditions except for Tap-7 males, which showed the highest percentages of pheromone calling males under laboratory cage conditions. For mature males of all strains, field-cage calling behavior increased during the last hour before sunset, with almost a 2 fold increase exhibited by wild males during the last half hour. The highest peak mating activity of the 4 mass-reared strains occurred 30 min earlier than for wild males. By means of solid phase microextraction (SPME) plus gas chromatography-mass spectrometry (GC-MS), the composition of volatiles released by males was analyzed and quantified. Wild males emitted significantly less amounts of (E,E)-farnesene but emitted significantly more amounts of (E,E)-suspensolide as they aged than mass-reared males. Within the 4 mass-reared strains, Tap-7 released significantly more amounts of (E,E)-farnesene and hybrid more of (E,E)-suspensolide. Differences in chemical composition could be explained by the intrinsic characteristics of the strains and the colony management regimes. Characterization of calling behavior and age changes of volatile composition between wild and mass-reared strains could explain the differences in mating competitiveness and may be useful for optimizing the sterile insect technique in *A. ludens*.

Natural *Leishmania* (Viannia) spp. infections in phlebotomine sand flies (Diptera: Psychodidae) from the Brazilian Amazon region reveal new putative transmission cycles of American cutaneous leishmaniasis

de Souza, AAA; dos Santos, TV; Jennings, YLL; Ishikawa, EAY; Barata, ID; Silva, MDS; Lima, JAN; Shaw, J; Lainson, R; Silveira, FT. 2016

In Amazonian Brazil the etiological agents of American cutaneous leishmaniasis (ACL) belong to at least seven *Leishmania* species but little is known about the putative phlebotomine sand fly vectors in different biomes. In 2002-2003 a survey of the phlebotomine fauna was undertaken in the "Floresta Nacional do Tapajós", Belterra municipality, in the lower Amazon region, western Para State, Brazil, where we recently confirmed the presence of a putative hybrid parasite, *L. (V.) guyanensis* x *L. (V.) shawi shawi*. Sand flies were collected from Centers for Disease Control (CDC) light traps, Shannon traps and by aspiration on tree bases. Females were dissected and attempts to isolate any flagellate infections were made by inoculating homogenized midguts into Difco B-45 medium. Isolates were characterized by monoclonal antibodies and isoenzyme electrophoresis. A total of 9,704 sand flies, belonging to 68 species or subspecies, were collected. Infections were found in the following sand flies: *L. (V.) naiffi* with *Psychodopygus hirsutus hirsutus* (1) and *Ps. davisi* (2); and *L. (V.) shawi shawi* with *Nyssomyia whitmani* (3) and *Lutzomyia gomezi* (1). These results provide strong evidence of new putative transmission cycles for *L. (V.) naiffi* and *L. (V.) shawi*.

The Effect of Parasitism and Interpopulation Hybridization on *Aedes albopictus* (Diptera: Culicidae) Fitness

Tseng, M. 2017

Recent research in mosquito population genetics suggests that interpopulation hybridization has likely contributed to the rapid spread of the container-breeding mosquitoes. Here, I used laboratory experiments to investigate whether interpopulation *Aedes* (*Stegomyia*) *albopictus* (Skuse) F1 and F2 hybrids exhibit higher fitness than parental populations, and whether hybrid mosquito performance is related to infection by the coevolved protozoan parasite *Ascogregarina taiwanensis* (Lien and Levine). Overall, there were significant differences in development time, wing length, and survival between the two parental mosquito populations, but no difference in per capita growth rate *r*. Hybrid mosquitoes were generally intermediate in phenotype to the parentals, except that F2 females were significantly larger than the midparent average. In addition, *As. taiwanensis* parasites produced fewest oocysts when they were reared in hosts of hybrid origin. These data suggest that hybridization between previously isolated mosquito populations can result in slight increases in potential mosquito reproductive success, via increased hybrid body size, and via the temporary escape from coevolved parasites. These findings are significant because studies have shown that even slight hybrid vigor can have positive fitness consequences for population persistence. Although this was a laboratory experiment extending only to the F2 generation, many other invasive insects also carry coevolved parasites, and thus the patterns seen in this mosquito system may be broadly relevant.

Behavioural mechanisms of reproductive isolation between two hybridizing dung fly species

Giesen, A; Blanckenhorn, WU; Schafer, MA. 2017

Characterization of the phenotypic differentiation and genetic basis of traits that can contribute to reproductive isolation is an important avenue to understand the mechanisms of speciation. We quantified the degree of prezygotic isolation and geographical variation in mating behaviour among four populations of *Sepsis neocynipsea* that occur in allopatry, parapatry or sympatry with four populations of its sister species *Sepsis cynipsea*. To obtain insights into the quantitative genetic basis and the role of selection against hybrid phenotypes we also investigated mating behaviour of F-1 hybrid offspring and corresponding backcrosses with the parental populations. Our study documents successful hybridization under laboratory conditions, with low copulation frequencies in heterospecific pairings but higher frequencies in pairings of F-1 hybrids signifying hybrid vigour. Analyses of F-1 offspring and their parental backcrosses provided little evidence for sexual selection against hybrids. Longer copulation latencies in heterospecific pairings indicate species recognition, probably due to surface or volatile chemicals. The frequency of male mating attempts did not differ greatly between species or hybrid pairings, suggesting no male discrimination of mating partners. Female shaking duration, signifying female choice and/or reluctance to mate, differed strongly between the species and appears to contribute to avoiding heterospecific males; this trait is partially maternally inherited. Importantly, females of both species discriminated more strongly against males in areas of sympatry than allopatry indicating reinforcement. Shorter copulations in heterospecific parental pairings and longer copulations in F-1 hybrids suggest mechanistic difficulties with sperm transfer. Overall, our study highlights an important role of character displacement affecting mating behaviour of hybridizing sepsid species in geographical areas of coexistence. (C) 2017 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Invasion of Japan by exotic leafminers *Liriomyza* spp. (Diptera: Agromyzidae) and its consequences

Abe, Y. 2017

Identifying patterns and causes of species displacement is important from the viewpoints of ecology and evolutionary biology as this phenomenon affects community structure. Here I review the species displacement between *Liriomyza trifolii* (Burgess) and *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Japan. These two species and *Liriomyza huidobrensis* (Blanchard) originated from the New World and are considered to have invaded Japan from around 1990 to the early 2000s. During this period, *L. trifolii* was apparently displaced by *L. sativae*, but the direction of displacement in Japan has been contrary to that observed between the same two species in the USA and China. While the displacement of *L. sativae* by *L. trifolii* in these two countries can be attributed to the lower insecticide susceptibility of *L. trifolii* there, species displacement in the opposite direction in Japan is probably due to the relatively high fecundity of *L. sativae* and differential effects of the introduced parasitoid *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) on both *Liriomyza* species, except in the south of the country.

Ecophysiological characterization and molecular differentiation of *Culex pipiens* forms (Diptera: Culicidae) in Tunisia

Beji, M; Rhim, A; Roiz, D; Bouattour, A. 2017

Background: The *Culex pipiens* complex (Diptera: Culicidae) includes the most widespread mosquito species in the world. Members of this complex are the primary enzootic and epidemic vectors of the West Nile virus (genus *Flavivirus*) in several countries. The two recognized forms of *Cx. pipiens* (Linnaeus, 1758) - *pipiens* and *molestus* - exhibit behavioral and physiological differences. Natural populations of *Cx. pipiens* were investigated in several sites in Tunisia to evaluate the ecophysiological and molecular characteristics of their forms. Results: The analysis showed the sympatric presence of *Cx. pipiens* forms and hybrids in all studied sites. Of all the tested larvae of *Cx. pipiens*, 33.5% were identified as *pipiens*, 30.8% were identified as *molestus*, and 35.6% were identified as hybrids. The *molestus* and hybrid forms were positively correlated with urban habitats and belowground sites while the *pipiens* form was positively correlated with rural habitats and aboveground sites. Autogeny was expressed in all types of habitats and breeding sites. By contrast with the microsatellite CQ11, the two molecular markers, *ace-2* and *cytb*, did not allow differentiation between the *Cx. pipiens* forms. Conclusions: Our study shows the ubiquitous distribution and the plasticity of the different forms of *Cx. pipiens* in a wide range of ecological conditions. It suggests that the behavioral traits assigned to the forms of *Cx. pipiens* seem to be more flexible than previously assumed. Our analysis also proves that the microsatellite CQ11 remains an efficient tool for distinguishing between *Cx. pipiens* forms.

An Additional Phytosanitary Cold Treatment Against *Ceratitis capitata* (Diptera: Tephritidae) in 'Oroblanco' Citrus Fruit

Gazit, Y; Kaspi, R. 2017

For 'Oroblanco' ('Sweetie'), the sweet seedless pummelo-grapefruit hybrid, when exported from Israel to Japan, the standard cold treatment against *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) is conducted at ≤ 1.5 degrees C, for 16 d. In recent years, the transportation means of exported citrus was changed from reefer vessels to individual refrigerated containers, where the fruit bulk is relatively small and may be exposed to temperature fluctuations and to the risk of chilling injuries. To reduce this risk, Israel proposed to Japan to increase the treatment temperature and extend its duration to 2.2 degrees C and 18 d, respectively. This study shows that the proposed treatment effectively kills the third instar larva of *C. capitata*, in Oroblanco.

Incipient speciation in the *Anastrepha fraterculus* cryptic species complex: reproductive compatibility between *A. sp.1* aff. *fraterculus* and *A. sp.3* aff. *fraterculus*

Roriz, AKP; Japyassu, HF; Joachim-Bravo, IS. 2017

Reproductive isolation among biological strains can be detected by analyzing reproductive barriers between populations. The *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae) cryptic species complex comprises eight morphotypes defined by morphometric characteristics. The present study analyzed the existence of pre- and post-zygotic isolation among populations of *A. sp.1* aff. *fraterculus* (*A. sp.1*) and *A. sp.3* aff. *fraterculus* (*A. sp.3*) determined by tests of sexual compatibility, analyses of the temporal pattern of calling behavior, and the reproductive performance of the offspring of homotypic and heterotypic crosses. Pre-zygotic tests indicated asymmetric matings with preferences for homotypic crosses. The *A. sp.3* population demonstrated distinct characteristics, including low copulation percentages and a high proportion of homotypic copulations under crowded conditions. The females of the *A. sp.1* population demonstrated lower copulation latency. The analyzed morphotypes demonstrated two peaks in pheromone emission activity, at the beginning and at the end of the morning and were differentiated in the abundance of males. The calling behavior of the hybrids between *A. sp.1* male and *A. sp.3* female crosses had an intermediate pattern compared to either parental type. Post-zygotic compatibility tests indicated low viability of the cross between *A. sp.3* males and *A. sp.1* females. Pronounced asymmetries were found in the sex ratios of the offspring of heterotypic crosses, and only hybrids from *A. sp.1* male vs. *A. sp.3* female crosses produced descendants. The observed pre- and post-zygotic incompatibilities reflect the probable occurrence of incipient speciation between *A. sp.1* and *A. sp.3*. A more adequate taxonomic classification of this species complex that considers the distinct characteristics of each morphotype will be needed to improve environmentally wise control methods against this insect pest.

Sugarcane Aphid Population Growth, Plant Injury, and Natural Enemies on Selected Grain Sorghum Hybrids in Texas and Louisiana

Brewer, MJ; Gordy, JW; Kerns, DL; Woolley, JB; Rooney, WL; Bowling, RD. 2017

In response to the 2013 outbreak of sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), on sorghum, *Sorghum bicolor* (L.), in North America, experiments were conducted at three southern U.S. grain sorghum production locations (Corpus Christi, TX; Winnsboro, LA; Rosenberg, TX). The objectives were to authenticate yield decline on susceptible hybrids (2014 and 2015) and to measure aphid population growth and natural enemy prevalence on susceptible and resistant hybrids with similar genetic background (2014). Yield decline on susceptible hybrids (Tx 2752/Tx430 and DKS53-67) was more substantial when aphid population growth accelerated quickly and peaked above 300 aphids per leaf (50 to nearly 100% yield decline). Location and year variation in maximum aphid density and cumulative aphid-days was high, with doubling time values on the susceptible hybrids ranging between 3.9 and 7.9 d. On resistant Tx2752/Tx2783, leaf injury and yield decline were not seen or less severe than on its paired susceptible Tx2752/Tx430. Aphids declined on Tx2752/Tx2783 after initial colony establishment (Corpus Christi) or took about 60% longer to double in population size when compared with Tx2752/Tx430 (Winnsboro). The predominant natural enemy taxa were aphelinid mummies (Hymenoptera: Aphelinidae), ladybird beetles (Coleoptera: Coccinellidae), and syrphid flies (Diptera: Syrphidae), and they were more prevalent during flowering than prior to flowering. They were generally responsive to changes in aphid density of both susceptible and resistant hybrids, but variability points to need for further study. In future research, full season observations should continue as well as more detailed study of potential compatibility of sorghum resistance and biological control.

Direct Multiplex PCR (dmPCR) for the Identification of Six Phlebotomine Sand Fly Species (Diptera:Psychodidae), Including Major Leishmania Vectors of the Mediterranean

Giantsis, IA; Chaskopoulou, A; Bon, MC. 2017

Sand flies (Diptera: Psychodidae, subfamily Phlebotominae) are hematophagous insects that are known to transmit several anthroponotic and zoonotic diseases. Reliable identification of sand flies at species level is crucial for their surveillance, the detection and spread of their pathogens, and the implementation of targeted pest control strategies. Here, we designed a novel, time-saving, cost-effective and easy-to-apply molecular methodology, which avoids sequencing, for the identification of the following six Eastern Mediterranean sand fly species: *Phlebotomus perfiliewi* Parrot, *Phlebotomus simici* Theodor, *Phlebotomus tobii* Adler and Theodor, *Phlebotomus papatasi* Scopoli, *Sergentomyia dentata* Sinton, and *Sergentomyia minuta* Theodor. This methodology, which is a multiplex PCR assay using one common and six diagnostic primers, is based on species-specific single-nucleotide polymorphisms of the nuclear 18S rRNA gene. Amplification products were easily and reliably separated in agarose gel yielding one single clear band of diagnostic size for each species. Further, we verified its successful application on tissue samples that were immersed directly to the PCR mix, skipping DNA extraction. The direct multiplex PCR can be completed in <3h, including all operating procedures, and costing no more than a simple PCR. The applicability of this methodology in the detection of hybrids is an additional considerable benefit.

The *Drosophila melanogaster* Eip74EF-PA transcription factor directly binds the sciarid BhC4-1 promoter

Frank, HO; Sanchez, DG; Oliveira, LD; Kobarg, J; Monesi, N. 2017

The DNA puff BhC4-1 gene of *Bradysia hygida* (Diptera, Sciaridae) is amplified and expressed in the salivary glands at the end of the last larval instar. Even though there are no BhC4-1 orthologs in *Drosophila melanogaster*, the mechanisms that regulate BhC4-1 gene expression in *B. hygida* are for the most part conserved in *D. melanogaster*. The BhC4-1 promoter contains a 129bp (-186/-58) cis-regulatory module (CRM) that drives developmentally regulated expression in transgenic salivary glands at the onset of metamorphosis. Both in the sciarid and in transgenic *D. melanogaster*, BhC4-1 gene expression is induced by the increase in ecdysone titers that triggers metamorphosis. Genetic interaction experiments revealed that in the absence of the Eip74EF-PA early gene isoform BhC4-1-lacZ levels of expression in the salivary gland are severely reduced. Here we show that the overexpression of the Eip74EF-PA transcription factor is sufficient to anticipate BhC4-1-lacZ expression in transgenic *D. melanogaster*. Through yeast one-hybrid assays we confirm that the Eip74EF-PA transcription factor directly binds to the 129 bp sciarid CRM. Together, these results contribute to the characterization of an insect CRM and indicate that the ecdysone gene regulatory network that promotes metamorphosis is conserved between *D. melanogaster* and the sciarid *B. hygida*.

Rift Valley fever virus and European mosquitoes: vector competence of *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*)

Brustolin, M; Talavera, S; Nunez, A; Santamaria, C; Rivas, R; Pujol, N; Valle, M; Verdun, M; Brun, A; Pages, N; Busquets, N. 2017

Rift Valley fever (RVF) is a mosquito-borne disease caused by the Rift Valley fever virus (RVFV). Rift Valley fever affects a large number of species, including human, and has severe impact on public health and the economy, especially in African countries. The present study examined the vector competence of three different European mosquito species, *Culex pipiens* (Linnaeus, 1758) form *molestus* (Diptera: Culicidae), *Culex pipiens* hybrid form and *Stegomyia albopicta* (= *Aedes albopictus*) (Skuse, 1894) (Diptera: Culicidae). Mosquitoes were artificially fed with blood containing RVFV. Infection, disseminated infection and transmission efficiency were evaluated. This is the first study to assess the transmission efficiency of European mosquito species using a virulent RVFV strain. The virus disseminated in *Cx.pipiens* hybrid form and in *S.albopicta*. Moreover, infectious viral particles were isolated from saliva of both species, showing their RVFV transmission capacity. The presence of competent *Cx.pipiens* and *S.albopicta* in Spain indicates that an autochthonous outbreak of RVF may occur if the virus is introduced. These findings provide information that will help health authorities to set up efficient entomological surveillance and RVFV vector control programmes.

Molecular phylogeny of *Anopheles hyrcanus* group (Diptera: Culicidae) based on mtDNA COI

Fang, Y; Shi, WQ; Zhang, Y. 2017

Background: The *Anopheles hyrcanus* group, which includes at least 25 species, is widely distributed in the Oriental and Palearctic regions. Some group members have been incriminated as vectors of malaria and other mosquito-borne diseases. It is difficult to identify *Hyrcanus* Group members by morphological features. Thus, molecular phylogeny has been proposed as an important complementary method to traditional morphological taxonomy. Methods: Based on the GenBank database and our original study data, we used 466 mitochondrial DNA COI sequences belonging to 18 species to reconstruct the molecular phylogeny of the *Hyrcanus* Group across its worldwide geographic range. Results: The results are as follows. 1) The average conspecific K2P divergence was 0.008 (range 0.002-0.017), whereas sequence divergence between congeneric species averaged 0.064 (range 0.026-0.108). 2) The topology of COI tree of the *Hyrcanus* Group was generally consistent with classical morphological taxonomy in terms of species classification, but disagreed in subgroup division. In the COI tree, the group was divided into at least three main clusters. The first cluster contained *An. nimpe*; the second was composed of the *Nigerrimus* Subgroup and *An. argyropus*; and the third cluster was comprised of the *Lesteri* Subgroup and other unassociated species. 3) Phylogenetic analysis of COI indicated that ancient hybridizations probably occurred among the three closely related species, *An. sinensis*, *An. belenrae*, and *An. kleini*. 4) The results supported *An. paraliae* as a probable synonym of *An. lesteri*, and it was possible that *An. pseudopictus* and *An. hyrcanus* were the same species, as evident from their extremely low interspecific genetic divergence (0.020 and 0.007, respectively) and their phylogenetic positions. Conclusions: In summary, we reconstructed the molecular phylogeny and analysed genetic divergence of the *Hyrcanus* Group using mitochondrial COI sequences. Our results suggest that in the future of malaria surveillance, we should not only pay much attention to those known vectors of malaria, but also their closely related species.

