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(AB=(*"reproductive barriers"* OR *"reproductive isolation"*) OR AK=(*"reproductive barriers"* OR *"reproductive isolation"*) OR TI=(*"reproductive barriers"* OR *"reproductive isolation"*)) AND (ALL=(*Lepidoptera*))

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Reproductive morphology, copulation, and inter-population variation in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera : Plutellidae)

Justus, KA; Mitchell, BK. 1999

Copulatory mechanisms and internal reproductive systems of male and female *Plutella xylostella* (L.) were investigated. Both male and female specimens exhibited characteristics typical of ditrysian Lepidoptera, with some peculiarities. Female structures appear to be relatively simple: the bursa copulatrix lacks cuticular signa within, the spermatheca lacks lagenar arms exhibited in some Lepidoptera, and colleterial glands have secretory system and reservoir combined. Male accessory gland ducts are joined distally, a condition that has not been described in other Lepidoptera. Genitalia of both sexes appear simple; females possess a posterior cuticular extension of abdominal sternites that houses the bursal duct and accommodates the needle-like aedeagus of a male during copulation. Despite the apparent simplicity, configuration of genitalia is highly specific in shape, angle, and size, which is likely to be important in maintaining reproductive isolation within the species. However, a population of *P. xylostella* from Australia showed some modification to both aedeagal and bursal structures. These variations suggest some important implications for considering species with worldwide distributions. (C) 1999 Elsevier Science Ltd. All rights reserved.

Rapid Divergence of Wing Volatile Profiles Between Subspecies of the Butterfly *Pieris rapae* (Lepidoptera: Pieridae)

McQueen, EW; Morehouse, NI. 2018

Complex signaling traits such as pheromone profiles can play an important role in the early stages of reproductive isolation between populations. These signals can diverge along multiple trait axes, and signal receivers are often sensitive to subtle differences in signal properties. In the Lepidoptera, prior research has highlighted that natural selection can drive rapid chemical signal divergence, for instance via mate recognition to maintain species boundaries. Much less is known about the occurrence of such changes for predominantly sexually selected chemical signals, such as those released by many male lepidopterans. We evaluated the divergence in male and female wing volatile profiles between two recently isolated subspecies of the pierid butterfly *Pieris rapae* Linnaeus (Lepidoptera: Pieridae): *P. rapae rapae* and *P. rapae crucivora*. In laboratory settings, these subspecies exhibit strong premating isolation, with females rejecting males of the opposite subspecies despite the fact that males direct equivalent courtship effort toward females of either subspecies. Using gas chromatography-mass spectrometry, we analyzed the volatile chemical profiles of individual males and females of each subspecies. We find that males of each subspecies differ in their wing volatile profiles, including quantitative differences in a male sex pheromone, ferrulactone. In contrast, female wing volatile profiles have diverged significantly less. These sex-specific patterns suggest that male chemical profiles may play a role in the observed premating isolation between these two subspecies, providing support for future investigations of sexually selected chemical traits in population divergence.

Speciation through chromosomal fusion and fission in Lepidoptera

de Vos, JM; Augustijn, H; Batscher, L; Lucek, K. 2020

Changes in chromosome numbers may strongly affect reproductive barriers, because individuals heterozygous for distinct karyotypes are typically expected to be at least partially sterile or to show reduced recombination. Therefore, several classic speciation models are based on chromosomal changes. One important mechanism generating variation in chromosome numbers is fusion and fission of existing chromosomes, which is particularly likely in species with holocentric chromosomes, i.e. chromosomes that lack a single centromere. Holocentric chromosomes evolved repeatedly across the tree of life, including in Lepidoptera. Although changes in chromosome numbers are hypothesized to be an important driver of the spectacular diversification of Lepidoptera, comparative studies across the order are lacking. We performed the first comprehensive literature survey of karyotypes for Lepidoptera species since the 1970s and tested if, and how, chromosomal variation might affect speciation. Even though a meta-analysis of karyological differences between closely related taxa did not reveal an effect on the degree of reproductive isolation, phylogenetic diversification rate analyses across the 16 best-covered genera indicated a strong, positive association of rates of chromosome number evolution and speciation. These findings suggest a macroevolutionary impact of varying chromosome numbers in Lepidoptera and likely apply to other taxonomic groups, especially to those with holocentric chromosomes. This article is part of the theme issue 'Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers'.