Interaction of seed coat color and seed hardness: An effective relationship which can be exploited to enhance resistance to the safflower fly (*Acanthiophilus*

helianthi) in *Carthamus* spp.

Karami, S; Sabzalian, MR; Rahimmalek, M; Saeidi, G; Ghasemi, S. 2017

A choice test study was conducted in the field from 2014 to 2016 to determine the mechanism(s) involved in resistance to the safflower fly (*Acanthophilus helianthi*, Diptera, Tephritidae) in germplasm of the wild safflower species with pigmented (*Carthamus oxyacanthus*, *C. lanatus*, and *C. glaucus*) and white (*C. palaestinus*) seed coats, some white-seeded cultivated genotypes, and a novel breeding line (A82) with black seeds obtained through interspecific hybridization of *C. tinctorius* x *C. oxyacanthus*. The choice test in 2014 revealed the significantly higher values of infested heads per plant and seed yield loss per plant of the white-seeded cultivated safflower (263-48.7% and 373-73.2%, respectively) compared with those recorded for the color-seeded wild accessions (3.0-10.9% and 3.8-11.4%, respectively) and line A82 (15.9% and 19.3%, respectively). Similar results were obtained in the two consecutive years of 2015 and 2016 as the black seed coated genotype A82 exhibited significantly lower seed loss per head and seed yield loss per plant than the other four cultivated genotypes. The highly significant correlations found between resistance to the safflower fly and each of the two parameters of seed hardness and seed coat color indicated that these latter parameters strongly affected plant response to the safflower fly; that is, harder seeds with darker seed coat colors made greater contributions to plant's resistance against the safflower fly while the white-seeded lines were generally found to be more susceptible to the fly. In short, the color-seeded wild species and the black-seeded line A82 exhibited a higher resistance while the white-seeded cultivars showed little or no resistance at all. Scanning electron microscopy was also used to investigate the morphological differences in seed coat among the color-seeded wild genotypes, black-seeded line A82 (resistant), and white-seeded cultivars (susceptible). The color-seeded genotypes and the white-seeded cultivars exhibited differences in their seed coat surface structure and thickness, suggesting that morphological structure and seed coat color concomitantly contribute to the fly resistance in some safflower genotypes. Based on the results obtained, genotype A82 may be suggested as a preferable and superior genotype for cultivation in areas under infestation by the safflower fly. (C) 2017 Elsevier Ltd. All rights reserved.

Avian Plasmodium in Eastern Austrian mosquitoes

Schoener, E; Uebleis, SS; Butter, J; Nawratil, M; Cuk, C; Flechl, E; Kothmayer, M; Obwallner, AG; Zechmeister, T; Rubel, F; Lebl, K; Zित्रa, C; Fuehrer, HP. 2017

Background: Insect vectors, namely mosquitoes (Diptera: Culicidae), are compulsory for malaria parasites (*Plasmodium* spp.) to complete their life cycle. Despite this, little is known about vector competence of different mosquito species for the transmission of avian malaria parasites. Methods: In this study, nested PCR was used to determine *Plasmodium* spp. occurrence in pools of whole individuals, as well as the diversity of mitochondrial cytochrome b gene sequences in wild-caught mosquitoes sampled across Eastern Austria in 2013-2015. Results: A total of 45,749 mosquitoes in 2628 pools were collected, of which 169 pools (6.43%) comprising 9 mosquito species were positive for avian *Plasmodium*, with the majority of positives in mosquitoes of *Culex pipiens* s.l./*Culex torrentium*. Six different avian *Plasmodium* lineages were found, the most common were *Plasmodium vaughani* SYAT05, *Plasmodium* sp. Linn1 and *Plasmodium relictum* SGS1. In 2014, mosquitoes of the *Culex pipiens* complex were genetically identified and *Culex pipiens* f. *pipiens* presented with the highest number of avian *Plasmodium* positives (n = 37; 16.74%). Despite this, the minimum infection rate (MIR) was highest in *Culex torrentium* (5.36%) and *Culex pipiens* f. *pipiens*/ f. *molestus* hybrids (5.26%). During 2014 and 2015, seasonal and annual changes in *Plasmodium* lineage distribution were also observed. In both years *P. vaughani* SYAT05 dominated at the beginning of the sampling period to be replaced later in the year by *P. relictum* SGS1 (2014) and *Plasmodium* sp. Linn1 (2015). Conclusions: This is the first large-scale study of avian *Plasmodium* parasites in Austrian mosquitoes. These results are of special interest, because molecular identification of the taxa of the *Cx. pipiens* complex and *Cx. torrentium* enabled the determination of *Plasmodium* prevalence in the different mosquito taxa and hybrids of this complex. Since pools of whole insects were used, it is not possible to assert any vector competence in any of the examined mosquitoes, but the results are nonetheless valuable in providing an overview of avian *Plasmodium* species and lineages present in Austria.

Genetic diversity and population structure of the tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossinidae) in Northern Uganda: Implications for vector control

Opiro, R; Saarman, NP; Echodu, R; Opiyo, EA; Dion, K; Halyard, A; Dunn, AW; Aksoy, S; Caccone, A. 2017

Uganda is the only country where the chronic and acute forms of human African Trypanosomiasis (HAT) or sleeping sickness both occur and are separated by < 100 km in areas north of Lake Kyoga. In Uganda, *Glossina fuscipes fuscipes* is the main vector of the *Trypanosoma* parasites responsible for these diseases as well for the animal African Trypanosomiasis (AAT), or Nagana. We used highly polymorphic microsatellite loci and a mitochondrial DNA (mtDNA) marker to provide fine scale spatial resolution of genetic structure of *G. f. fuscipes* from 42 sampling sites from the northern region of Uganda where a merger of the two disease belts is feared. Based on microsatellite analyses, we found that *G. f. fuscipes* in northern Uganda are structured into three distinct genetic clusters with varying degrees of interconnectivity among them. Based on genetic assignment and spatial location, we grouped the sampling sites into four genetic units corresponding to northwestern Uganda in the Albert Nile drainage, northeastern Uganda in the Lake Kyoga drainage, western Uganda in the Victoria Nile drainage, and a transition zone between the two northern genetic clusters characterized by high level of genetic admixture. An analysis using HYBRIDLAB supported a hybrid swarm model as most consistent with tsetse genotypes in these admixed samples. Results of mtDNA analyses revealed the presence of 30 haplotypes representing three main haplogroups, whose location broadly overlaps with the microsatellite defined clusters. Migration analyses based on microsatellites point to moderate migration among the northern units located in the Albert Nile, Achwa River, Okole River, and Lake Kyoga drainages, but not between the northern units and the Victoria Nile drainage in the west. Effective population size estimates were variable with low to moderate sizes in most populations and with evidence of recent population bottlenecks, especially in the northeast unit of the Lake Kyoga drainage. Our microsatellite and mtDNA based analyses indicate that *G. f. fuscipes* movement along the Achwa and Okole rivers may facilitate northwest expansion of the Rhodesiense disease belt in Uganda. We identified tsetse migration corridors and recommend a rolling carpet approach from south of Lake Kyoga northward to minimize disease dispersal and prevent vector re-colonization. Additionally, our findings highlight the need for continuing tsetse monitoring efforts during and after control.

Experimental hybridization and reproductive isolation between two sympatric species of tephritid fruit flies in the *Anastrepha fraterculus* species group

Rull, J; Tadeo, E; Lasa, R; Rodriguez, CL; Altuzar-Molina, A; Aluja, M. 2018

Among tephritid fruit flies, hybridization has been found to produce local adaptation and speciation, and in the case of pest species, induce behavioral and ecological alterations that can adversely impact efficient pest management. The *fraterculus* species group within *Anastrepha* (Diptera: Tephritidae), is a rapidly radiating aggregate, which includes cryptic species complexes, numerous sister species, and several pest species. Molecular studies have highlighted the possibility of introgression between *A. fraterculus* and *A. obliqua*. Reproductive isolation has been studied among morphotypes of the *A. fraterculus* species complex as a tool for species delimitation. Here we examined the existence and strength of prezygotic and postzygotic isolation between sympatric populations of two closely related species within the highly derived *fraterculus* group (*A. fraterculus* and *A. obliqua*), coexisting in nature. Although adults of both species showed a strong tendency for assortative mating, a small proportion of hybrid pairings in both directions were observed. We also observed asymmetric postzygotic isolation, with one hybrid cross displaying a strong reduction in fecundity and F1 egg fertility. Survival was greater for the progeny of homotypic and hybrid crosses in the maternal host. There was a marked female biased sex ratio distortion for both F1 hybrid adults. Hybridization between *A. fraterculus* and *A. obliqua* in nature may be difficult but possible; these two species display stronger reproductive isolation than all pairs of species previously examined in the *A. fraterculus* species complex. Asymmetric postzygotic isolation is suggestive of Wolbachia mediated cytoplasmic incompatibilities that may be exploited in area-wide pest management.

Recurrent Amplification of the Heterochromatin Protein 1 (HP1) Gene Family across Diptera

Helleu, Q; Levine, MT. 2018

The heterochromatic genome compartment mediates strictly conserved cellular processes such as chromosome segregation, telomere integrity, and genome stability. Paradoxically, heterochromatic DNA sequence is wildly unconserved. Recent reports that many hybrid incompatibility genes encode heterochromatin proteins, together with the observation that interspecies hybrids suffer aberrant heterochromatin-dependent processes, suggest that heterochromatic DNA packaging requires species-specific innovations. Testing this model of coevolution between fast-evolving heterochromatic DNA and its packaging proteins begins with defining the latter. Here we describe many such candidates encoded by the Heterochromatin Protein 1 (HP1) gene family across Diptera, an insect Order that encompasses dramatic episodes of heterochromatic sequence turnover. Using BLAST, synteny analysis, and phylogenetic tree building across 64 Diptera genomes, we discovered a staggering 121 HP1 duplication events. In contrast, we observed virtually no gene duplication in gene families that share a common "chromodomain" with HP1s, including Potycomb and Su(var)3-9. The remarkably high number of Dipteran HP1 paralogs arises from distant clades undergoing convergent HP1 family amplifications. These independently derived, young HP1s span diverse ages, domain structures, and rates of molecular evolution, including episodes of positive selection. Moreover, independently derived HP1s exhibit convergent expression evolution. While ancient HP1 parent genes are transcribed ubiquitously, young HP1 paralogs are transcribed primarily in male germline tissue, a pattern typical of young genes. Pervasive gene youth, rapid evolution, and germline specialization implicate heterochromatin-encoded selfish elements driving recurrent HP1 gene family expansions. The 121 young genes offer valuable experimental traction for elucidating the germline processes shaped by Diptera's many dramatic episodes of heterochromatin turnover.

Comparative studies of stenogamous behaviour in the mosquito *Culex pipiens* complex

Kim, S; Trocke, S; Sim, C. 2018

Understanding the processes of reproductive behaviour in mosquitoes is crucial for improving mating competitiveness and mating specificity for sterile insect release programmes. The *Culex pipiens* (Linnaeus) (Diptera: Culicidae) forms *pipiens* and *molestus* (Forsk.) two biotypes of the *Cx. pipiens* complex, are vectors for West Nile virus, St Louis encephalitis virus and lymphatic filariases. Hybridization of these biotypes is known to occur in nature, although form *pipiens* mates above ground in large spaces (eurygamy) and form *molestus* preferentially mates in small spaces (stenogamy) such as sewage tunnels. Hybridization may allow gene flow of biotype-specific characteristics that are crucial in the disease transmission cycle. The present study examined and compared mating behaviours, insemination rates, fecundity and fertility in parental and F1 hybrids between *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus* in conditions of stenogamy. Unique mating behaviour sequences were identified in *Cx. pipiens* f. *molestus*, including tapping, mounting, co-flying and copulation. Despite the considerably high insemination rates in hybrid crosses, fertility and fecundity rates were varied. This observation could suggest reproductive isolation in the hybrid zone. The study also documents a failure of heterospecific males to produce fertile eggs in *Cx. pipiens* f. *pipiens* females, which may be attributable to gametic incompatibilities and may represent an additional barrier to gene exchange.

Experimental hybridization in allopatric species of the *Drosophila repleta* group (Diptera: Drosophilidae): implications for the mode of speciation

Colines, B; Soto, IM; de Panis, DN; Padro, J. 2018

The Pleistocene refugia theory proposes that recurrent expansions and contractions of xerophytic vegetation over periods of climate change affected the evolution of cactophilic *Drosophila* in South America. The resulting demographic fluctuations linked to the available patches of vegetation should have been prone to bottlenecks and founder events, affecting the fate of gene pool dynamics. However, these events also promoted the diversification of cacti, creating an ecological opportunity for host specialization. We tested the hypothesis of ecological speciation in the *Drosophila buzzatii* group. We assessed adaptive footprints and examined the genetic architecture of fitness-related traits in the sibling allopatric species *D. koepferae* and *D. antonietae*. The results are in line with the idea that these species evolved under different ecological scenarios. Joint-scaling analysis comparing both species and their hybrids revealed that additive genetic variance was the major contributor to phenotypic divergence, but dominance, epistasis and maternal effects were also important factors. Correlation analysis among functionally related traits suggested divergent selection on phenotypic integration associated with fitness. These findings support the hypothesis of adaptive evolution driving the phylogenetic radiation of the group through independent events of host shifts to chemically complex columnar cacti.

DNA Barcoding Reveals Hidden Diversity of Sand Flies (Diptera: Psychodidae) at Fine and Broad Spatial Scales in Brazilian Endemic Regions for Leishmaniasis

Rodrigues, BL; Carvalho-Costa, LF; Pinto, ID; Rebelo, JMM. 2018

Sand fly (Diptera: Psychodidae) taxonomy is complex and time-consuming, which hampers epidemiological efforts directed toward controlling leishmaniasis in endemic regions such as northeastern Brazil. Here, we used a fragment of the mitochondrial cytochrome c oxidase I (COI) gene to identify sand fly species in Maranhao State (northeastern Brazil) and to assess cryptic diversity occurring at different spatial scales. For this, we obtained 148 COI sequences of 15 sand fly species (10 genera) from Maranhao (fine spatial scale), and joined them to COI sequences from other Brazilian localities (distant about 2,000 km from Maranhao, broad spatial scale) available in GenBank. We revealed cases of cryptic diversity in sand flies both at fine (*Lutzomyia longipalpis* (Lutz and Neiva) and *Evandromyia termitophila* (Martins, Falcao and Silva)) and broad spatial scales (*Migonemyia migonei* (Franca), *Pressatia choti* (Floch and Abonnenc), *Psychodopygus davisii* (Root), *Sciopemyia sordellii* (Shannon and Del Ponte), and *Bichromomyia flaviscutellata* (Mangabeira)). We argue that in the case of *Bi. flaviscutellata*, the cryptic diversity is associated with a putative new species. Cases in which DNA taxonomy was not as effective as morphological identification possibly involved recent speciation and/or introgressive hybridization, highlighting the need for integrative approaches to identify some sand fly species. Finally, we provide the first barcode sequences for four species (*Brumptomyia avellari* (Costa Lima), *Evandromyia infraspinoza* (Mangabeira), *Evandromyia evandroi* (Costa Lima and Antunes), and *Psychodopygus complexus* (Mangabeira)), which will be useful for further molecular identification of neotropical species.

Reproductive compatibility among Mexican populations of *Anastrepha obliqua*: theoretical and management implications

Rull, J; Tadeo, E; Lasa, R; Diaz-Fleischer, F; Arredondo, J; Aluja, M. 2018

The *fraterculus* species group, composed of 34 species in the genus *Anastrepha* (Diptera: Tephritidae), includes the *fraterculus* cryptic species complex formed by eight reproductively isolated morphotypes. A previous study revealed six genetic mitochondrial types of *Anastrepha obliqua*, suggesting the existence of a second cryptic species complex. However, marked discrepancies between nuclear and mitochondrial loci rather suggest incomplete lineage sorting or introgression between *A. obliqua* and *A. fraterculus*. Such hybridization could nevertheless result in reproductive isolation, an outcome that could affect efficacy of area-wide management for the most important pest of mangos in America. Two mitochondrial types occur in Mexico, and the limits of a third one, encompassing Central American populations, have not been clearly established. Here, we tested reproductive compatibility among three *A. obliqua* populations from the Pacific and a population from the Gulf of Mexico. No evidence of pre-zygotic isolation was found. Flies from the Atlantic mated randomly for equal duration with individuals from three Pacific populations. Homotypic and heterotypic crosses produced similar numbers of eggs, with heterotypic crosses of Pacific males and Atlantic females hatching in lower proportions. Larvae of all cross types developed equally in mangos and exhibited no sex ratio distortion of hybrid F1. The three mitochondrial types identified in Mexico and Central America do not appear to be cryptic species and can be managed using one single strain for the sterile insect technique.

Genetic variation in foundation species governs the dynamics of trophic interactions

Valencia-Cuevas, L; Mussali-Galante, P; Cano-Santana, Z; Pujade-Villar, J; Equihua-Martinez, A; Tovar-Sanchez, E. 2018

Various studies have demonstrated that the foundation species genetic diversity can have direct effects that extend beyond the individual or population level, affecting the dependent communities. Additionally, these effects may be indirectly extended to higher trophic levels throughout the entire community. *Quercus castanea* is an oak species with characteristics of foundation species beyond presenting a wide geographical distribution and being a dominant element of Mexican temperate forests. In this study, we analyzed the influence of population (He) and individual (HL) genetic diversity of *Q. castanea* on its canopy endophagous insect community and associated parasitoids. Specifically, we studied the composition, richness (S) and density of leaf-mining moths (Lepidoptera: Tischeriidae, Citheraniidae), gall-forming wasps (Hymenoptera: Cynipidae), and canopy parasitoids of *Q. castanea*. We sampled 120 trees belonging to six populations (20/site) through the previously recognized gradient of genetic diversity. In total, 22 endophagous insect species belonging to three orders (Hymenoptera, Lepidoptera, and Diptera) and 20 parasitoid species belonging to 13 families were identified. In general, we observed that the individual genetic diversity of the host plant (HL) has a significant positive effect on the S and density of the canopy endophagous insect communities. In contrast, He has a significant negative effect on the S of endophagous insects. Additionally, indirect effects of HL were observed, affecting the S and density of parasitoid insects. Our results suggest that genetic variation in foundation species can be one of the most important factors governing the dynamics of tritrophic interactions that involve oaks, herbivores, and parasitoids.

Population genetic structure of the *Culex pipiens* (Diptera: Culicidae) complex, vectors of West Nile virus, in five habitats

Joyce, AL; Melese, E; Ha, PT; Inman, A. 2018

Background: The *Culex pipiens* complex consists of several morphologically similar, closely related species. In the United States, *Cx. pipiens* L. is distributed North of 39 degrees latitude, while *Cx. quinquefasciatus* Say occurs South of 36 degrees latitude; a hybrid zone occurs between these two latitudes including in the Central Valley of California. Members of the *Cx. pipiens* complex and their hybrids are vectors for West Nile virus (WNV). Hybrid offspring of *Cx. pipiens* and *Cx. quinquefasciatus* have been found to have enhanced transmission rates of WNV over those of pure populations of each species. We investigated whether hybrids of *Cx. pipiens* and *Cx. quinquefasciatus* occurred more frequently in any of five habitats which were dairies, rural, suburban, and urban areas, and wetlands. In addition, the proportion of alleles unique to *Cx. quinquefasciatus* and *Cx. pipiens* found in each habitat-associated population were determined. Methods: Amplified fragment length polymorphism (AFLP) markers were used to compare the population structure of the *Cx. pipiens* complex from each habitat to geographically distant populations considered pure *Cx. pipiens* and *Cx. quinquefasciatus*. Structure analyses were used to assign individuals to either *Cx. pipiens*, *Cx. quinquefasciatus*, or hybrids of the *Cx. pipiens* complex. The ancestry of hybrids (F1, F2, or backcrossed) in relation to the two parent populations was estimated for each Central Valley population. Loci unique to the pure *Cx. pipiens* population and the pure *Cx. quinquefasciatus* population were determined. The proportion of loci unique to *Cx. pipiens* and *Cx. quinquefasciatus* populations were subsequently determined for each population from the five Merced habitats and from the Oroville California population. The unique loci found in Merced populations and not in *Cx. pipiens* or *Cx. quinquefasciatus* were also determined. A principal components analysis was run, as was an analysis to determine loci under putative selection. Results: The Structure Harvester analysis found $K = 3$, and the *Culex pipiens* complex mosquitoes formed a genetic cluster distinct from *Cx. quinquefasciatus* and *Cx. pipiens*. Individuals collected from each habitat were nearly all hybrids. However, *Cx. pipiens* complex collected near dairies had more individuals categorized as *Cx. pipiens* than collections from the other habitats. None of the mosquitoes collected in Merced or Oroville were considered pure *Cx. quinquefasciatus*. Significant genetic divergence was detected among the *Cx. pipiens* complex from the five habitats in Merced; *Cx. pipiens* complex mosquitoes from dairies were divergent from the urban and suburban populations. New Hybrids analysis found that individuals from all five Merced habitat-associated populations and the population from Oroville were primarily categorized as hybrids backcrossed to the *Cx. pipiens* population. Finally, all five habitat-associated populations shared more alleles with *Cx. pipiens* than with *Cx. quinquefasciatus*, even though the pure *Cx. quinquefasciatus* population was more geographically proximate to Merced. Results from the principal component analysis, and the occurrence of several unique loci in Merced populations, suggest that *Cx. pipiens molestus* may also occur in the habitats sampled. Conclusions: Nearly all mosquitoes in the five habitats in Merced in the Central Valley of California area were hybrids of *Cx. pipiens* and *Cx. quinquefasciatus*, consisting of hybrids backcrossed to *Cx. pipiens*. Habitat-associated mosquitoes collected near dairies had more individuals consisting of pure *Cx. pipiens*, and no mosquitoes from Merced or Oroville CA classified as pure *Cx. quinquefasciatus*. The genetic distances among *Cx. pipiens* and *Cx. quinquefasciatus*, and hybrid populations agree with previous studies using other molecular markers. *Cx. pipiens* hybrids in Merced shared more alleles with *Cx. pipiens* than *Cx. quinquefasciatus* which was unexpected, since Merced is geographically closer to the northern limit of *Cx. quinquefasciatus* distribution. *Culex pipiens molestus* may occur in more habitats in the Central Valley than previously suspected, which warrants further investigation. Future studies could investigate the vector competence of hybrids backcrossed to either *Cx. pipiens* or *Cx. quinquefasciatus* parent for their ability to transmit West Nile virus.

Precopulatory mating and postzygotic isolation between two walnut-infesting species of *Rhagoletis* from Mexican highlands

Tadeo, E; Aluja, M; Rull, J. 2018

Pleistocene glacial and postglacial cycles producing contraction and expansion of temperate habitats have resulted in substantial diversification among several plant and animal taxa of Nearctic origin undergoing periods of isolation and secondary contact in high-elevation areas of Mexico. One of such groups are walnut-infesting fruit flies in the genus *Rhagoletis* (Diptera: Tephritidae), comprised of six recently derived species among which phylogenetic relationships have been difficult to unravel using conventional molecular methods. Here, we examined pre- and postzygotic isolation between two genetically similar and morphologically distinct species which are currently parapatric in central Mexico. Local adaptation driven by differences in host plant phenology between *Rhagoletis zoqui* Bush and *Rhagoletis ramosae* Hernandez-Ortiz resulted in allochronic isolation. Despite the existence of precopulatory mating isolation, there was a substantial number of hybrid matings in field cages where conspecific and heterospecific males and females were simultaneously released. The bulk of mating activity took place on host fruit. *Rhagoletis zoqui* females were more reluctant to mate with *R. ramosae* males than with males of their own species. Distinctive behavioral differences were observed between males during contests, fruit guarding, and approach to conspecifics on fruit. There was also some asymmetric postzygotic isolation, with the hybrid combination of *R. zoqui* males and *R. ramosae* females resulting in lower egg hatch than other mating combinations. Results were consistent with those of a phylogenetic study suggesting recent divergence of *R. ramosae* from *Rhagoletis completa* Cresson and *R. zoqui* in the Sierra Madre Oriental and the Mexican Trans Volcanic Belt.

New species of gall midge (Diptera: Cecidomyiidae) damaging flower buds of ornamental *Alstroemeria* plants

Kolesik, P; Baker, G; Hill, K; Manners, AG; Dijkstra, E. 2018

Alstroemeria (Liliales: Alstroemeriaceae) is a plant genus native to South America with many interspecific hybrids cultivated as ornamentals worldwide. Several yellow larvae of an undescribed gall midge (Diptera: Cecidomyiidae) were found feeding inside flower buds of *Alstroemeria* plants grown in production greenhouses in South Australia in 2013; Queensland, Australia, in 2015 and the Netherlands in 2016. Infested buds became malformed, did not produce flowers and turned necrotic later. In Queensland and the Netherlands, the necrotised buds became additionally infected by a saprophytic fungus *Cladosporium* sp. With up to 80% of flower buds infested, the new gall midge decreased the value of plants aimed for the cut flower market and reduced seed of reproduction plants. The new species, named *Contarinia jongi* Kolesik sp. nov., is described, and a segment of its Cytochrome Oxidase unit I mitochondrial gene is sequenced. This is the first gall midge known to feed on a host plant from the family Alstroemeriaceae. Previously, *Alstroemeria* cut flowers imported from Australia and New Zealand to Japan were found to contain viable larvae of the new species, demonstrating that the new species can spread with the flower trade. It is possible that *Contarinia jongi* is native to South America, the homeland of its host plant.

Anchored phylogenomics unravels the evolution of spider flies (Diptera, Acroceridae) and reveals discordance between nucleotides and amino acids

Gillung, JP; Winterton, SL; Bayless, KM; Khouri, Z; Borowiec, ML; Yeates, D; Kimsey, LS; Misof, B; Shing, S; Zhou, X; Mayer, C; Petersen, M; Wiegmann, BM. 2018

The onset of phylogenomics has contributed to the resolution of numerous challenging evolutionary questions while offering new perspectives regarding biodiversity. However, in some instances, analyses of large genomic datasets can also result in conflicting estimates of phylogeny. Here, we present the first phylogenomic scale study of a dipteran parasitoid family, built upon anchored hybrid enrichment and transcriptomic data of 240 loci of 43 ingroup acrocerid taxa. A new hypothesis for the timing of spider fly evolution is proposed, wielding recent advances in divergence time dating, including the fossilized birth-death process to show that the origin of Acroceridae is younger than previously proposed. To test the robustness of our phylogenetic inferences, we analyzed our datasets using different phylogenetic estimation criteria, including supermatrix and coalescent-based approaches, maximum-likelihood and Bayesian methods, combined with other approaches such as permutations of the data, homogeneous versus heterogeneous models, and alternative data and taxon sets. Resulting topologies based on amino acids and nucleotides are both strongly supported but critically discordant, primarily in terms of the monophyly of Panopinae. Conflict was not resolved by controlling for compositional heterogeneity and saturation in third codon positions, which highlights the need for a better understanding of how different biases affect different data sources. In our study, results based on nucleotides were both more robust to alterations of the data and different analytical methods and more compatible with our current understanding of acrocerid morphology and patterns of host usage.

OVIPOSITION PREFERENCE AND BIOLOGY OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) ON GRAPE VINE GENOTYPES

Correa, SC; Wille, CL; Hoffer, H; Boff, MIC; Franco, CR. 2018

Grape orchards are highly affected by oviposition of fruit flies on grape berries, which compromises the productivity and quality of the grapes. The goal of this study was to evaluate the susceptibility of American, European, and hybrid grape genotypes to *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) under laboratory conditions (25 +/- 2 degrees C, 60 +/- 10% RH, and 14-hour photophase). The assays were conducted by evaluating oviposition preference through choice and no-choice (antibiosis) tests. The choice test was set up using circular arenas (diameter: 300 mm) with 10 grapes per genotype. The no-choice test was set up using 150 grapes per genotype which were placed inside plastic boxes (417 x 297 x 289 mm). After exposure of the grapes to one or two couples of fruit flies per genotype in choice and no-choice tests, respectively, the grapes were transferred to transparent plastic containers (750 mL). The number of eggs per grape and its viability were evaluated. The no-choice test also evaluated the biological cycle of the fruit flies. The assays were conducted in a completely randomized design with 15 replicates. The most preferred grapes for oviposition by *A. fraterculus* were Cabernet Sauvignon, Niagara Rosada, and BRS Cora, while *C. capitata* mainly preferred Isabel Precoce grapes. We observed the complete development of fruit flies in the Moscato Embrapa grapes, but this only occurred with *C. capitata*. All genotypes evaluated were considered susceptible to *A. fraterculus* and *C. capitata*. However, the fruit flies expressed differences in preference for oviposition and host quality.

Exploring species boundaries with multiple genetic loci using empirical data from non-biting midges

Lin, XL; Stur, E; Ekrem, T. 2018

Over the past decade, molecular approaches to species delimitation have seen rapid development. However, species delimitation based on a single locus, for example, DNA barcodes, can lead to inaccurate results in cases of recent speciation and incomplete lineage sorting. Here, we compare the performance of Automatic Barcode Gap Discovery (ABGD), Bayesian Poisson tree processes (PTP), networks, generalized mixed Yule coalescent (GMYC) and Bayesian phylogenetics and phylogeography (BPP) models to delineate cryptic species previously detected by DNA barcodes within *Tanytarsus* (Diptera: Chironomidae) non-biting midges. We compare the results from analyses of one mitochondrial (cytochrome c oxidase subunit I [COI]) and three nuclear (alanine-tRNA synthetase 1 [AATS1], carbamoyl phosphate synthetase 1 [CAD1] and 6-phosphogluconate dehydrogenase [PGD]) protein-coding genes. Our results show that species delimitation based on multiple nuclear DNA markers is largely concordant with morphological variation and delimitations using a single locus, for example, the COI barcode. However, ABGD, GMYC, PTP and network models led to conflicting results based on a single locus and delineate species differently than morphology. Results from BPP analyses on multiple loci correspond best with current morphological species concept. In total, 10 lineages of the *Tanytarsus curticornis* species complex were uncovered. Excluding a Norwegian population of *Tanytarsus brundini* which might have undergone recent hybridization, this suggests six hitherto unrecognized species new to science. Five distinct species are well supported in the *Tanytarsus heusdensis* species complex, including two species new to science.

First record of *Culex* (*Culex*) *bidens* (Diptera: Culicidae) in Colombia: Taxonomic and epidemiological implications

Laurito, M; Hoyos-Lopez, R. 2018

Arbovirus transmission cycles must be studied locally since both vectors and hosts vary in different regions. Colombia has a highly diverse mosquito fauna. *Culex* (*Culex*) *bidens* is reported here for the first time in Colombia. Because *Cx. bidens* Dyar and Knab and *Cx. declarator* Dyar and Knab share a close taxonomic history and because it is difficult to differentiate between them, a morphological and molecular comparison was performed. The male genitalia of three specimens of *Cx. bidens* from Colombia were mounted on microscope slides and morphologically compared with the male genitalia of *Cx. declarator* also from Colombia. In *Cx. bidens*, the individual teeth of the lateral plate are long, straight, laterally directed and sharply pointed; in *Cx. declarator* these teeth are robust, curved, with convex margins and bluntly rounded. Moreover, DNA was extracted from the same specimens and a fragment of the cytochrome c oxidase subunit I mitochondrial gene was amplified and sequenced. Neither *Cx. bidens* nor *Cx. declarator* were clustered in the Neighbour-joining topology, with K2P interspecific divergence between 0.15-1.45%. The circulation of Eastern Equine Encephalitis Virus in Colombia was reported since 1957 and *Cx. bidens* was suspected to be the vector of this virus during an epizootic in Argentina in 1988. Hybridization between species of the subgenus *Culex* has been demonstrated, hence the degree of reproductive isolation between *Cx. bidens* and *Cx. declarator* should be investigated, as well as their taxonomic status, because they only can be discriminated by a single male genitalic feature and not by nuclear or mitochondrial markers.

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; McMurtry, 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 fraterculus* 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. fraterculus*. 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.

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, E.JG. 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.

The Role of *Culex pipiens* L. (Diptera: Culicidae) in Virus Transmission in Europe

Brugman, VA; Hernandez-Triana, LM; Medlock, JM; Fooks, AR; Carpenter, S; Johnson, N. 2018

Over the past three decades, a range of mosquito-borne viruses that threaten public and veterinary health have emerged or re-emerged in Europe. Mosquito surveillance activities have highlighted the *Culex pipiens* species complex as being critical for the maintenance of a number of these viruses. This species complex contains morphologically similar forms that exhibit variation in phenotypes that can influence the probability of virus transmission. Critical amongst these is the choice of host on which to feed, with different forms showing different feeding preferences. This influences the ability of the mosquito to vector viruses and facilitate transmission of viruses to humans and domestic animals. Biases towards blood-feeding on avian or mammalian hosts have been demonstrated for different *Cx. pipiens* ecoforms and emerging evidence of hybrid populations across Europe adds another level of complexity to virus transmission. A range of molecular methods based on DNA have been developed to enable discrimination between morphologically indistinguishable forms, although this remains an active area of research. This review provides a comprehensive overview of developments in the understanding of the ecology, behaviour and genetics of *Cx. pipiens* in Europe, and how this influences arbovirus transmission.

One for all: Mating compatibility among various populations of olive fruit fly (Diptera: Tephritidae) for application of the sterile insect technique

Ahmad, S; ul Haq, I; Caceres, C; Tomas, US; Dammalage, T; Gembinsky, K; Paulus, H; Vreysen, MJB; Rempoulakis, P. 2018

The olive fruit fly, *Bactrocera oleae* (Rossi), is the most important insect pest for the cultivation of olives worldwide. Considerable research efforts have been invested in the past decades to develop eradication or suppression tactics for use within an area-wide integrated pest management (AW-IPM) approach that includes a sterile insect technique (SIT) component. One of the major obstacles encountered in the development of SIT for olive fruit fly was the inferior quality of the mass-reared flies, expressed among others evident primarily by sterile males having a different timing of peak mating and a lower mating propensity in comparison with their wild counterparts. In this study we assessed the mating behaviour and mating compatibility of olive fruit flies originating from four countries of the Mediterranean region (Croatia, France, Italy, Spain) in walk-in field cages and post zygotic compatibility (expressed as% egg hatch) under laboratory conditions. Furthermore, we tested the hypothesis whether a hybrid strain (Greece (domesticated)/Israel (wild)) adapted to laboratory rearing conditions showed any mating barriers with all the four "wild" populations. Finally, we examined the effect of colonization on the mating compatibility of the four newly established populations over three consecutive generations. The results showed no prezygotic (mating barriers) or post-zygotic isolations (measured by egg hatch%) among the olive fruit fly populations from the four countries tested. Also, there was no evidence of mating barriers between the hybrid strain and the wild populations of the Mediterranean region.

Species Composition and Distribution of Sibling Species of the Genus *Chironomus* Meigen (Diptera: Chironomidae) in the Curonian and Vistula Lagoons, Baltic Sea

Markiyanova, MF. 2018

The species compositions of the genus *Chironomus* in the Curonian and Vistula lagoons are very similar, represented by *Ch. plumosus* and *Ch. balatonicus*. In the Curonian Lagoon, in addition to these two species, *Ch. muratensis* and the first-generation interspecific hybrid *Ch. muratensis* x *Ch. plumosus* are also found. In this lagoon, *Ch. plumosus* occurs most frequently (84%); *Ch. balatonicus* is considered a subdominant species (24%). In the Vistula Lagoon, on the contrary, the high frequency of occurrence is typical of *Ch. balatonicus* (89%); for *Ch. plumosus*, it reaches only 11%. The *Ch. plumosus* population occupies most of the Curonian Lagoon; *Ch. balatonicus* is present only in its northern part, from the village of Juodkrante to the city of Klaipeda. In the Vistula Lagoon, *Ch. balatonicus* is distributed over almost its entire area, while *Ch. plumosus* is found locally, only near the mouth of the Nogat River. The distribution of the sibling species in the lagoons is determined by the salinity gradient.