A chromosomal investigation of two closely related species of Spodoptera

Monti, L; Lemeunier, F; Lalanne-Cassou, B. 1998

This paper reports the first cytogenetic study of the two closely related species of noctuid moths, *Spodoptera latitarsia* and *S. descoinsi*. Chromosomes were prepared using a spreading technique on warm slides. Both gonads and larval brains, which are innovating in the Lepidoptera, were used. Out of 100 specimens observed, 35 showed mitotic metaphases, which allowed chromosomes to be counted. For both species and F1 **hybrids**, the diploid chromosome number was $2n = 62$. The chromosomes of the two species appeared dot-shaped, more rarely rod-shaped, showing little variation in size or morphology. The preparations from larval brains also suggested the existence of two levels of ploidy (haploidy and diploidy) in some nuclei. This result will need further investigation. For the first time in the Lepidoptera, in situ **hybridization** with *Drosophila* rDNA as a probe was carried out on *S. latitarsia* and *S. descoinsi*. It revealed the presence of nucleolar organizing regions located at the distal part of one chromosome pair. Though no sophisticated characterization of *S. latitarsia* and *S. descoinsi* was possible, it seemed that there was no major chromosomal difference. No karyotypic element could be identified as being involved in reproductive isolation between the two species. ((C) Academie des sciences/Elsevier, Paris.)

SEX-LINKED GENES AND SPECIES-DIFFERENCES IN LEPIDOPTERA

SPERLING, FAH. 1994

I review reports on the genetic basis for species differences in the Lepidoptera. In the six best-studied species complexes, more than half of all ecological, behavioral, or physiological differences among species are controlled by X-linked genes. Because Lepidoptera have about 30 pairs of chromosomes, this finding clearly indicates strong bias toward X-linkage of genes for species differences. The proportion of X-linked species differences ranges from complete X-linkage in *Colias* butterflies, to almost none in *Yponomeuta* moths. Four other complexes all have at least one X-linked gene that is crucial to species differences, including the *Choristoneura fumiferana* Clemens, *Papilio glaucus* L., and *Papilio machaon* L. species groups, and *Ostrinia nubilalis* Hubner pheromone strains. The mechanisms that account for this phenomenon are open to speculation. Nonetheless, an interesting implication of disproportionate X-linkage is that reproductive isolation may frequently arise by selection on linkage complexes, rather than as a random byproduct of evolution in geographically isolated populations. If confirmed, the bias toward X-linked species differences may also help efforts to find characters that distinguish host races and sibling species, as well as provide an avenue by which genes crucial to speciation can be more easily mapped and characterized at the molecular level.

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

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

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

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

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

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) is a Neotropical moth that has diverged into corn, *Zea mays* L., and rice, *Oryza sativa* L., host strains because these plants are their most frequently used hosts. The corn strain also has been found in cotton, *Gossypium hirsutum* L., and sorghum, *Sorghum bicolor* (L.) Moench, and the rice strain in small grasses and pasture grasses. Studies of the reproductive isolation between these two strains have provided ambiguous results from populations in the United States. In Colombia, we tested pre- and postzygotic isolation in these strains. Both strains showed postzygotic isolation for several life-history traits, including number of egg masses, number of larvae, number of females, pupal developmental time, female and male longevity, and female and male pupal weight. We observed a reduction of the number of **hybrid** females and a reduction in fertility in **hybrids** in *S. frugiperda*. These results suggest the possibility of Haldane's rule. Heterosis in the F₁(2) and F₂(1) generations was observed for number of larvae and adult longevity. This line presented a high standard deviation, suggesting instability in this cross. A possible effect of the X chromosome may explain the reduction in viability and sterility in F₁ **hybrids** of host strains of *S. frugiperda*. No temporal isolation was observed between the corn and rice strains. Differences in longevity between corn and rice strains might be another form of temporal isolation between these strains, because differences in adulthood time might reduce the encounters between them and thus **hybridization**.

Lack of reproductive isolation in *Helicoverpa gelatopoeon* (Lepidoptera: Noctuidae) populations from different host plant species in Argentina

Herrero, MI; Fogliata, SV; Dami, LC; Casmuz, A; Gastaminza, G; Murua, MG. 2019

Helicoverpa gelatopoeon (Dyar) (Lepidoptera: Noctuidae) is a polyphagous pest that belongs to the *Heliiothinae* complex, and has been described as feeding on numerous crops. Larvae damage mainly pods and seeds of soybean and other legumes. Adaptation to different host plants might reduce gene flow between phytophagous insect populations, which could lead to reproductive isolation. Therefore, studies on gene flow among host-associated populations are necessary to design management strategies. The objective of this study was to evaluate reproductive compatibility between 2 populations of *H. gelatopoeon* coming from different host plants. We collected *H. gelatopoeon* larvae in alfalfa and soybean crops, and studied biological and reproductive parameters, and mating compatibility. *Helicoverpa gelatopoeon* populations from soybean and alfalfa reared on artificial diet showed similar biological and reproductive characteristics. Intra- and inter-population crosses revealed that there are no signs of host-associated isolation, allowing gene exchange between these populations, which can be explained by the high mobility and generalist behavior of this species. This information will be useful to establish control measures for this pest.