Floral synomone diversification of *Bulbophyllum* sibling species (Orchidaceae) in attracting fruit fly pollinators

Nakahira, M; Ono, H; Wee, SL; Tan, KH; Nishida, R. 2018

Floral scent is one of the crucial cues to attract specific groups of insect pollinators in angiosperms. We examined the semiochemical diversity in the interactions between "fruit fly orchids" and their pollinator fruit fly species in two genera, *Bactrocera* and *Zeugodacus* (Tephritidae: Diptera). Two known attractants for the Dacini fruit flies, methyl eugenol (ME) and raspberry ketone (RK), have been identified from the *Bulbophyllum* orchids. Additionally, zingerone (ZN), with a hybrid chemical structure between ME and RK, and attracts both ME- and RK-sensitive fly species, was also identified. Male flies utilize the floral scent as sex pheromone precursor or components to attract conspecific females and gain mating advantage. We analyzed the floral components of two sibling orchids, *Bulbophyllum macranthum* collected from Southeast-Asian countries and *Bu. praetervisum* collected from Sabah, Malaysia. For *Bu. macranthum*, the major floral component from Malaysia and Thailand was identified as ZN; whereas that from the Philippines was ME. For *Bu. praetervisum*, RK was found as the major constituent, but chemical profiles of the attractants were different among individuals, i.e. in addition to RK, ZN was also found in some of the *Bu. praetervisum* flowers; and one of the specimens contained ME besides RK and ZN. These differences in fruit fly-attracting floral scents shown by the sympatric chemotypes of *Bu. praetervisum* are contrary to that shown by the allopatric chemotypes of *Bu. macranthum*, demonstrating the versatility in the floral synomone biosynthetic processes. Phylogenetic analysis using chloroplast DNA shows that the Malaysia and Thailand-chemotypes of *Bu. macranthum* and *Bu. praetervisum* belong to the same lineage, although their chemical profiles are distinctly different. This demonstrates that diversification of floral synomone may happen even within a putative orchid species in order to attract a wider community of fruit fly pollinators from different genera to maximize pollination success.

An experimental assessment of reproductive isolation and its consequences for seasonal hybridization dynamics

Foucault, Q; Wieser, A; Heumann-Kiesler, C; Diogo, J; Cocchiarraro, B; Nowak, C; Waldvogel, AM; Pfenninger, M. 2019

While the spatial aspect of hybridization has been investigated in depth, the temporal aspect has not. We analysed the seasonal hybridization dynamics of a

multivoltine non-biting midge sister species pair, *Chironomus riparius* and *C. piger*. We investigated the fertility and fitness of F-1 hybrids, and all possible first-generation backcross hybrids, under different temperature conditions. Based on microsatellite markers, we then inferred the presence of different hybrid classes in seasonal field samples (spring and autumn) from a site of co-occurrence over two consecutive years. All experimental hybrids showed reduced fertility albeit without a specific temperature effect. Fitness was zero in one F-1 direction, but not reduced in the other. No sex ratio distortion was observed. However, fitness was reduced in all backcrosses, indicating that hybridization should be rare in the field. In the seasonal samples, *C. piger* was found only in autumn individuals. All 359 genotyped individuals were attributed to either of the pure species. However, several individuals of the autumn samples carried signs of later generation introgression, indicating that actual hybridization must take place recurrently during summer. We thus present evidence that seasonal environmental changes can drive hybridization dynamics and that long-term recurrent hybridization need not affect species integrity.

Comparative Analysis of Variation of the BOLD Fragment of Mitochondrial *cox1* Gene and Y Chromosome *kl-2* 1-beta dynein heavy chain Gene in *Drosophilavirilis* Species Group (Diptera: Drosophilidae)

Andrianov, BV; Romanov, DA; Sorokina, SY; Gorelova, TV. 2019

The species of the virilis group of *Drosophila* are one of the well-studied models of speciation and microevolution. A comparative analysis of variability of the marker genes for two non-recombining regions of the genome was conducted: the BOLD fragment of the mitochondrial *cox1* gene and the fragment of the dynein gene in order to identify events of interspecific hybridization in 11 *Drosophila* species of the virilis group. We identified single events of the mitochondrial DNA transfer from *Drosophila montana* to *Drosophila laticola* and of the Y chromosome transfer from *Drosophila ezoana* to *Drosophila montana*. The probable connection of the modern speciation process in *Drosophila montana* with genomic instability and interspecific hybridization in nature is discussed.

Geographic patterns of postzygotic isolation between two closely related widespread dung fly species (*Sepsis cynipsea* and *Sepsis neocynipsea*; Diptera: Sepsidae)

Giesen, A; Schafer, MA; Blanckenhorn, WU. 2019

Identifying the contribution of pre- and postzygotic barriers to gene flow is a key goal of speciation research. The widespread dung fly species *Sepsis cynipsea* and *Sepsis neocynipsea* offer great potential for studying the speciation process over a range of opportunities for gene exchange within and across sister species (cross-continental allopatry, continental parapatry and sympatry). We examined the role of postcopulatory isolating barriers by comparing female fecundity and egg-to-adult viability of F-1 and F-2 hybrids, as well as backcrosses of F-1 hybrids with the parental species, via replicated crosses of sym-, para- and allopatric populations. Egg-to-adult viability was strongly but not totally suppressed in hybrids, and offspring production approached nil in the F-2 generation (hybrid breakdown), indicating yet unspecified intrinsic incompatibilities. Viable F-1 hybrid offspring showed almost absolute male (the heterogametic sex) sterility while females remained largely fertile, in accordance with Haldane's rule. Hybridization between the two species in European areas of sympatry (Swiss Alps) indicated only minor reinforcement based on fecundity traits. Crossing geographically isolated European and North American *S. neocynipsea* showed similar albeit weaker isolating barriers that are most easily explained by random genetic drift. We conclude that in this system with a biogeographic continuum of reproductive barriers, speciation is mediated primarily by genetic drift following dispersal of flies over a wide (allopatric) geographic range, with some role of natural or sexual selection in incidental or direct reinforcement of incompatibility mechanisms in areas of European sympatry. *S(ubs)pecies* status of continental *S. neocynipsea* appears warranted.

First report on effective pollination of *Masdevallia floribunda*, *M. tuerckheimii* and their hybrid (Orchidaceae-Pleurothallidinae) by *Zygothrica* fruit flies (Diptera-Drosophilidae) in Guatemala

Lipinska, MM; Morales, FLA; Gilka, W; Beuk, PLT; Szlachetko, DL. 2019

The first report on pollination of two Neotropical orchid species, *Masdevallia floribunda* LINDL., *M. tuerckheimii* AMES and their hybrid by fruit flies is presented. Two presumably undescribed species of the genus *Zygothrica*, tentatively named as *Zygothrica* spec. 1 and spec. 2, were observed as pollinators. The distinct hypercephaly in the examined males, the transparent wing membrane lacking infuscations or markings in both sexes indicate that *Zygothrica* spec. 1 is a member of the caudata subgroup of the dispar species group. The exact affinities of *Zygothrica* spec. 2 are uncertain, but this species resembles *Z. mesopoei* BURLA. Our observations evidenced that the *Masdevallia* flowers attract both males and females of *Zygothrica*, and that both can carry the pollinia. The Estacion Experimental de Orquideas de la Familia Archila, a seminatural plantation located in a cloud forest of Guatemala, is a place of intense pollination activity by *Zygothrica* adults on the flowers of the two *Masdevallia* species, which also leads to effective and frequent hybridization.

***Lutzomyia longipalpis* (Diptera: Psychodidae) Argentina-Bolivia border: new report and genetic diversity**

Quintana, MG; Pech-May, A; Fuenzalida, AD; Mancini, JMD; Barroso, PA; Yadon, ZE; Zaidenberg, M; Salomon, OD. 2019

American visceral leishmaniasis (AVL) has two main scenarios of transmission as follows: scattered cases in rural areas and urban outbreaks. Urban AVL is in active dispersion from the northeastern border of Argentina-Paraguay-Brazil to the South. The presence of *Lutzomyia longipalpis* was initially reported in urban environments in the northwestern border of the country. The presence of *Lu. longipalpis*, environmental variables associated with its distribution, and its genetic diversity were assessed in Salvador Mazza, Argentina, on the border with Bolivia. The genetic analysis showed high haplotype diversity, low nucleotide diversity, and low nucleotide polymorphism index. We discuss the hypothesis of an expanding urban population with introgressive hybridisation of older haplogroups found in their path in natural forest or rural environments, acquiring a new adaptability to urban environments, and the possibility of changes in vector capacity.

***Haematobia irritans* parasitism of F1 yak x beef cattle (*Bos grunniens* x *Bos taurus*) hybrids**

Calkins, CM; Scasta, JD; Smith, T; Stayton, MM; Lake, SL. 2019

The horn fly *Haematobia irritans* (Diptera: Muscidae) is a blood obligate ectoparasite of bovids that causes annual losses to the U.S. beef cattle industry of over US\$1.75 billion. Climate warming, the anthropogenic dispersion of bovids and the cross-breeding of beef cattle with other bovid species may facilitate novel horn fly-host interactions. In particular, hybridizing yaks [*Bos grunniens* (Artiodactyla: Bovidae)] with beef cows (*Bos taurus*) for heterosis and carcass improvements may increase the exposure of yak x beef hybrids to horn flies. The present paper reports on the collection of digital images of commingled beef heifers (n = 12) and F1 yak x beef hybrid bovids (heifers, n = 7; steers, n = 5) near Laramie, Wyoming (similar to 2200 m a.s.l.) in 2018. The total numbers of horn flies on beef heifers and F1 yak x beef heifers [mean +/- standard error (SE): 88 +/- 13 and 70 +/- 17, respectively] did not differ significantly; however, F1 yak x beef steers had greater total horn fly abundance (mean +/- SE: 159 +/- 39) than female bovids. The present report of this experiment is the first such report in the literature and suggests that F1 yak x beef bovids are as susceptible as cattle to horn fly parasitism. Therefore, similar monitoring and treatment practices should be adopted by veterinarians, entomologists and producers.

Bottom-up effect of host plants on life-history characteristics of *Aphidoletes aphidimyza* feeding on *Aphis gossypii*

Madahi, K; Sahragard, A; Hosseini, R; Baniameri, V. 2019

The predatory midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) is widely used for the control of *Aphis* spp. in many agricultural systems. We aimed to determine the most suitable host plant for rearing the predatory midges on the prey *Aphis gossypii* Glover (Hemiptera: Aphididae). Six host plants were selected: cucumber (*Cucumis sativus* L. cv. Beith Alpha), tomato (*Solanum lycopersicum* L. cv. Falat111), eggplant (*Solanum melongena* L. cv. Yummy), pepper (*Capsicum annuum* L. cv. Bertene) (all Solanaceae), okra [*Abelmoschus esculentus* (L.) Moench cv. Clemson Spineless] (Malvaceae), and squash (*Cucurbita pepo* L. cv. Hybrid rajai) (Cucurbitaceae). Some physical traits (length and density of trichomes) and chemical attributes (nitrogen content) of prey host plants were investigated. The results showed that prey host plants differed significantly in their effect on fitness of the predator. The shortest immature development time (18.07 \pm 0.257 days), the longest female adult longevity (7.5 \pm 0.18 days), and the highest fecundity (89 eggs/female) of *A. aphidimyza* were found with squash as prey food. The highest intrinsic rate of increase (0.171 \pm 0.009 day⁻¹) and also the shortest mean generation time (22.4 \pm 0.32 days) were also obtained when *A. aphidimyza* fed on *A. gossypii* reared on squash. Canonical correlation analysis (CCA) approved the correlation between life-history traits of *A. aphidimyza* and characteristics of prey host plants. The suitability of squash for rearing *A. aphidimyza* can be attributed to the higher nitrogen content, longer trichomes, and relatively high density of trichomes, which provided a better environment for *A. gossypii* and indirectly favored *A. aphidimyza*. This study showed that squash is the most suitable host plant for rearing *A. aphidimyza* feeding on *A. gossypii*.

European corn borer and its parasites overwintering abundance and damages on different corn FAO maturity groups

Lemic, D; Mandic, J; Cacicja, M; Drmic, Z; Mrganic, M; Cavlovicak, S; Bazok, R; Viric Gasparic, H. 2019

European corn borer (ECB) is one of the most significant maize pests in the world and also in Croatia. ECB causes yield reduction from 2 to 25%, even more in years favorable for its development. According to estimations, these losses are around 7%. About 90% of the hybrids had some resistance to whorl-leaf feeding (first-generation ECB) and 75% had some resistance to sheath and sheath-collar feeding (second-generation ECB). Along with resistance, modern maize hybrids possess certain level of tolerance. The main aim of this paper was to determine moth eclosion of the overwintering generation and presence of the parasites of ECB larvae during the overwintering as well as to estimate population density of ECB on maize growing area in Croatia. Also, the aim was to establish the differences among maize FAO maturity groups in damage caused by ECB larvae. Estimated overwintering population was over 8,000 moths/ha i.e. more than 4 million larvae of first generation. During the overwintering four different parasites attacked the larvae. Two species belong to the order Hymenoptera (*Cotesia marginiventris* Cresson and *Eriborus terebrans* Gravenhorst) and two species belong to the order Diptera (*Ramonda spathulata* Fallen and *Lydella thompsoni* Herting). One caterpillar predator species *Paragymnomerus spiricornis* Spinola (Hymenoptera) overwinters in maize stalks as well. The highest attack of the first ECB generation was recorded on FAO maturity group 500. The damage from second ECB generation was the highest on FAO groups 400 and 500. The maize of the higher FAO groups has high and robust stems with large number of big leaves. That intensive vegetative growth is a biological characteristic that attracts first generation of ECB to intensifying egg laying. High population level of the first generation may lead to high level of second ECB generation which ultimately caused yield reduced on the hybrids of longer vegetation period (medium-late FAO maturity groups).

Is Genetic Continuity Between *Anopheles sinensis* (Diptera: Culicidae) and its Sibling Species Due to Gene Introgression or Incomplete Speciation?

Wang, G; Guo, XX; Song, FL; Zheng, W; Tan, WL; Huang, EJ; Wu, JH; Wang, CC; Yang, QG; Li, CX; Zhao, TY. 2019

The *Anopheles* mosquito *Hyrceanus* Group is widely distributed geographically across both Palearctic and Oriental regions and comprises 26 valid species. Although the species *Anopheles sinensis* Wiedemann (1828) is the most common in China and has a low potential vector rank, it has nevertheless long been thought to be an important natural malaria vector within the middle and lower reaches of the Yangtze River. A number of previous research studies have found evidence to support the occurrence of natural hybridization between *An. sinensis* and *Anopheles kleini* Rueda, 2005 (a competent malaria vector). We, therefore, collected a sample series of *An. sinensis* and morphologically similar species across China and undertook ribosomal and mitochondrial DNA analyses in order to assess genetic differentiation (Fst) and gene flow (Nm) amongst different groups. This enabled us to evaluate divergence times between morphologically similar species using the cytochrome oxidase I (COI) gene. The results of this study reveal significant genetic similarities between *An. sinensis*, *An. kleini*, and *Anopheles belenrae* Rueda, 2005 and therefore imply that correct molecular identifications will require additional molecular markers. As results also reveal the presence of gene flow between these three species, their taxonomic status will require further work. Data suggest that *An. kleini* is the most basal of the three species, while *An. sinensis* and *An. belenrae* share the closest genetic relationship.

DNA-aided identification of *Culex* mosquitoes (Diptera: Culicidae) reveals unexpected diversity in underground cavities in Austria

Zittra, C; Moog, O; Christian, E; Fuehrer, HP. 2019

Subterranean cavities serve as resting places and hibernation shelters for mosquitoes. In Europe, members of the genus *Culex* are often the most abundant insects on cave walls. *Culex pipiens* L., the common house mosquito, exists in two physically very similar, yet genetically and ecologically distinct biotypes (or forms, f.), namely *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus*. Autogeny and stenogamy of the latter form have been interpreted as adaptations to underground habitats. The epigeal occurrence of the two biotypes and their hybrids was recently examined in Eastern Austria, but the hypogean distribution of the *Cx. pipiens* complex and morphologically similar non-members such as *Cx. torrentium* is unknown. Considering the key role of *Culex* mosquitoes in the epidemiology of certain zoonotic pathogens, the general paucity of data on species composition and relative abundance in subterranean shelters appears unfortunate. For a first pertinent investigation in Austria, we collected mosquitoes in four eastern federal states. Based on analyses of the ACE2 gene and the CQ11 microsatellite locus, 150 female and three male mosquitoes of the genus *Culex*, two females of the genus *Culiseta* and a single female of the genus *Anopheles* were determined to species level or below. In our catches, *Cx. pipiens* f. *pipiens* exceeded the apparent abundance of the purportedly cave-adapted *Cx. pipiens* f. *molestus* many times over. Records of *Cx. hortensis* and *Cx. territans*, two species rarely collected in Austria, lead us to infer that underground habitats host a higher diversity of culicine mosquitoes than previously thought.

Evaluation of Monitoring Traps and Lures for *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Plantings in Florida

Harmon, DS; Haseeb, M; Kanga, LHB; Liburd, OE. 2019

Drosophila suzukii (Diptera: Drosophilidae) is an invasive insect pest that was detected in Florida in August 2009 in Hillsborough County. Very limited information is available for berry growers to properly detect and monitor this serious pest in southern highbush blueberry (hybrids of *Vaccinium corymbosum* L. x *V. darrowi* Camp), rabbiteye blueberry (*Vaccinium virgatum* L.), and blackberry (*Rubus fruticosus* L.) production systems. We compared several *D. suzukii* traps and lures/baits at two sites in Florida. The traps evaluated included Trece, Scentry, and a standard homemade cup trap. These traps were compared with various baits and lures, including Trece lure, Scentry lure, yeast bait, and *Suzukii* trap, under Florida production systems. Early detection is important to develop an effective monitoring system so management action can be taken before economic damage occurs. Data were recorded as overall trends, as well as in 4-5 trapping periods from early to late season. Overall, the Scentry trap baited with Scentry lure, the Trece trap baited with Trece lure + yeast, and the Trece trap baited with Scentry lure were the best performing traps. Yeast-based traps were also attractive to *D. suzukii* early in the season, but they did not provide consistent captures as the season progressed. The Scentry trap with yeast bait, the Scentry trap with Scentry lure, the Trece trap with Trece lure + yeast bait, and a cup trap with yeast bait caught most of the flies during the first

trapping period in 2015 and 2016 in the rabbiteye blueberry. In the southern highbush blueberry, the population of *D. suzukii* was much lower than in the rabbiteye blueberry planting, and the Scentry trap with Scentry lure captured the highest number of flies during the first trapping period in 2016. In the blackberry, the Scentry trap with Scentry lure numerically had the highest captures during the first trapping period, but this was not significantly different from the cup trap with yeast bait, the Trece trap baited with *Suzukii* trap, and the Trece trap with Trece lure. Overall, the Scentry trap with Scentry lure was the most consistent trap that captured *D. suzukii* flies throughout the season in the three production systems-rabbiteye blueberry, southern highbush blueberry, and blackberry. Growers in low pressure systems that are similar to Florida can use the Scentry trap with Scentry lure to monitor *D. suzukii* populations.

SPECIES COMPOSITION AND ABUNDANCE OF THE NATURAL ENEMIES OF SUGARCANE APHID, *MELANAPHIS SACCHARI* (ZEHNTNER) (HEMIPTERA: APHIDIDAE), ON SORGHUM IN TEXAS

Maxson, EL; Brewer, MJ; Rooney, WL; Woolley, JB. 2019

The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an emergent sorghum pest in the United States. This study was designed to identify which natural enemy species are present in aphid populations in sorghum in Texas, and to track the seasonal population trends of the aphid and its natural enemies on sorghum hybrids that differ in susceptibility to the aphid. From 2015 through 2016, sugarcane aphid and its natural enemies were sampled weekly in plots of aphid-susceptible and partially aphid-resistant sorghum hybrids at two field sites in Nueces County and Burleson County, Texas. In 2015, aphids and natural enemies had greater peak abundance on the susceptible hybrid than on the resistant hybrid. Peak abundance of most natural enemies tended to lag behind that of aphids by one to two weeks. Natural enemy taxa observed at both field sites included two primary parasitoid wasp species (Hymenoptera: Aphelinidae: *Aphelinus nigritus* Howard stat. rev.; Braconidae: *Lysiphlebus testaceipes* (Cresson)), one species of hyperparasitoid (Hymenoptera: Encyrtidae), ten lady beetle species (Coleoptera: Coccinellidae) and three morphospecies of dusky lady beetle (Coccinellidae: Scymninae), three hoverfly species (Diptera: Syrphidae), five green lacewing species (Neuroptera: Chrysopidae), brown lacewings (Neuroptera: Hemerobiidae: Hemerobius), and minute pirate bugs (Hemiptera: Anthocoridae: *Orius insidiosus* (Say)). *Aphelinus* and Coccinellidae were the numerically dominant natural enemy groups in all sorghum hybrids, followed by Chrysopidae and Syrphidae. Aphids mummified by *Aphelinus* were hyperparasitized by *Syrphophagus aphidivorus* at a rate of approximately 90%. Natural enemy densities were similarly proportionate to aphid densities on both aphid-susceptible and aphid-resistant plants. Overall, the continuity of natural enemy species composition and population trends supports that these natural enemies responded positively to sugarcane aphid on sorghum.

High-Elevation Chromosomal Diversity of Black Flies (Diptera: Simuliidae) in Thailand

Adler, PH; Srisuka, W; Van Lun, LL; Takaoka, H; Saeung, A. 2019

The highest elevations of tropical mountains, much like oceanic islands, often support disproportionately high levels of endemism, some of which is concealed as cryptic species. In a search for additional species, we explored the chromosomal diversity of three morphologically defined species of black flies known only from the upper elevations of Doi Inthanon, Thailand's highest mountain. Analysis of the polytene chromosomes facilitated the discovery of the previously unknown larva of *Simulium kiewmaepanense* Takaoka & Srisuka, permitted linkage of the formal name of *S. undecimum* Takaoka, Srisuka & Saeung with its previous cytological identity, and revealed cryptic species, designated A and B, within *S. tenebrosus* Takaoka, Srisuka & Atiporn. A probable new species of microsporidium was discovered in the larval fat body of *S. kiewmaepanense*, suggesting that at least some symbiotic organisms of endemic hosts are also restricted to Doi Inthanon. A mitochondrial DNA analysis did not distinguish the two cryptic species of *S. tenebrosus* despite complete reproductive isolation chromosomally inferred from an absence of hybrids, emphasizing the need for integrated approaches to species problems. The chromosomes of all four analyzed species were monomorphic, or nearly so, perhaps reflecting the suppression of polymorphic inversions that otherwise would restrict recombination needed to generate adequate variation in the peripheral environment of the mountain summit.

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.

The diagnostic utility of sequence-based assays for the molecular delimitation of the epidemiologically relevant *Culex pipiens pipiens* taxa (Diptera: Culicidae)

Francuski, L; Gojkovic, N; Krtinic, B; Milankov, V. 2019

The northern house mosquito (*Culex pipiens pipiens* L.) is a vector of several important pathogens and comprises two epidemiologically distinct ecotypes (*molestus* Forskal and *pipiens*). The delimitation of its ecotypes is a crucial, yet controversial step in vector surveillance due to varying diagnostic values of different characters. Therefore, we reviewed the success of a diagnostic assay based on the mitochondrial cytochrome c oxidase subunit I locus (COI) by analyzing previously published sequences of *molestus* and *pipiens* sampled in different geographical areas. Next, by genotyping individuals from Northern Serbia at this locus, we additionally assessed whether genetic structure of urban and rural *Cx. p. pipiens* ecotypes corresponded to the admixture pattern. Finally, to account for the different susceptibility of genetic markers to introgression, we also analyzed genetic structuring based on the ribosomal internal transcribed spacer 2 (ITS2). No latitude-dependent differentiation of *Cx. p. pipiens* ecotypes was found at a global level, with the COI assay further failing to accurately identify *molestus* and *pipiens* ecotypes. Likewise, both individual-(BAPS) and population-based (analysis of molecular variance and F-ST estimates) methods showed no significant urban/rural genetic differentiation in Serbia, indicating unhindered gene flow between different *Cx. p. pipiens* habitat types. The findings challenge the previous instances of *Cx. p. pipiens* ecotype identification, while also spotlighting the vectorial capacity of their hybrid offspring.

CHROMOSOMAL RELATIONSHIPS OF BLACK FLIES (DIPTERA: SIMULIIDAE) IN THE *SIMULIUM AUREUM* FRIES SPECIES GROUP ON THE GREEK ISLAND OF RHODES

Adler, PH; Seitz, G. 2020

Larval black flies in the *Simulium* (*Eusimulium*) *aureum* Fries species group from the Greek island of Rhodes were chromosomally analyzed to clarify species limits

and relationships with mainland taxa. Three species were identified, corresponding with earlier morphological identifications: *S. flexibranchium* Crosskey, *S. petricolum* (Rivosecchi), and *S. rubzovianum* (Sherban). The morphologically established endemic species *S. flexibranchium* shows chromosomal banding equivalency with *S. angustipes* Edwards, a widespread Palearctic species; the pair, therefore, are homosequential species, the ninth such example in the Simuliidae. The species status of *S. flexibranchium* is maintained on the basis of structural characters that allow diagnosis across the four islands from which it is known in the Aegean Sea. The chromosomal banding sequences of Rhodian populations of *S. petricolum* and *S. rubzovianum*, as well as a population of the latter that we examined from Cyprus, conform to the classic sequences of these species across their ranges, but tend toward the monomorphic condition typical of island black flies. The first definitive hybrid in the *S. aureum* group, a larva of *S. flexibranchium* x *S. petricolum*, was discovered chromosomally, and suggests an overall hybridization rate in the *S. aureum* group, with hybrid survival to the late larval stage, of less than 0.1%.

Hybridization in Phlebotominae (Diptera: Psychodidae): A mini-review

dos Reis, YV; Alevi, KCC. 2020

Taxonomy based only on morphology, although extremely important for the classification of sandflies, has been shown to be insufficient for the delimitation of some taxa. Thus, integrative taxonomy could play a fundamental role in clarifying these and other taxonomic issues, since data from different areas are used to aggregate greater reliability in species classification. Experimental crosses are important taxonomic tools, since the presence of reproductive barriers when associated with divergence between two evolutionary lineages, confirms the specific status of taxa based on the biological species concept. In the subfamily Phlebotominae, experimental crosses were mostly focused on the study of the *Lutzomyia longipalpis* complex, which helped to identify different evolutionary lineages for that group of vectors. Considering the difficulty of classifying some Phlebotominae species and the importance of hybridization studies for taxonomy, we grouped all the information associated with experimental crosses in sandflies in a mini-review. In view of the results grouped in this review, it is evident that i) experimental crossings are important tools to aggregate studies of integrative taxonomy in the Phlebotominae subfamily; ii) these analyses should be applied in the taxonomic studies of cryptic species; iii) *Lu. longipalpis* populations have pre and/or post-zygotic reproductive barriers; iv) *Lu. longipalpis* represents more than one species and efforts must be applied to differentiate the taxa of the *Lu. longipalpis* complex; v) *Phlebotomus* populations do not present intraspecific reproductive barriers; vi) the absence of reproductive barriers between *Ph. sergenti* from Israel and Turkey (representing populations of the same evolutionary lineage) does not rule out the possible existence of cryptic species, it being necessary to perform experimental crosses between the different strains indicated by the molecular markers; and finally, vii) different species of *Phlebotomus* have post-zygotic barriers, confirming the specific status of *Ph. duboscqi*, *Ph. papatasi*, and *Ph. bergeroti*.

Long-term coexistence of a hybridization-derived population of *Drosophila parapallidosa* with closely related *Drosophila ananassae* (Diptera: Drosophilidae)

Hama, Y; Lee, CY; Matsuda, M; Kamimura, Y; Sawamura, K. 2020

A 2012-13 survey on Penang Island, Malaysia, revealed the existence of both *Drosophila ananassae* and *Drosophila parapallidosa*, the latter of which carries chromosomes Y and 4 from *D. ananassae* and thus is of hybrid origin. We collected the flies again from the same location in 2018. The hybrid population remained present, which suggests that the *D. parapallidosa* of hybrid origin does not represent a mere transient population but is stable. Why do these two species coexist irrespective of gene flow? We realized that body size is generally larger in *D. ananassae* than in *D. parapallidosa*, which constitutes a new character with which to discriminate these species; previously the number of sex comb teeth was the only diagnostic trait. Character displacement was not detected, however, for those traits. We crossed these two species, which resulted in offspring that had an altered genomic constitution. The body size of *D. ananassae* was dominant, and the presence of chromosomes Y and 4 did not have a significant effect on body size. By contrast, the presence of chromosome 4 from *D. ananassae* significantly affected the number of sex comb teeth. Even flies having a genomic constitution similar to that of the Penang *D. parapallidosa* exhibited a number of sex comb teeth that was intermediate between the two species. We propose that the *D. parapallidosa* sex comb character underwent selection during evolution of the Penang Island population. Reproductive interference between the species, presumably caused by signal jamming, was detected.

Species Composition of Anopheles (Diptera: Culicidae) in Selected Forested Tourist Areas of Nigeria Endemic for Malaria

Oduwale, OA; Oringanje, CM; Oduola, AO; Nwachuku, NS; Meremikwu, MM; Alaribe, AAA. 2020

The study was carried out to determine relative abundance, species diversity, of *Anopheles* species (Diptera: Culicidae) in selected forested areas in Cross River State, Nigeria and the prevalence of malaria infection in the specimens. Mosquitoes were collected using pyrethrum spray catch and Centre for Disease Control light traps modified with yeast and sugar to generate carbon dioxide (CO₂) and identified using morphological identification keys. We used a multiplex polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) to simultaneously distinguish sibling species of the *An. gambiae* s.l., including separation of *An. gambiae* s.s. and *An. coluzzii* (Diptera: Culicidae). The samples were also screened for *Plasmodium* infection using the enzyme-linked immunosorbent assay. One hundred and four *Anopheles* specimens were collected during the study of which 97% was *An. gambiae* complex and 3% was *An. rufipes* (Diptera: Culicidae). Only 77% of the *An. gambiae* s.l. was identified to species level. The result shows that 41.6% was *An. gambiae* s.s. and 34.6% was *An. coluzzii*. No sporozoite of *Plasmodium* was detected in the *Anopheles* species. The study also found a hybrid form of *An. gambiae* s.s. and *An. coluzzii*. These findings suggest the first documented evidence of hybrid forms of *An. gambiae* s.s./*An. coluzzii* in South Eastern Nigeria although its epidemiological implication is still not clear.

Bloodmeal, Host Selection, and Genetic Admixture Analyses of *Culex pipiens* Complex (Diptera: Culicidae) Mosquitoes in Chicago, IL

Kothera, L; Mutebi, JP; Kenney, JL; Saxton-Shaw, K; Ward, MP; Savage, HM. 2020

The area in and around Chicago, IL, is a hotspot of West Nile virus activity. The discovery of a *Culex pipiens* form *molestus* Forsk. population in Chicago in 2009 added to speculation that offspring from hybridization between *Cx. pipiens* f. *pipiens* L. and f. *molestus* could show a preference for feeding on humans. We collected blood-fed female mosquitoes (N = 1,023) from eight residential sites and one public park site in Chicago in July and August 2012. Bloodmeal analysis using the COI (cytochrome c oxidase subunit I) gene was performed to ascertain host choice. Almost all (99%) bloodmeals came from birds, with American Robins (*Turdus migratorius* L.) and House Sparrows (*Passer domesticus* L.) making up the largest percentage (74% combined). A forage ratio analysis comparing bird species fed upon and available bird species based on point count surveys indicated Northern Cardinals (*Cardinalis cardinalis*) and American Robins (*Turdus migratorius*) appeared to be over-utilized, whereas several species were under-utilized. Two human bloodmeals came from *Culex pipiens* complex mosquitoes. Admixture and population genetic analyses were conducted with 15 microsatellite loci on head and thorax DNA from the collected blood-fed mosquitoes. A modest amount of hybridization was detected between *Cx. pipiens* f. *pipiens* and f. *molestus*, as well as between f. *pipiens* and *Cx. quinquefasciatus* Say. Several pure *Cx. quinquefasciatus* individuals were noted at the two Trumbull Park sites. Our data suggest that *Cx. pipiens* complex mosquitoes in the Chicago area are not highly introgressed with f. *molestus* and appear to utilize avian hosts.

Chemistry matters: biological activity of Eucalyptus essential oils on mosquito larval mortality

Gallon, C; Martello, RH; Cozzer, G; Rezende, CAL; Calisto, JFF; Floss, PA; Oliveira, JV; Rezende, RD; Dal Magro, J; Albeny-Simoes, D. 2020

The mosquito *Aedes aegypti* L. (Diptera: Culicidae) is a vector of arboviral diseases such as dengue fever. Currently, the main approach to mosquito control is the

application of synthetic insecticides, which can lead to negative environmental impacts and insecticide resistance in mosquito populations. As such, there has been increased interest in developing alternative methods for control of vector populations such as utilizing plant compounds that act as larvicides. The aim of this work is to evaluate the effectiveness of *Eucalyptus* sp. (Myrtaceae) essential oils for control of *Ae. aegypti* larvae. The essential oils of seven *Eucalyptus* species and hybrids were extracted by hydrodistillation and analyzed by gas chromatography coupled to mass spectrometry. The essential oils were further diluted in water with acetone (0.40%) at the following concentrations: 100, 50, 25, and 10 $\mu\text{g ml}^{-1}$. Mortality trials were conducted in plastic containers with a solution of ultrapure water and 200 μl of diluted oil for a total volume of 50 ml per treatment. The experiments for each *Eucalyptus* species/hybrid and concentration were performed in triplicate, using a control containing only water and acetone. Twenty larvae were added to each container and mortality was recorded at 1, 2, 4, and 24 h. The *Eucalyptus* essential oils showed larvicidal activity in most of the evaluated concentrations, mainly at 50 and 100 $\mu\text{g ml}^{-1}$. *Eucalyptus benthamii* Maiden & Cambage and the hybrid *Urograndis* displayed the highest larvicidal potential (100% at 24 h) in the 100 $\mu\text{g ml}^{-1}$ treatment. Larval mortality of *Ae. aegypti* showed a positive correlation with the compounds gamma-, o-cymol, o-cymene, terpineol, 3-odecylfuran-2,5-dione, alpha-pinene, globulol, and ledol. The most abundant compounds identified in the essential oils were 1,8-cineole and alpha-pinene. These results highlight the potential of using *Eucalyptus* essential oils for the isolation of natural larvicidal products.

Morphological and molecular identification of Culicidae mosquitoes (Diptera: Culicidae) in Lorestan province, Western Iran

Kayed, MH; Sepahvand, F; Mostafavi, E; Chinikar, S; Mokhayeri, H; Sharafi, AC; Wong, G; Shahhosseini, N; Kazemi, SHM. 2020

Culicidae mosquitoes are main vectors of arboviruses that cause arboviral diseases in humans. Studies on fauna, ecology, biology, resting behaviors of Culicidae mosquitoes are important and greatly impacts the control of arboviral diseases that are transmitted by vectors. The aim of the present study was to determine fauna of mosquitoes (Diptera: Culicidae) based on morphological and molecular (genomic) identification and their habitats in Lorestan province, Western Iran. Meanwhile mosquito samples were examined for arbovirus infection. Culicidae mosquitoes were caught in 2015 and 2016 from human homes, animal dwellings, storehouses and pit shelters in Lorestan province, Western Iran, using an oral aspirator (hand catch), total catch, human and animal bait and light trap methods. The samples were identified on the genus and species. Six species of *Culex* and eight species of *Anopheles* were caught. One complex species (*Cx. pipiens* complex) and a hybrid between *Cx. pipiens* *pipiens* biotype *pipiens* and *Cx. pipiens* *pipiens* biotype *molestus* were identified. Among all of the trapped mosquitoes (4211), 94.68% were from genus *Culex* mosquitoes (3987), which indicate that this genus is the dominant in Lorestan province, Western Iran. *Anopheles* comprised of 201 individuals out of the total catch. Arboviruses were not detected in these samples.