Identification of the Female Sex Pheromone of the Leafroller *Proeulia triquetra* Obraztsov (Lepidoptera: Tortricidae)

Bergmann, J; Reyes-Garcia, L; Ballesteros, C; Cuevas, Y; Flores, MF; Curkovic, T. 2016

Proeulia triquetra Obraztsov (Lepidoptera: Tortricidae) is an occasional pest in fruit orchards in central-southern Chile. In order to develop species-specific lures for detection and monitoring of this species, we identified the female-produced sex pheromone. (Z)-11-Tetradecenyl acetate (Z11-14:OAc), (E)-9-dodecenyl acetate (E9-12:OAc), and (E)-11-Tetradecenyl acetate (E11-14:OAc) were identified as biologically active compounds present in female pheromone glands by solvent extraction of the gland and analysis of the extracts by gas chromatography-electroantennographic detection and gas chromatography-mass spectrometry. In field tests, lures baited with synthetic Z11-14:OAc and E9-12:OAc in a 10:1 ratio were highly attractive to males of the species.

Reproductive incompatibility and fitness components in *Neoleucinodes elegantalis* races (Lepidoptera, Crambidae) from three Solanaceae hosts

Diaz-Montilla, AE; Baena-Bejarano, N; Montoya-Lerma, J; Saldamando-Benjumea, CI. 2018

Neoleucinodes elegantalis (Lepidoptera, Crambidae) is a Neotropical Solanaceae pest that has evolved into four host races due to host plant association. In this study, prezygotic and postzygotic isolation were evaluated in parental and F₁ generations collected from *Solanum lycopersicum* (S. 1), *S. quitoense* (S. q) and *S. betaceum* (S. b), representing medium size (S. 1, S. q) and large size genitalia races (S. b). 617 adults were obtained in the laboratory and 106 copulated. One spermatophore per female was found in the bursa copulatrix, suggesting monoandry. Crosses between adults mostly occurred assortatively. A third of the female's eggs laid occurred, but females from female S. b x male S. 1, female S. 1 x male S. b and S. 1 x S. 1 did not. In the cross, female S. 1 x male S. q and its reciprocal F-1 progeny developed into larvae. Reproductive success only occurred in three crosses: S. q x S. q, female S. q x male S. b and its reciprocal progeny. All the progeny from these crosses reached adulthood. The parental population showed differences in pupae and adult measurements according to host. The results on reproductive isolation obtained here suggest speciation in *N. elegantalis* due to host race differentiation which is relevant for the improvement of the management of this species, especially when the species has evolved reproductive isolation among races. We suggest that the use of their host plants in sympatry might reduce *N. elegantalis* densities in the field since **hybrids** between its races have low survival rates and to evaluate the possibility of pheromone composition differentiation among races given the results on assortative mating obtained here.

CHEMICAL COMMUNICATION AND REPRODUCTIVE ISOLATION IN 2 TYPES OF THE FALL WEBWORM (LEPIDOPTERA, ARCTIIDAE)

MCLELLAN, KAM; NORDIN, GL; HAYNES, KF. 1991

Two sympatric types of fall webworm, *Hyphantria cunea* (Drury), were not cross-attractive in a laboratory wind tunnel and showed little propensity for intertype courtship in small arena bioassays. Both types of females called at dawn for about 1 h. Some adult males responded to calling females of the same type in the wind tunnel by wing fanning, taking flight, flying upwind, and making contact with cages containing calling females. Males did not complete this sequence of mate-finding behavior when responding to females of the other type. Under competitive and noncompetitive mating conditions, intratype matings occurred more frequently than intertype matings. If mating occurred, eggs were often laid and could be viable. Seasonal differences between the types are well known, but the present study indicates that pheromonal differences may contribute to reproductive isolation between the two types.