Low Levels of Pyrethroid Resistance in Hybrid Offspring of a Highly Resistant and a More Susceptible Mosquito Strain

Pinch, M; Rodriguez, SD; Mitra, S; Kandel, Y; Moore, E; Hansen, IA. 2020

The use of insecticides has been a central approach to control disease-transmitting mosquitoes for the last century. The high prevalence of pyrethroid use as public health insecticides has resulted in the evolution of pyrethroid resistance in many populations of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae), throughout its global distribution range. Insecticide resistance is often correlated with an associated fitness cost. In this project, we studied the phenotypes of hybrid mosquitoes derived from crossing a pyrethroid-resistant strain of *Ae. aegypti* (Puerto Rico [PR]) with a more susceptible one (Rockefeller [ROCK]). We first sequenced and compared the para gene of both original strains. We then crossed males from one strain with females of the other, creating two hybrids (Puertoeller, Rockorico). We used aY-tube choice assay to measure the attraction of these strains towards a human host. We then compared the levels of pyrethroid resistance in the different strains. We found three known resistance mutations in the para gene sequence of the PR strain. In our attraction assays, PR females showed lower attraction to humans, than the ROCK females. Both hybrid strains showed strong attraction to a human host. In the insecticide resistance bottle assays, both hybrid strains showed marginal increases in resistance to permethrin compared to the more susceptible ROCK strain. These results suggest that hybrids of sensitive and permethrin-resistant mosquitoes have an incremental advantage compared to more susceptible mosquitoes when challenged with permethrin. This explains the rapid spread of permethrin resistance that was observed many times in the field.

Anchored hybrid enrichment challenges the traditional classification of flesh flies (Diptera: Sarcophagidae)

Buenaventura, E; Szpila, K; Cassel, BK; Wiegmann, BM; Pape, T. 2020

Sarcophagidae is one of the most species-rich families within the superfamily Oestroidea. This diversity is usually represented by three lineages: Miltogramminae, Paramacronychiinae and Sarcophaginae. Historically, the phylogenetic relationships among these lineages have been elusive, due to poorly supported hypotheses or small taxon sets, or both. This study provides a dramatic increase in molecular data, more balanced sampling of all three lineages from all biogeographical regions and a reassessment of morphological characters using scanning electron microscopy in the most comprehensive assessment of subfamily-level phylogeny in Sarcophagidae to date. This analysis of the largest molecular dataset ever produced for a phylogenetic analysis of a fly lineage, with 950 loci from anchored hybrid enrichment comprising 435 930 bp from 101 species, revealed Paramacronychiinae as sister to Miltogramminae, not to Sarcophaginae, as suggested by adult morphology. Maximum likelihood analysis produced a well-supported topology, with 91% of the nodes receiving strong bootstrap proportions (> 97%). In contrast to the molecular data, three out of nine morphological characters studied point to a sister-group relationship of (Sarcophaginae + Paramacronychiinae) and the remaining six characters are either silent on subfamily relationships or in need of further study. Re-examination of morphological structures provides new insights into the evolution of male genitalic traits within Sarcophagidae and highlights their convergence producing conflicting phylogenetic signal. Our phylogeny reconciles older and widely used systems of classification with tree-based thinking and sets up a classification of flesh flies that is more aligned with their evolutionary history.

A High-Quality Genome Assembly from Short and Long Reads for the Non-biting Midge *Chironomus riparius* (Diptera)

Schmidt, H; Hellmann, SL; Waldvogel, AM; Feldmeyer, B; Hankeln, T; Pfenninger, M. 2020

Chironomus riparius is of great importance as a study species in various fields like ecotoxicology, molecular genetics, developmental biology and ecology. However, only a fragmented draft genome exists to date, hindering the recent rush of population genomic studies in this species. Making use of 50 NGS datasets, we present a hybrid genome assembly from short and long sequence reads that make *C. riparius*' genome one of the most contiguous Dipteran genomes published, the first complete mitochondrial genome of the species, and the respective recombination rate among the first insect recombination rates at all. The genome assembly and associated resources will be highly valuable to the broad community working with dipterans in general and chironomids in particular. The estimated recombination rate will help evolutionary biologists gaining a better understanding of commonalities and differences of genomic patterns in insects.

The puzzling mitochondrial phylogeography of the black soldier fly (*Hermetia illucens*), the commercially most important insect protein species

Stahls, G; Meier, R; Sandrock, C; Hauser, M; Zoric, LS; Laiho, E; Aracil, A; Doderovic, J; Badenhorst, R; Unadirekkul, P; Adom, NABM; Wein, L; Richards, C; Tomberlin, JK; Rojo, S; Veselic, S; Parviainen, T. 2020

Background The black soldier fly (Diptera: Stratiomyidae, *Hermetia illucens*) is renowned for its bioconversion ability of organic matter, and is the worldwide most widely used source of insect protein. Despite varying extensively in morphology, it is widely assumed that all black soldier flies belong to the same species, *Hermetia*

illucens. We here screened about 600 field-collected and cultured flies from 39 countries and six biogeographic regions to test this assumption based on data for three genes (mitochondrial COI, nuclear ITS2 & 28S rDNA) and in order to gain insights into the phylogeography of the species. Results Our study reveals a surprisingly high level of intraspecific genetic diversity for the mitochondrial barcoding gene COI (divergences up to 4.9%). This level of variability is often associated with the presence of multiple species, but tested nuclear markers (ITS2 and 28S rDNA) were invariant and fly strain hybridization experiments under laboratory conditions revealed reproductive compatibility. COI haplotype diversity is not only very high in all biogeographic regions (56 distinct haplotypes in total), but also in breeding facilities and research centers from six continents (10 haplotypes: divergences up to 4.3%). The high genetic diversity in fly-breeding facilities is mostly likely due to many independent acquisitions of cultures via sharing and/or establishing new colonies from field-collected flies. However, explaining some of the observed diversity in several biogeographic regions is difficult given that the origin of the species is considered to be New World (32 distinct haplotypes) and one would expect severely reduced genetic diversity in the putatively non-native populations in the remaining biogeographic regions. However, distinct, private haplotypes are known from the Australasian (N = 1), Oriental (N = 4), and the Eastern Palearctic (N = 4) populations. We reviewed museum specimen records and conclude that the evidence for introductions is strong for the Western Palearctic and Afrotropical regions which lack distinct, private haplotypes. Conclusions Based on the results of this paper, we urge the black soldier fly community to apply molecular characterization (genotyping) of the fly strains used in artificial fly-breeding and share these data in research publications as well as when sharing cultures. In addition, fast-evolving nuclear markers should be used to reconstruct the recent invasion history of the species.

A rural-urban latitudinal study of the distributions of *Culex quinquefasciatus* and *Culex pipiens* bioforms in their southernmost sympatric fringe

Cardo, MV; Rubio, A; Junges, MT; Vezzani, D; Carbajo, AE. 2020

Mosquitoes grouped in the complex *Culex pipiens* L. (Diptera: Culicidae) are important vectors of medical and veterinary diseases. In the South American sympatric region, *Cx. pipiens* and *Culex quinquefasciatus* Say coexist and potentially hybridize. To identify key drivers of their geographical distribution, mosquito immatures were collected from flower vases of eight urban/rural cemetery pairs within a 5 degrees latitudinal transect along Buenos Aires Province, Argentina. The specimens were identified by molecular methods and their relative proportion modelled as a function of environmental variables. At the beginning of the warm season, northern and southern cemeteries presented exclusively *Cx. quinquefasciatus* and *Cx. pipiens*, respectively, with different proportions of both at mid latitudes. By the end of the summer, *Cx. quinquefasciatus* was present throughout the study area, exclusively in 11 of the 16 cemeteries both rural and urban, whereas *Cx. pipiens* was predominant only in the southernmost pair. Mean annual temperature, photoperiod variability and time of the season were key drivers of their distributions. All specimens of *Cx. pipiens* were identified as form *molestus* and no hybrids were recognized. The reported distribution patterns and the potential absence of *Cx. pipiens f. pipiens* and hybrids are discussed, along with their implications in disease transmission.

West Nile Virus Mosquito Vectors (Diptera: Culicidae) in Germany

Kampen, H; Holicki, CM; Ziegler, U; Groschup, MH; Tews, BA; Werner, D. 2020

In 2018, West Nile virus (WNV) broke out for the first time in Germany, with continuation of the epidemic in 2019, involving birds, horses and humans. To identify vectors and characterize the virus, mosquitoes were collected in both years in zoological gardens and on a horse meadow immediately following the diagnosis of disease cases in birds and horses. Mosquitoes were identified and screened for WNV by qRT-PCR, with virus-positive samples being sequenced for the viral envelope protein gene. While no positive mosquitoes were found in 2018, seven mosquito pools tested positive for WNV in 2019 in the Tierpark (Wildlife Park) Berlin. The pools consisted of *Cx. pipiens* biotype *pipiens* (n = 5), and a mixture of *Cx. p. biotype pipiens* and *Cx. p. biotype molestus* (n = 2), or hybrids of these, and were collected between 13 August and 24 September 2019. The virus strain turned out to be nearly identical to two WNV strains isolated from birds diseased in 2018 in eastern Germany. The findings represent the first demonstration of WNV in mosquitoes in Germany and include the possibility of local overwintering of the virus.

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).

Genetic analysis and population structure of the *Anopheles gambiae* complex from different ecological zones of Burkina Faso

Zoure, AA; Noel, G; Sombie, A; Somda, Z; Badolo, A; Francis, F. 2020

The *Anopheles gambiae* complex (Diptera: Culicidae) is the most important vector for malaria in Sub-Saharan Africa, besides other vectors such as *Anopheles funestus*. Malaria vector control should encompass specific identification, genetic diversity and population structure of *An. gambiae* to design vector control strategies. The aim of this study was to determine the distribution of sibling species of the *An. gambiae* complex according to climatic regions related to cotton-growing or cotton-free areas by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Then, variation in mitochondrial cytochrome c oxidase 1 (COI) was used to assess the genetic structure within and between populations from our selected ecological zones. At the sibling species level, the following proportions were found across all samples (n = 180): *An. coluzzii* 65.56%, *An. gambiae* stricto sensu (s.s.) 21.11%, and *An. arabiensis* 3.33%. Hybrids between *An. gambiae* s.s. and *An. coluzzii* (7.78%) and hybrids between *An. coluzzii* and *An. arabiensis* (2.22%) were found. The phylogenetic tree and Integer Neighbour-Joining (IntNJ) haplotype network did not reveal any distinct genetic structure pattern related to climatic or agricultural conditions in Burkina Faso. The *F_{st}* (Wright's F-statistic) values close to zero showed a free gene flow and no differentiation in *An. gambiae* complex populations. Furthermore, neutrality indices calculated by Tajima's *D*, *F_u* and Li's *D**, *F_u* and Li's *F**, *F_u*'s *F_s* tests suggested an excess of rare mutations in the populations. Overall, variation in the proportions of *An. gambiae* s.s., *An. coluzzii* and *An. arabiensis* was found according to climatic regions, but COI analysis did not evidence any population structuring of the *An. gambiae* complex. These scientific contributions can be used as a basis for further in-depth study of the genetic diversity of the *An. gambiae* complex for epidemiological risk assessment of malaria in Burkina Faso.

Sexual and natural selection on pollen morphology in *Taraxacum*

Lynn, A; Piotter, E; Harrison, E; Galen, C. 2020

Premise Spiny pollen has evolved independently in multiple entomophilous lineages. Sexual selection may act on exine traits that facilitate male mating success by influencing the transfer of pollen from the anther to the body of the pollinator, while natural selection acts to increase pollen survival. We postulated that relative to sexual congeners, apomictic dandelions undergo relaxed selection on traits associated with male mating success. Methods We explored sexual selection on exine traits by measuring the propensity for *Taraxacum* spp. pollen to attach to hairs of flower-visiting bumblebees (*Bombus* spp.) or flies (Diptera: Syrphidae and Muscoidea) and assessed natural selection by testing whether pollen traits defend against consumption. Results Pollen picked up by bumblebees exhibited a narrower subset of spine-spacing phenotypes, consistent with stabilizing selection. Flies picked up larger pollen from flowers than expected at random. Surveys of corbiculae

(pollen basket) contents from foraging bumblebees and feces of flies showed that pollen grains consumed by both kinds of visitors are similar in spine characteristics and size to those produced by the donor. When bees visit inflorescences of apomictic *T. officinale*, they pick up pollen with spine-spacing phenotypes above the mean and shifted toward those of sexual *T. ceratophorum*. Conclusions We demonstrate that traits under sexual selection during pollen pickup vary among pollinators, while natural selection for pollen defense is nil in *T. ceratophorum*. In hybrid zones between apomictic and sexual dandelions, pollen traits place apomictic donors at a dispersal disadvantage, potentially reinforcing reproductive isolation.

Phytophagy in a *Polypedilum* (Diptera: Chironomidae) species new to Australia and Japan: taxonomy and expanded Asian distribution

Tang, HQ; Cranston, PS. 2020

Polypedilum johannseni Sublette & Sublette (= *Chironomus* (*Polypedilum*) *anticus* Johannsen), described from Indonesia, is recorded from Australia, where larvae are newly reported to feed on species of the aquatic fern *Azolla* Lam. (Salvinaceae). We extend the Australian distribution for the species that was reported previously as *Polypedilum* 'nr seorsus' from northern Australia. Review of the species in Asia shows previously known presence in Singapore as *Polypedilum anticus* and in Thailand and the Philippines, where the larva was reported to feed on *Azolla*. In China, *Polypedilum johannseni* is reported from a diverse range of aquatic host plants, including *Azolla*. We also newly report the species from Japan, where larvae feed on hybrid *Azolla cristata* x *Azolla filiculoides* and *Trapa japonica* Flerow (Lythraceae). We review the taxonomy, provide new illustrations, record and map the wider distribution and comment on morphological variation. Here, we also redescribe the Australian *Polypedilum seorsus* (Skuse) with which *P. johannseni* has been confused, and from China, we redescribe *Polypedilum tigrinum* Hashimoto, the larva of which is a proven aquatic phytophage that co-occurs with several species of *Polypedilum*, including *P. johannseni*.

Cryptic species of *Anopheles messeae* sensu lato (Diptera: Culicidae), their identification, features and nomenclature

Novikov, YM; Shevchenko, AI. 2020

The paper describes the change in perspective in the composition of the *A. messeae* taxonomic unit. Initially, based on the disequilibrium of natural populations, the species was differentiated into A and B forms using chromosomal inversions as markers. The positive assortative mating, as well as the ecological features and geographical distribution of these forms, made it possible to give them the status of species in statu nascendi. Later, we additionally investigated the EcoRI restriction fragments of the genomic DNA and the ITS2 nucleotide sequences in the A and B *A. messeae* species. Unambiguous differences between the species in the former marker and semi-quantitative differences in the latter one, alongside with the absence of hybrids in the populations studied, led us to conclude that *A. messeae* s.l. is comprised of two homosequential cryptic species with parallel chromosomal polymorphisms. Unequivocal parallels between *A. lewisi* Ludlow, 1920 and *A. messeae* B in regards to their features, as well as the identity of *A. daciae* Linton et al., 2004 to *A. messeae* A in its ITS2 sequence, and to *A. messeae* Fall. in diagnostic chromosomal inversions, allowed us to consider *A. lewisi* Ludlow, 1920 and *A. messeae* B as two names of the same biological species, and *A. messeae* Fall., 1926, *A. messeae* A, and *A. daciae* Linton et al., 2004 as three names of the other one. Both are members of the palaearctic group of the *Maculipennis* complex under the names *Anopheles* (Ano.) *lewisi* Ludlow, 1920 and *Anopheles* (Ano) *messeae* Falleroni, 1926, respectively.

Yield of southern highbush blueberry (*Vaccinium corymbosum*) using the fly *Calliphora albifrontalis* (Diptera: Calliphoridae) as a pollinator

Cook, DF; Deyl, RA; Mickan, BS; Howse, ET. 2020

Southern highbush blueberry plants (*Vaccinium corymbosum* hybrid) from a commercial blueberry farm were placed in quarantine glasshouses and either exposed to adults of the western golden-haired blowfly *Calliphora albifrontalis* (Malloch) or no insects at all over a 21-week period. Laboratory reared *C. albifrontalis* were regularly released into one house to maintain a population of 1000-1500 flies. Flies could only obtain sugar from the plant flowers, and there was sufficient water from the leachate fraction draining out of each bagged plant. Berries were harvested at least twice weekly from the bushes over 21 weeks (46 harvest dates), and yield (both total berry weight and numbers) recorded. Temperature and humidity were very similar between the glasshouses, and plants were irrigated daily with 2.5-4 L/plant as per the producer's recommendations. Adult *C. albifrontalis* required at least 1.5 flowers/fly to survive. Both more berries (17.14 kg from 9108 berries vs. 10.43 kg from 6379 berries) and larger berries (1.88 vs. 1.63 g/berry) were produced by the bushes with adult *C. albifrontalis* present. Mature berries are ready to pick approximate to 10 weeks after flower opening. This study showed that yield between the two treatments began to differ 11 weeks after the flies were first released. Berry yield remained higher in the house with flies (11.29 kg from 6177 berries at 1.83 g/berry) compared with those plants without flies (4.98 kg from 3427 berries at 1.45 g/berry). Berry size was positively correlated with seed numbers. This is the first demonstration under controlled conditions of the ability of an Australian calliphorid blowfly to pollinate and increase yield of commercial blueberry bushes.

Gonoactivity of *Culex* (*Culex*) (Diptera: Culicidae) Mosquitoes During Winter in Temperate Argentina

Branda, MF; Laurito, M; Visintin, AM; Almiron, WR. 2021

The subgenus *Culex* L. includes species involved in summer-autumn arbovirus transmission but studies during winter are scarce in temperate Argentina. Female specimens were collected host-seeking at dry-ice-baited traps during autumn-winter-spring at two sites in Cordoba City during 2016 and 2017. The specimens were morphologically identified and dissected to determine the follicular developmental stage (gonotrophic activity). Females with advanced follicular stages (>= III) were subjected to molecular procedures to confirm or re-identify previous morphological identification. Five species (*Culex apicinus* Philippi (Diptera: Culicidae), *Culex dolosus* (Lynch-Arribalzaga) (Diptera: Culicidae), *Culex maxi* Dyar (Diptera: Culicidae), *Culex pipiens pipiens* L. (Diptera: Culicidae), and *Culex quinquefasciatus* Say (Diptera: Culicidae)) were collected and found gonoactive during winter; showing that a high proportion of *Culex* (*Culex*) females remain reproductively active during the unfavorable season for mosquito populations. Among them, it is worth noting the collection of *Cx. quinquefasciatus*, vector of the St. Louis encephalitis virus (endemic in the city), a specimen of *Cx. p. pipiens*, and a hybrid of *Cx. p. pipiens*/*Cx. quinquefasciatus* (during autumn). The study of this community during winter should continue because a high gonoactive female proportion with advanced follicular stages was found: 29.12 and 13.07% in 2016 and 2017, respectively. Local studies such as this one provide evidence about ornithophilic *Culex* species with active year-round life cycles, species that could favor arbovirus overwintering.

Chronological Incongruences between Mitochondrial and Nuclear Phylogenies of *Aedes* Mosquitoes

Zadra, N; Rizzoli, A; Rota-Stabelli, O. 2021

One-third of all mosquitoes belong to the Aedini, a tribe comprising common vectors of viral zoonoses such as *Aedes aegypti* and *Aedes albopictus*. To improve our understanding of their evolution, we present an updated multigene estimate of Aedini phylogeny and divergence, focusing on the disentanglement between nuclear and mitochondrial phylogenetic signals. We first show that there are some phylogenetic discrepancies between nuclear and mitochondrial markers which may be caused by wrong taxa assignment in samples collections or by some stochastic effect due to small gene samples. We indeed show that the concatenated dataset is model and framework dependent, indicating a general paucity of signal. Our Bayesian calibrated divergence estimates point toward a mosquito radiation in the mid-Jurassic and an *Aedes* radiation from the mid-Cretaceous on. We observe, however a strong chronological incongruence between mitochondrial and nuclear data, the latter providing divergence times within the Aedini significantly younger than the former. We show that this incongruence is consistent over different datasets and taxon sampling and that may be explained by either peculiar evolutionary event such as different levels of saturation in certain lineages or a past history of

hybridization throughout the genus. Overall, our updated picture of Aedini phylogeny, reveal a strong nuclear-mitochondrial incongruence which may be of help in setting the research agenda for future phylogenomic studies of Aedini mosquitoes.

A new and a described species of *Cystiphora* (Diptera: Cecidomyiidae) from Japan, with reference to geographically diversified intraspecific populations of *C. taraxaci* and its host range expansion from native Japanese to alien and hybrid species of *Taraxacum* (Asteraceae)

Yukawa, J; Kim, W; Nishino, T; Minami, T; Yamauchi, S; Ogawa, M; Ohara, K. 2021

Two species of *Cystiphora* (Diptera: Cecidomyiidae) are newly recorded from Japan. A gall midge that induces leaf galls on *Taraxacum* species (Asteraceae) is identified as *Cystiphora taraxaci* (Kieffer). Another gall midge that is responsible for leaf galls on *Sonchus brachyotus* (Asteraceae) is described as a new species under the name *Cystiphora hachijounae* Yukawa and Kim based on its incised hypoproct of male terminalia and some of the pupal characteristics. Genetic analysis supported the species identifications and indicated that the Palearctic *C. taraxaci* has long existed in Japan. It has been diversifying geographically into intraspecific populations since at least 2.57 Ma. Our current study argues against the recent invasion of Japan by alien *C. taraxaci* at the time of multiple introductions of European *Taraxacum* species to Japan for use as food (seed oil), forage and greening material during the late 19th century. Genetic analysis of gall-bearing *Taraxacum* plants collected from various localities in Japan indicates that most of them were alien or hybrid plants, while native Japanese species were rare, and these plants exhibited polyploidy from 2x to 5x. *Cystiphora taraxaci* is considered to have expanded its host range from native Japanese to alien and hybrid species of *Taraxacum* without regard to polyploidy.

Land use and terrestrial arthropods at the Colombian Pacific coast

Lohr, B; Narvaez, A. 2021

Diversity, permanence, and activity of terrestrial arthropods were investigated in four areas of different land use in the lowlands of the Pacific coast of Colombia with the aim to identify potential predator species for the palm root borer, *Sagilassa valida*. Ten pitfall traps were established along a 100 m transect in four areas: a secondary forest, a 20 year.-old peach palm plantation, and two hybrid oil palm plantations of three and seven years of age, respectively. Twenty-two collections were made covering a whole year. All ants were identified to species or morphospecies level, the other arthropods to order or where possible to family level. In total, 50,603 arthropods were captured, the most abundant were ants (37.0 %), followed by *Collembola* (35.4 %), *Acari* (10.6 %), *Coleoptera* (7.0 %) and *Diptera*, *Hemiptera* and *Araneae* in almost equal numbers (around 2.5 %). *Orthoptera* (92 % *Gryllidae*) were present in all collections, always at low numbers. The highest number of ants were recorded in the oil palm transects; *Diptera*, *Hemiptera* and *Orthoptera* were more numerous in the secondary forest, *Acari*, *Araneae* and *Collembola* in the palm transects. *Ectatomma ruidum* was by far the dominant ant species (84.9 % of all specimens) and absent from only 20 of the 880 captures. The second most frequent ant genus were army ants with two species, *Labidus praedator* and *L. coecus*. Rainfall, even area-wide flooding, and temperature did not explain variability in captures of any taxonomic group satisfactorily. We conclude that *E. ruidum* might be the predator to provide control of the root borer and recommend further studies on its efficiency.

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.

Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host species

Common, LK; Sumasgutner, P; Dudaniec, RY; Colombelli-Negrel, D; Kleindorfer, S. 2021

In invasive parasites, generalism is considered advantageous during the initial phase of introduction. Thereafter, fitness costs to parasites, such as host-specific mortality, can drive parasites towards specialism to avoid costly hosts. It is important to determine changes in host specificity of invasive populations to understand host-parasite dynamics and their effects on vulnerable host populations. We examined changes in mortality in the introduced avian vampire fly (*Philornis downsi*) (Diptera: Muscidae), a generalist myiasis-causing ectoparasite, between 2004 and 2020 on Floreana Island (Galapagos). Mortality was measured as the proportion of immature larvae found upon host nest termination. Over the time period, the avian vampire fly was most abundant and had low mortality in nests of the critically endangered medium tree finch (*Camarhynchus pauper*) and had the highest mortality in nests of hybrid tree finches (*Camarhynchus* spp.). Low larval mortality was also found in small tree (*Camarhynchus parvulus*) and small ground finch (*Geospiza fuliginosa*) nests. Selection could favour avian vampire flies that select medium tree finch nests and/or avoid hybrid nests. Overall, the finding of differences in avian vampire fly survival across host species is parsimonious with the idea that the introduced fly may be evolving towards host specialisation.

Importation biological control of invasive fire ants with parasitoid phorid flies-progress and prospects

Chen, L; Morrison, LW. 2021

Twenty-two known species of *Pseudacteon* flies (Diptera: Phoridae) are parasitoids of South American fire ants in the *Solenopsis saevissima* (Smith) complex. Phorid flies of different sizes and with differing activity patterns have been released in the United States to control two imported fire ant species-*Solenopsis richteri* and *Solenopsis invicta*-and their hybrid. Six highly host-specific *Pseudacteon* species have been successfully established at dozens of release sites and most are now widely distributed across areas infested by imported fire ants. This complex of released fly species is expected to weaken the competitive vigor of fire ant colonies through both direct and indirect effects, and eventually reduce the abundance of imported fire ants. To date, however, few studies have attempted to document the effect of these parasitoids on host ants in the field, and future research should focus on the overall magnitude of reduction in host ant populations. Knowledge gained from the successful importation and establishment of South American phorid flies in the US can provide guidance for utilization of these parasitoid flies for biological control of *S. invicta* in other introduced ranges, and aid the search for additional importation biological control agents of pest ants in general.

Field evaluation of roosting plants with food bait spray in managing melon fly, *Zeugodacus cucurbitae* in cucumber, *Cucumis sativus*

Ramasamy, M. 2021

Melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae) (Coquillett) is the major pest of cucumber, *Cucumis sativus*. We investigated the attractiveness of roosting plants with food bait spray to manage melon fly in cucumber under open field conditions. Cucumber was planted over two successive Kharif seasons together with maize (*Zea mays*), castor bean (*Ricinus communis*), sorghum (*Sorghum bicolor*), and Napier grass (Hybrid Co-3) as border plants. Border plants were sprayed weekly with an azadirachtin based food bait. The study revealed that castor bean was the most attractive roosting plant to melon fly followed by maize, sorghum, and Napier grass. The adult melon flies preferred to roost on the lower sides of the maize, sorghum, and Napier grass leaves and on the stems of castor bean. There were significant differences in the per cent fruit infestation of cucumber in all roosting host treatments except Napier grass compared to the control, with the lowest fruit infestation of 11.75% recorded in castor bean treatment. The highest cucumber yield 17.90 t/ha and the highest number of cucumber harvests 13.75 per season were observed in the castor bean treatment. Establishment of preferred roosting hosts could improve the bait application technique.

Diversity of mosquito fauna (Diptera, Culicidae) in higher-altitude regions of Croatia

Busic, N; Kucinic, M; Merdic, E; Bruvo-Madaric, B. 2021

Global climate change and the accompanying rise in temperature could affect the biology and ecology of a number of vectors, including mosquitoes. High altitude areas that were previously unsuitable for the spread of mosquito vector populations could become suitable. The aim of this research was to study the distribution of mosquito species in higher altitude regions of Croatia. Samples were collected in three areas: Slavonian Mountains, Gorski Kotar, and Middle Velebit. Specimens were morphologically determined and confirmed by DNA barcoding and other genetic markers and showed the presence of 16 species belonging to six genera. The most abundant species were the *Culex pipiens* complex with 50% of the collected specimens. Both *pipiens* (Linnaeus, 1758) and *molestus* (Forsk., 1775) biotypes and their hybrids were identified within the complex, followed by *Culex torrentium* (Martini, 1925) (20.2%), *Culiseta longiareolata* (Macquart, 1838) (8.5%), and the invasive species *Aedes japonicus* (Theobald, 1901) (7.8% of the total number of collected specimens). The remaining 12 species made up 14.7% of the collected specimens. Intraspecific COI p-distances were within the standard barcoding threshold for OTUs, while interspecific genetic distances were much higher, confirming the existence of barcoding gaps. Mosquito fauna of Croatian mountains showed a moderate variety and made 30.8% of the total number of recorded mosquito species in Croatia thus far.

Towards a new classification of Muscidae (Diptera): a comparison of hypotheses based on multiple molecular phylogenetic approaches

Grzywacz, A; Trzeciak, P; Wiegmann, BM; Cassel, BK; Pape, T; Walczak, K; Bystrowski, C; Nelson, L; Piwczynski, M. 2021

Muscidae are a megadiverse dipteran family that exhibits extraordinary diversity in morphology and life history as both immatures and adults. The classification of Muscidae has been long debated, and most higher-level relationships remain unknown. In this study, we used multilocus Sanger sequencing (mS-seq), anchored hybrid enrichment (AHE) and restriction-site associated DNA sequencing (RAD-seq) approaches to examine relationships within Muscidae. The results from AHE and RAD-seq largely correspond to those obtained from mS-seq in terms of overall topology, yet phylogenomic approaches received much higher nodal support. The results from all molecular approaches contradict the traditional classification based predominantly on adult morphology, but provide an opportunity to re-interpret the morphology of immature stages. Rearrangements in Muscidae classification are proposed as follows: (i) *Mesembrina* Meigen and *Polietes* Rondani are transferred from *Muscinae* to *Azeliinae*; (ii) *Reinwardtiinae* stat. rev. is resurrected as a subfamily distinct from *Azeliinae*; (iii) *Eginia* Robineau-Desvoidy, *Neohelina* Malloch, *Syngamoptera* Schnabl and *Xenotachina* Malloch are transferred to *Reinwardtiinae* stat. rev.

Gene-rich germline-restricted chromosomes in black-winged fungus gnats evolved through hybridization

Hodson, CN; Jaron, KS; Gerbi, S; Ross, L. 2022

Germline-restricted DNA has evolved in diverse animal taxa and is found in several vertebrate clades, nematodes, and flies. In these lineages, either portions of chromosomes or entire chromosomes are eliminated from somatic cells early in development, restricting portions of the genome to the germline. Little is known about why germline-restricted DNA has evolved, especially in flies, in which 3 diverse families, Chironomidae, Cecidomyiidae, and Sciaridae, carry germline-restricted chromosomes (GRCs). We conducted a genomic analysis of GRCs in the fungus gnat *Bradysia* (*Sciara*) *coprophila* (Diptera: Sciaridae), which has 2 large germline-restricted "L" chromosomes. We sequenced and assembled the genome of *B. coprophila* and used differences in sequence coverage and k-mer frequency between somatic and germline tissues to identify GRC sequence and compare it to the other chromosomes in the genome. We found that the GRCs in *B. coprophila* are large, gene rich, and have many genes with divergent homologs on other chromosomes in the genome. We also found that 2 divergent GRCs exist in the population we sequenced. GRC genes are more similar in sequence to genes from another Dipteran family (Cecidomyiidae) than to homologous genes from Sciaridae. This unexpected finding suggests that these chromosomes likely arose in Sciaridae through hybridization with a related lineage. These results provide a foundation from which to answer many questions about the evolution of GRCs in Sciaridae, such as how this hybridization event resulted in GRCs and what features on these chromosomes cause them to be restricted to the germline.

Improving the DNA Barcode Library of Mosquito Species With New Identifications and Discoveries in North-Central Argentina

Laurito, M; Ayala, AM; Arias-Builes, DL; Almiron, WR. 2022

The family Culicidae is represented by 244 species in Argentina, many of them with epidemiological importance. DNA barcodes are effective tools for identifying mosquito species, for knowing genetic variability, and for establishing phylogenetic relationships. This work aims to explore mosquito diversity employing different species delimitation approaches and to establish formally a DNA barcode library for the Argentinian mosquito fauna. Barcode fragments of 80 specimens of Argentinian mosquitoes of 28 species of the genera *Aedeomyia* Theobald (Diptera: Culicidae), *Anopheles* Meigen (Diptera: Culicidae), *Coquillettidia* Dyar (Diptera: Culicidae), *Culex* L. (Diptera: Culicidae), *Haemagogus* Williston (Diptera: Culicidae), *Mansonia* Blanchard (Diptera: Culicidae), *Nyssorhynchus* Blanchard (Diptera: Culicidae), *Ochlerotatus* Lynch-Arribalzaga (Diptera: Culicidae), *Psorophora* Robineau-Desvoidy (Diptera: Culicidae) and *Uranotaenia* Lynch-Arribalzaga (Diptera: Culicidae) were sequenced. Another 82 sequences were obtained from public databases to establish the phylogenetic relationships using Maximum Likelihood and Bayesian Inference, and the species boundaries based on three approaches (ABGD, GMYC, and mPTP). Sixteen of the 28 species sequenced were recovered as monophyletic, of which 12 were also recognized as molecular operational taxonomic units according to the three methodologies. The disparity between morphology and barcode-based identifications could be explained by synonymy, species complexes occurrence, hybridization, incomplete lineage sorting, or the effect of the geographical scale of sampling. Twenty of the 28 sequenced species are new barcodes for Argentina and 11 are the first for science. This increases from 31 to 52 (12.7 to 21.31%) and from six to 10 (28.57 to 47.62%) the number of species and genera, respectively, with barcode sequences in Argentina. New species records are provided.

A near-chromosome level genome assembly of the European hoverfly, *Sphaerophoria rueppellii* (Diptera: Syrphidae), provides comparative insights into insecticide resistance-related gene family evolution

Bailey, E; Field, L; Rawlings, C; King, R; Mohareb, F; Pak, KH; Hughes, D; Williamson, M; Ganko, E; Buer, B; Nauen, R. 2022

Background *Sphaerophoria rueppellii*, a European species of hoverfly, is a highly effective beneficial predator of hemipteran crop pests including aphids, thrips and coleopteran/lepidopteran larvae in integrated pest management (IPM) programmes. It is also a key pollinator of a wide variety of important agricultural crops. No genomic information is currently available for *S. rueppellii*. Without genomic information for such beneficial predator species, we are unable to perform comparative analyses of insecticide target-sites and genes encoding metabolic enzymes potentially responsible for insecticide resistance, between crop pests and their predators. These metabolic mechanisms include several gene families - cytochrome P450 monooxygenases (P450s), ATP binding cassette transporters (ABCs), glutathione-S-transferases (GSTs), UDP-glycosyltransferases (UGTs) and carboxyl/choline esterases (CCEs). Methods and findings In this study, a high-quality near-chromosome level de novo genome assembly (as well as a mitochondrial genome assembly) for *S. rueppellii* has been generated using a hybrid approach with PacBio long-read and Illumina short-read data, followed by super scaffolding using Hi-C data. The final assembly achieved a scaffold N50 of 87Mb, a total genome size of 537.6Mb and a level of completeness of 96% using a set of 1,658 core insect genes present as full-length genes. The assembly was annotated with 14,249 protein-coding genes. Comparative analysis revealed gene expansions of CYP6Zx P450s, epsilon-class GSTs, dietary CCEs and multiple UGT families (UGT37/302/308/430/431). Conversely, ABCs, delta-class GSTs and non-CYP6Zx P450s showed limited expansion. Differences were seen in the distributions of resistance-associated gene families across subfamilies between *S. rueppellii* and some hemipteran crop pests. Additionally, *S. rueppellii* had larger numbers of detoxification genes than other pollinator species. Conclusion and significance This assembly is the first published genome for a predatory member of the Syrphidae family and will serve as a useful resource for further research into selectivity and potential tolerance of insecticides by beneficial predators. Furthermore, the expansion of some gene families often linked to insecticide resistance and selectivity may be an indicator of the capacity of this predator to detoxify IPM selective insecticides. These findings could be exploited by targeted insecticide screens and functional studies to increase effectiveness of IPM strategies, which aim to increase crop yields by sustainably and effectively controlling pests without impacting beneficial predator populations.

Spatial Distribution of PCR-Identified Species of *Anopheles gambiae* sensu lato (Diptera: Culicidae) Across Three Eco-Vegetational Zones in Cross River State, Nigeria

Thabet, HS; TagEldin, RA; Fahmy, NT; Diclaro, JW; Alaribe, AA; Ezedinachi, E; Nwachuku, NS; Odey, FO; Arimoto, H. 2022

Anopheles gambiae sensu lato complex (*An. gambiae* s.l.) describes a group of nine morphologically indistinguishable members that vary in their distribution, ability to transmit malaria, and susceptibility to pyrethroids. Here, we recorded the spatial patterns of PCR-identified *An. gambiae* s.l. complex species collected from four sites in Cross River State, Nigeria that represented three different ecological zones. Trapping was conducted between October 2015 and June 2016. *Anopheles gambiae* s.l. complex species identification was performed using species-specific primers followed by *An. gambiae* and *An. coluzzii* differentiation using the restriction fragment length polymorphism (RFLP) method. Bivariate and multivariate logistic regression models were used to identify ecological and seasonal variables closely associated with *An. coluzzii* and *An. gambiae* distribution. Out of 1,388 *An. gambiae* s.l. successfully amplified, 1,074 (77.4%) were *An. coluzzii*, 278 (20%) were *An. gambiae*, and 25 (1.8%) were hybrids (*An. coluzzii*/*An. gambiae*). A very small number of *An. arabiensis* (0.8%, n = 11) were also collected. Statistical analysis indicated that *An. coluzzii* is predominant in Guinea-savannah and tropical rainforest, and is highly associated with rainy seasons, while, *An. gambiae* is prevalent in mangrove swamp forest during dry seasons. Only 13 *An. gambiae* s.l. females were infected with *Plasmodium falciparum* (*P. falciparum*). The sporozoite infection rate was higher in mangrove swamp forest (53.8%, n = 7) than in rain forest (38.5%, n = 5) followed by Guinea-savannah (7.7%, n = 1) ecological zones. These results provide important insights for strategic planning of malaria control programs in Nigeria.