Evolution of insect pheromones and their role in reproductive isolation and speciation

Wicker-Thomas, C. 2011

The importance for reproductive isolation of a change in the pheromone biosynthetic pathway, resulting in a different pheromone blend, is discussed in Lepidoptera and Diptera. The different sites of pheromone production and the biosynthetic enzymes are briefly reviewed. Two examples of a modification in the pheromone blend leading to reproductive isolation in Lepidoptera are taken as examples: the first, in *Ostrinia nubilalis*, is involved in the formation of two different populations showing reproductive isolation; the second, in the genus *Ostrinia*, might be at the origin of the formation of two different species. In both examples, a modification in the function of a desaturase involved in pheromone biosynthesis brings about change in the pheromone blend. In the fruitfly, *Drosophila melanogaster*, a mutation at a desaturase locus leads to the formation of two populations which differ in their pheromone mixtures and have developed premating isolation. The closely related species, *D. melanogaster* and *D. simulans*, differ in their female pheromonal cuticular hydrocarbons. This pheromonal difference is due to two species- and female-specific genes, *desatF* and *eloF*. The activity of *desatF* could account for an effective barrier between these species. All these examples show that a birth and death process of desaturases is at the origin of major shifts in the pheromone blend leading to sexual isolation and speciation.

PEROMONAL PRODUCTION OF AND RESPONSE TO OPTICALLY-ACTIVE EPOXYDIENES IN SOME GEOMETRID MOTHS (LEPIDOPTERA, GEOMETRIDAE)

TOTH, M; SZOCS, G; FRANCKE, W; SCHMIDT, F; PHILIPP, P; LOFSTEDT, C; HANSSON, BS; FARAG, AI. 1994

In pheromone extracts of calling female *Chiasma clathrata* L. (Lepidoptera: Geometridae), a defoliator pest of alfalfa, (Z,Z,Z)-3,6,9-heptadecatriene and (Z,Z)-6,9-cis-3,4-epoxyheptadecadiene was identified. Chiral gas chromatography using a modified cyclodextrin and synthetic reference samples proved the natural epoxide to show (3R,4S)-configuration. In field trapping tests, only the pure (3R,4S)-enantiomer of the epoxide attracted males. The addition of the triene component was synergistic. Males of the sympatric species *Tephрина arenacearia* Hbn. (Lepidoptera:Geometridae) were caught only in traps with baits containing the (3S,4R)-enantiomer [together with a previously described minor component, (Z,Z)-3,9-cis-6,7-epoxyheptadecadiene]. In trapping tests conducted in a different biotope, *Abraxas grossu-lariata* L. (Lepidoptera: Geometridae) males were attracted by the (SS,4R)-enantiomer, whereas the (3R,4S)-enantiomer attracted a close relative *Abraxas sylvata* Scop. (Lepidoptera:Geometridae). The present results suggest that one of the key mechanisms responsible for pheromone specificity among both the two alfalfa geometrids and the two *Abraxas* species in their respective biotops, may be the use of different enantiomers of the same polyene-derived epoxide as a sex pheromone component. It is probable that this discrimination mechanism is widespread among moth species utilizing epoxide pheromone components.

Consistent seasonal polyphenism in male genitalia of three Leptidea butterfly species (Lepidoptera: Pieridae)

Fumi, M; Friberg, M. 2019

Insect genitalia are important for species identification and taxonomy and are key traits for the evolution of reproductive isolation through their 'lock-and-key mechanism'. Nevertheless, few studies have evaluated intraspecific variation and the impact of seasonal environmental variation on genital morphology. We used geometric morphometric analysis to quantify male genital morphology within and among the three taxa in the *Leptidea sinapis* cryptic species complex (Lepidoptera: Pieridae). We focused on the potential presence of seasonal polyphenism, i. e. consistent differences among temporally separated generations. As predicted, our analysis revealed that male genitalia differed in size, shape and form (the joint effect of size and shape) among species. More surprisingly, all species harboured consistent genital variation between the spring and summer generations. The interspecific variation in genital structures could function as a mechanical reproductive barrier, although contemporary populations of these butterflies do not engage in heterospecific matings. We suggest that the smaller, but significant, genital differences among conspecific generations provide an opportunity to test empirically whether a seasonal polyphenism in genital morphology could generate raw material for the build-up of reproductive isolation at an early stage of speciation. Collectively, our results provide evidence demonstrating that insect genitalia can indeed harbour ample intraspecific variation.

Sex pheromones and attractants in the Eucosmini and Grapholitini (Lepidoptera, Tortricidae)

Witzgall, P; Chambon, JP; Bengtsson, M; Unelius, CR; Appelgren, M; Makranczy, G; Muraleedharan, N; Reed, DW; Hellrigl, K; Buser, HR; Hallberg, E; Bergstrom, G; Toth, M; Lofstedt, C; Lofqvist, J. 1996

The geometric isomers (E, E)-, (E, Z)-, (Z, E)-, and (Z, Z)-8, 10-dodecadien-1-yl acetate were identified as sex pheromone components or sex attractants in the tribes Eucosmini and Grapholitini of the tortricid subfamily Olethreutinae. Species belonging to the more ancestral Tortricinae were not attracted. Each one isomer was behaviourally active in males of *Cydia* and *Grapholita* (Grapholitini), either as main pheromone compound, attraction synergist or attraction inhibitor. Their reciprocal attractive/antagonistic activity in a number of species enables specific communication with these four compounds. Pammene, as well as other *Grapholita* and *Cydia* responded to the monoenic 8- or 10-dodecen-1-yl acetates. Of the tribes Olethreutini and Eucosmini, *Hedya*, *Epiblema*, *Eucosma*, and *Notocelia trimaculana* were also attracted to 8, 10-dodecadien-1-yl acetates, but several other *Notocelia* to 10, 12-tetradecadien-1-yl acetates. The female sex pheromones of *C. fagiglandana*, *C. pyrivora*, *C. splendana*, *Epiblema foenella* and *Notocelia roborana* were identified. (E, E)- and (E, Z)- 8, 10-dodecadien-1-yl acetate are produced via a common E9 desaturation pathway in *C. splendana*. Calling *C. nigricana* and *C. fagiglandana* females are attracted to wingfanning males.

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

Lima, ER; McNeil, JN. 2009

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

Courtship behaviour of the African maize stem borer: *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) under laboratory conditions

Frerot, B; Felix, AE; Sarapuu, E; Calatayud, PA; Le Ru, B; Guenego, H. 2006

Busseola fusca (Fuller) (Lepidoptera: Noctuidae) is the most important African stem borer damaging maize and sorghum. Chemical mediators play an essential role in all life cycle of this moth, especially for mating recognition and host plant choice. The female sex pheromone, courtship and mating behaviours act on the reproductive isolation within insect populations. *B. fusca* courtship behaviour was studied to decipher each step that could account as a process for reproductive isolation. *B. fusca* males and females presented a very simple and fast courtship behaviour, without any particular events or male pheromone emission.

Limited intrinsic postzygotic reproductive isolation despite chromosomal rearrangements between closely related sympatric species of small ermine moths (Lepidoptera: Yponomeutidae)

Hora, KH; Marec, F; Roessingh, P; Menken, SBJ. 2019

In evolutionarily young species and sympatric host races of phytophagous insects, postzygotic incompatibility is often not yet fully developed, but reduced fitness of **hybrids** is thought to facilitate further divergence. However, empirical evidence supporting this hypothesis is limited. To assess the role of reduced **hybrid** fitness, we studied meiosis and fertility in **hybrids** of two closely related small ermine moths, *Yponomeuta padella* and *Yponomeuta cagnagella*, and determined the extent of intrinsic postzygotic reproductive isolation. We found extensive rearrangements between the karyotypes of the two species and irregularities in meiotic chromosome pairing in their **hybrids**. The fertility of reciprocal F-1 and, surprisingly, also of backcrosses with both parental species was not significantly decreased compared with intraspecific offspring. The results indicate that intrinsic postzygotic reproductive isolation between these closely related species is limited. We conclude that the observed chromosomal rearrangements are probably not the result of an accumulation of postzygotic incompatibilities preventing **hybridization**. Alternative explanations, such as adaptation to new host plants, are discussed.

Diel activity and reproductive isolation in a diverse assemblage of Neotropical skippers (Lepidoptera : Hesperiidae)

Devries, PJ; Austin, GT; Martin, NH. 2008

A sample of over 400 co-occurring species of Neotropical Hesperiidae attracted to artificial lures was used to test for temporal structure among species and explore potential consequences of temporal partitioning within assemblages. Five years of intermittent transect samples at the same site revealed that diel activity was significantly partitioned among subfamilies, genera and species within this species assemblage. Calculations of reproductive isolation indicate that partitions in activity times are sufficient to provide significant prezygotic reproductive isolation among some congeneric species pairs. Temporal patterns in this assemblage are considered in light of field observations and temporal partitions are examined as a potential isolating barrier among insects. (c) 2008 The Linnean Society of London.