The Effect of Hybridization on Dosage Compensation in Member Species of the *Anopheles gambiae* Species Complex

Deitz, KC; Takken, W; Slotman, MA. NA

Dosage compensation has evolved in concert with Y-chromosome degeneration in many taxa that exhibit heterogametic sex chromosomes. Dosage compensation overcomes the biological challenge of a "half dose" of X chromosome gene transcripts in the heterogametic sex. The need to equalize gene expression of a hemizygous X with that of autosomes arises from the fact that the X chromosomes retain hundreds of functional genes that are actively transcribed in both sexes and interact with genes expressed on the autosomes. Sex determination and heterogametic sex chromosomes have evolved multiple times in Diptera, and in each case the genetic control of dosage compensation is tightly linked to sex determination. In the *Anopheles gambiae* species complex (Culicidae), maleness is conferred by the Y-chromosome gene Yob, which despite its conserved role between species is polymorphic in its copy number between them. Previous work demonstrated that male *An. gambiae* s.s. males exhibit complete dosage compensation in pupal and adult stages. In the present study, we have extended this analysis to three sister species in the *An. gambiae* complex: *An. coluzzii*, *An. arabiensis*, and *An. quadriannulatus*. In addition, we analyzed dosage compensation in bi-directional F1 hybrids between these species to determine if hybridization results in the mis-regulation and disruption of dosage compensation. Our results confirm that dosage compensation operates in the *An. gambiae* species complex through the hypertranscription of the male X chromosome. Additionally, dosage compensation in hybrid males does not differ from parental males, indicating that hybridization does not result in the mis-regulation of dosage compensation.

Genetic population structure and hybridization in two sibling species, *Tomoplagia reticulata* and *Tomoplagia pallens* (Diptera: Tephritidae)

Abreu, AG; Solferini, VN. NA

Tomoplagia reticulata and *T. pallens* are sibling species that are specialists on *Eremanthus glomerulatus*. Besides adult terminalia, they show slight morphological differences and distinct geographic distributions. Once, however, they were found sympatrically. Using data from allozyme and mtDNA, we examined patterns of intra- and interspecific genetic structure, and investigated the possible occurrence of gene flow between them. Both species showed low diversity and high genetic structure, which can be linked to their high degree of specialization. Larval development occurs within flower heads, tissues that are available only during a short period of the year. Afterward, as they do not hibernate, they probably suffer a great reduction in population size, which leads to low genetic diversity. As monophagous insects, their population structure may correspond to the fragmented distribution of *E. glomerulatus*, which could isolate fly populations and increase inbreeding within them. One population exhibited a mixed genetic composition, compatible with one hybridization season when species were sympatric. This hybridization seems to be a rare event, due to *T. pallens* unusual range expansion.

Insecticide resistance monitoring of field-collected *Anopheles gambiae* s.l. populations from Jinja, eastern Uganda, identifies high levels of pyrethroid resistance

Mawejje, HD; Wilding, CS; Rippon, EJ; Hughes, A; Weetman, D; Donnelly, MJ. NA

Insecticide resistance in the malaria vector *Anopheles gambiae* s.l. (Diptera: Culicidae) threatens insecticide-based control efforts, necessitating regular monitoring. We assessed resistance in field-collected *An. gambiae* s.l. from Jinja, Uganda using World Health Organization (WHO) bioassays. Only *An. gambiae* s.s. and *An. arabiensis* (70%) were present. Female *An. gambiae* exhibited extremely high pyrethroid resistance (permethrin LT50 > 2 h; deltamethrin LT50 > 5 h). Female *An. arabiensis* were resistant to permethrin and exhibited reduced susceptibility to deltamethrin. However, while *An. gambiae* were DDT resistant, *An. arabiensis* were fully susceptible. Both species were fully susceptible to bendiocarb and fenitrothion. Kdr 1014S has increased rapidly in the Jinja population of *An. gambiae* s.s. and now approaches fixation (95%), consistent with insecticide-mediated selection, but is currently at a low frequency in *An. arabiensis* (0.07%). Kdr 1014F was also at a low frequency in *An. gambiae*. These frequencies preclude adequately-powered tests for an association with phenotypic resistance. PBO synergist bioassays resulted

in near complete recovery of pyrethroid susceptibility suggesting involvement of CYP450s in resistance. A small number (0.22%) of *An. gambiae* s.s. x *An. arabiensis* hybrids were found, suggesting the possibility of introgression of resistance alleles between species. The high levels of pyrethroid resistance encountered in Jinja threaten to reduce the efficacy of vector control programmes which rely on pyrethroid-impregnated bednets or indoor spraying of pyrethroids.

A Remnant of an Incipient Speciation Event in the *Simulium arcticum* Complex (Diptera: Simuliidae)

Shields, GF; Kratochvil, MJ. NA

By using cytogenetic analysis of larval polytene chromosomes from small samples of the *Simulium arcticum* complex of black flies (Diptera: Simuliidae) at the Coeur d'Alene River in northern Idaho in 2004 and 2005 we discovered a population that consisted of individuals having sex chromosomes characteristic of *S. saxosum*, *S. arcticum* s. s. and combinations of the two. Most taxa of the *S. arcticum* complex can be identified only on the basis of their well differentiated sex chromosomes, and the presence of larvae having species specific and combinational sex chromosome types presented us with a unique opportunity to further investigate this rare event. This variety of sex chromosome types could be explained if (1) sex-chromosomes in *S. saxosum* were operating autosomally in *S. arcticum* s. s. and vice-versa, (2) the combinational types were formed as a result of hybridization or (3) the population was in genetic equilibrium suggesting a unique entity. The latter possibility could be the remnant of an incipient speciation event. We returned to the Coeur d'Alene in the springs of 2009 and 2010 and made more extensive collections. We analyzed all types present for sex chromosome diversity, frequencies of sex chromosome types, tests of Hardy-Weinberg equilibrium for sex chromosomes and the autosomal polymorphism IS-1, and the extent of chromosome pairing and chromocenter morphology between *S. saxosum*, *S. arcticum* s.s. and combinational types. Finally, we analyzed additional larvae of the *S. arcticum* complex from three sites to the west and four sites to the east of the Coeur d'Alene River to determine the geographic distribution of all types. There is no evidence for alternative sex chromosome types of *S. saxosum* and *S. arcticum* s. s. acting autosomally, nor is there evidence for hybridization between *S. saxosum* and *S. arcticum* s. s. We therefore conclude that the population at the Coeur d'Alene River may be the remnant of a population that gave rise to *S. saxosum* to the west and to *S. arcticum* s. s. to the east. This may be a natural example of a remnant population whose types have experienced "mating trials" of different combinations of sex chromosome types that subsequently gave rise to the described siblings via incipient speciation.

Insecticide Susceptibility Screening Against *Culex* and *Aedes* (Diptera: Culicidae) Mosquitoes From the United States

Richards, SL; Balanay, JAG; White, AV; Hope, J; Vandock, K; Byrd, BD; Reiskind, MH. NA

Mosquitoes exposed to sublethal doses of insecticides may be selected for resistance to insecticide active ingredients (AIs). Mosquitoes are exposed to AIs through agricultural, public/private mosquito control programs, homeowners, and other sources. Hence, mosquito control programs should routinely measure the resistance/susceptibility status of mosquito populations of public health concern. The objectives here were to determine resistance status for six AIs used in adult mosquito control in the United States to assess how resistance/susceptibility differs between AI, mosquito species (states where > 1 species collected), and between years (some populations sampled for 2 yr). Field-collected eggs from 21 mosquito populations of six different species or hybrid species (*Aedes albopictus* Skuse [Diptera: Culicidae], *Aedes aegypti* L. [Diptera: Culicidae], *Culex nigripalpus* Theobald, *Culex pipiens* L. [Diptera: Culicidae], *Culex quinquefasciatus* Say [Diptera: Culicidae], *Cx. pipiens/quinquefasciatus*) were obtained. Centers for Disease Control and Prevention bottle bioassays were used to assess the resistance/susceptibility status for six AIs (bifenthrin, deltamethrin, etofenprox, malathion, permethrin, and phenothrin). World Health Organization guidelines were used to classify mosquitoes as susceptible (98-100% mortality at diagnostic time [DT]), possibly resistant (80-97% mortality at DT), or resistant (<80% mortality at DT). Significant differences were observed in mosquito susceptibility/resistance between species and AIs. In states where both *Aedes* and *Culex* were collected, the odds of exhibiting resistance in *Culex* were 68-69 times higher than *Aedes* (Texas odds ratio: 69.30; 95% confidence interval: 5.86, 819.44; $P = 0.001$; North Carolina odds ratio: 67.99; 95% confidence interval: 15.21, 303.94; $P < 0.0001$). Some level of resistance was detected against all tested AIs in several mosquito populations and some varied between 2015 and 2016.

Phylogenomics reveals accelerated late Cretaceous diversification of bee flies (Diptera: Bombyliidae)

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Bombyliidae is a very species-rich and widespread family of parasitoid flies with more than 250 genera classified into 17 extant subfamilies. However, little is known about their evolutionary history or how their present-day diversity was shaped. Transcriptomes of 15 species and anchored hybrid enrichment (AHE) sequence captures of 86 species, representing 94 bee fly species and 14 subfamilies, were used to reconstruct the phylogeny of Bombyliidae. We integrated data from transcriptomes across each of the main lineages in our AHE tree to build a data set with more genes (550 loci versus 216 loci) and higher support levels. Our overall results show strong congruence with the current classification of the family, with 11 out of 14 included subfamilies recovered as monophyletic. Heterotropinae and Mythicomyiinae are successive sister groups to the remainder of the family. We examined the evolution of key morphological characters through our phylogenetic hypotheses and show that neither the "sand chamber subfamilies" nor the "Tomophthalmae" are monophyletic in our phylogenomic analyses. Based on our results, we reinstate two tribes at the subfamily level (Phthiriinae stat. rev. and Ecliminae stat. rev.) and we include the genus *Sericosoma* Macquart (previously incertae sedis) in the subfamily Oniromyiinae, bringing the total number of bee fly subfamilies to 19. Our dating analyses indicate a Jurassic origin of the family (165-194 Ma), with the sand chamber evolving early in bee fly evolution, in the late Jurassic or mid-Cretaceous (100-165 Ma). We hypothesize that the angiosperm radiation and the hothouse climate established during the late Cretaceous accelerated the diversification of bee flies, by providing an expanded range of resources for the parasitoid larvae and nectarivorous adults.

Susceptibility of cold hardy grapes to *Drosophila suzukii* (Diptera: Drosophilidae)

Pelton, E; Gratton, C; Guedot, C. NA

Drosophila suzukii (Diptera: Drosophilidae), known commonly as spotted wing drosophila, is a vinegar fly originating from South-East Asia and a major pest to many soft-skinned fruits. Due to the species recent arrival in North America in 2008, many fruit varieties are yet untested for susceptibility to infestation. While previous work has focused on *Vitis vinifera*, this study aimed to determine grape susceptibility of cold hardy varieties based on hybrids of *V. labrusca*, *V. riparia* and *V. vinifera*. Field sampling was conducted in Southern Wisconsin (USA) vineyards to establish adult and larval abundance and determine whether the number of adults caught in traps correlates with fruit infestation. Host susceptibility was further assessed through no-choice bioassays of both intact and damaged fruits. The field study found *D. suzukii* adults present in all varieties, low larval abundance and no correlation between adult abundance and larval presence. Peak adult abundance occurred mid-season between veraison and harvest, while larval infestation rates were highest near harvest. In laboratory no-choice tests, significantly more eggs, larvae and adults occurred in damaged than undamaged grapes. In damaged grapes, larvae and adult abundance was comparable between varieties and to the highly susceptible control of undamaged raspberry; however, *D. suzukii* developed significantly faster in raspberry than grapes. Fruit characteristics (degrees Brix, titratable acidity, pH) in grapes were uncorrelated with *D. suzukii* performance. Together, these findings suggest that cold hardy grapes are overall resistant to *D. suzukii* if intact and highly susceptible if damaged.

A "complex" problem: delimiting sibling species boundaries in black flies (Diptera: Simuliidae)

Conflitti, IM; Shields, GF; Currie, DC. NA

Rapid and recent lineage radiations pose challenges to systematists. Using members of the highly diverse *Simulium arcticum* Malloch complex, we tested whether the cytochrome c oxidase subunit I (COI) barcoding gene can differentiate black fly sibling species. Members of the *S. arcticum* complex were monophyletic in relation to two morphospecies and two cryptic species of the *Simulium malyschevi* Dorogostaisky et al. and *Simulium noelleri* Friederichs species-groups, respectively. Of five *S. arcticum* sibling species analysed, only *Simulium negativum* Adler et al. was monophyletic. No other members of the complex could be distinguished using COI barcodes. The inability to resolve *S. arcticum* sibling species resulted because (1) haplotypes were shared between species and (2) the distribution of interspecific genetic distances completely overlapped the range of variation within species. Potential sources of incongruence between barcode data and species boundaries include imperfect taxonomy, inadequate genetic information, incomplete lineage sorting, and/or introgressive hybridization. We ruled out imperfect taxonomy because chromosomal, ecological, and distributional evidence support the validity of *S. arcticum* sibling species. Therefore, current nomenclature should be maintained pending further study. We conclude that one or more of the latter three sources of incongruence could be responsible for the lack of reciprocal monophyly among species of the *S. arcticum* complex.

Genetic panmixia within a narrow contact zone between chromosomally and ecologically distinct black fly sibling species (Diptera: Simuliidae)

Conflitti, IM; Shields, GF; Murphy, RW; Currie, DC. NA

Hybrid zones are windows into the speciation process, and their study can give clues into the maintenance and breakdown of species boundaries. Using both genetic and ecological tools, we investigate lineage diversification across a contact zone characterized by chromosome rearrangements. We show that black fly sibling species, *Simulium arcticum* sensu stricto (s.s.) and *Simulium saxosum*, lack genetic differentiation at both microsatellite and mtDNA loci in allopatry and sympatry, as well as exhibit high levels of gene flow and continuous chromosome variation in sympatry. Furthermore, hybrid frequencies at the contact zone are similar to those seen between races, rather than species. In contrast, *S. arcticum* s.s. and *S. saxosum* maintain ecological differences and distinct habitat associations - the contact zone situated at the margin of suitable habitat for each sibling species. Moreover, gene flow occurs only in a narrow band along an ecological transition. Except for the contact zone, *S. arcticum* s.s. and *S. saxosum* hybrids do not occur elsewhere within the sibling species' ranges. Although *S. arcticum* s.s. and *S. saxosum* maintain the potential to interbreed freely, we conclude that habitat associations and, perhaps, chromosome systems prevent expansion of ranges and assimilation of lineages.

Diet overlap among non-native trout species and native cutthroat Trout (*Oncorhynchus clarkii*) in two US ecoregions

Minder, M; Arsenault, ER; Erdenree, B; Maasri, A; Pyron, M. NA

Simple genetic inheritance conditions resistance to *Liriomyza sativae* in melon

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The leafminer *Liriomyza sativae* (Diptera: Agromyzidae) stands out as the main plant health problem in melon in the Northeast region of Brazil, which is the main region for production and export of the fruit. Genetic resistance of plants is an important strategy in management of this pest. The plant BAGMEL 56-R was selected as a new source of resistance to *L. sativae* through antibiosis; this resistance is characterized by the death of larvae soon after they begin feeding on the leaf mesophyll; the result is leaf mines that are small and insignificant in terms of yield reduction. Lines with contrasting levels of resistance were obtained from the progenies of this source of resistance through successive self-pollinations, conducted by the pedigree breeding method. Through the segregation pattern of the progenies and the test cross, the genetic nature of resistance was determined; one gene with complete dominance conditions resistance. The name *Liriomyza sativae* resistance and the symbol *Ls* are suggested to represent this new gene. In addition, through a nonpreference test with lines in contrast for antibiosis and the susceptible hybrid 'Goldex', the presence of antixenosis was observed in this source of resistance. Probably, these different types of resistance in the source BAGMEL 56-R are associated with distinct defense mechanisms. Therefore, with this new source, introgression of resistance to *L. sativae* in elite lines or commercial hybrids of melon is possible.

Baseline Insecticide Susceptibility Screening Against Six Active Ingredients for *Culex* and *Aedes* (Diptera: Culicidae) Mosquitoes in the United States

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Mosquitoes may develop resistance to insecticide active ingredients (AI). Thus, mosquitoes should be tested for resistance to confirm efficacy of insecticide-based control, inform management decisions, and protect public and environmental health. Our objectives were to determine a baseline of resistance for six AIs used in mosquito control in the United States to assess how resistance differs between mosquito collection location, AI, and mosquito species (container-ovipositing *Aedes* and *Culex* that may oviposit in containers or other sources). Field-collected eggs from 26 mosquito populations of five different species or hybrid species (*Aedes albopictus* Say, *Aedes triseriatus* Say, *Culex pipiens* L., *Culex quinquefasciatus* Say, *Culex pipiens/quinquefasciatus*) were obtained from four regions across the United States. Centers for Disease Control and Prevention bottle bioassays were used to determine baseline resistance and susceptibility status for six AIs (bifenthrin, deltamethrin, etofenprox, malathion, permethrin, and phenothrin). World Health Organization guidelines were used to classify mosquitoes as susceptible (98-100% mortality at diagnostic time [DT]), possibly resistant (80-97% mortality at DT), or resistant (<80% mortality at DT). *Aedes* spp. mosquitoes were less likely to exhibit resistance, compared with *Culex* spp. mosquitoes. A high degree of resistance to etofenprox and malathion was observed (4-26-fold greater resistance to these two AIs compared with the other examined AIs). Baseline data on resistance and susceptibility for mosquitoes exposed to commonly used insecticides may help us evaluate resistance trends and highlight the importance of assessing local resistance trends before insecticide-based control measures are implemented.