SEX-PHEROMONE COMPONENTS OF THE SPRING HEMLOCK LOOPER, *LAMBDA-ATHASARIA* (WALKER) (LEPIDOPTERA, GEOMETRIDAE)

GRIES, R; GRIES, G; LI, JX; MAIER, CT; LEMMON, CR; SLESSOR, KN. 1994

Two methylated hydrocarbons, 7-methylheptadecane (7) and 7,11-dimethylheptadecane (7,11), are female sex pheromone components of the spring hemlock looper (SHL), *Lambdina athasaria* (Walker). Compounds extracted from female pheromone glands were identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) and coupled GC-mass spectrometry (GC-MS) in selected ion monitoring mode. In field trapping experiments, (7) and (7,11) by themselves were behaviorally inactive, but in combination attracted numerous male moths. (5,11)-Dimethylheptadecane (5,11) was detected in female SHL pheromone gland extracts, but did not enhance attraction to the binary blend of (7) and (7,11). The sex pheromone of SHL is related to that of congeneric eastern hemlock looper (EHL), *Lambdina fiscellaria fiscellaria* (Guen.) [(5,11) and 2,5-dimethylheptadecane (2,5)] and western hemlock looper (WHL), *L.f. lugubrosa* (Hulst) [(5,11), (2,5) and (7)]. Specificity of the pheromonal blend, spatial separation of coseasonal EHL and WHL, and temporal separation of sympatric EHL and SHL contribute to reproductive isolation.

To be or not to be a species: use of reproductive isolation experiments and genetic analysis to clarify the taxonomic status of two *Busseola* (Lepidoptera: Noctuidae) species in Kenya

Felix, AE; Calatayud, PA; Le Ru, B; Capdevielle-Dulac, C; Ong'amo, G; Silvain, JF; Frerot, B. 2013

Phylogenetic analysis combined with chemical ecology can contribute to the delimitation of closely related insect species, particularly in Lepidoptera. In this study, the taxonomic status of a species in the genus *Busseola* (Lepidoptera: Noctuidae) was discussed using morphological data, cross-mating experiments, sex pheromone chemistry, field-trapping, and molecular classification. The results of the chemical ecology experiments corroborated those from the phylogeny studies. It was concluded that several reproductive isolation components, namely host plants, geography, pheromone emission time, pheromone blend, and post-zygotic isolation factors, led to the separation of *Busseola* n. sp. from its closely related species *B. segeta*. Molecular data showed a strong difference between these two species, regardless of the marker used. The new species named *Busseola nairobiica* was morphologically described and a hypothesis about the evolutionary history of the studied species was put forward.

Morphological Outcomes of Gynandromorphism in *Lycaeides* Butterflies (Lepidoptera: Lycaenidae)

Jahner, JP; Lucas, LK; Wilson, JS; Forister, ML. 2015

The genitalia of male insects have been widely used in taxonomic identification and systematics and are potentially involved in maintaining reproductive isolation between species. Although sexual selection has been invoked to explain patterns of morphological variation in genitalia among populations and species, developmental plasticity in genitalia likely contributes to observed variation but has been rarely examined, particularly in wild populations. Bilateral gynandromorphs are individuals that are genetically male on one side of the midline and genetically female on the other, while mosaic gynandromorphs have only a portion of their body developing as the opposite sex. Gynandromorphs might offer unique insights into developmental plasticity because individuals experience abnormal cellular interactions at the genitalic midline. In this study, we compare the genitalia and wing patterns of gynandromorphic Anna and Melissa blue butterflies, *Lycaeides anna* (Edwards) (formerly *L. idas anna*) and *L. melissa* (Edwards) (Lepidoptera: Lycaenidae), to the morphology of normal individuals from the same populations. Gynandromorph wing markings all fell within the range of variation of normal butterflies; however, a number of genitalic measurements were outliers when compared with normal individuals. From these results, we conclude that the gynandromorphs' genitalia, but not wing patterns, can be abnormal when compared with normal individuals and that the gynandromorphic genitalia do not deviate developmentally in a consistent pattern across individuals. Finally, genetic mechanisms are considered for the development of gynandromorphism in *Lycaeides* butterflies.

Pre- and post-mating reproductive barriers drive divergence of five sympatric species of Naryciinae moths (Lepidoptera: Psychidae)

Elzinga, JA; Mappes, J; Kaila, L. 2014

The biological species concept suggests that species can be separated on the basis of reproductive isolation. However, because natural interbreeding capabilities are often unknown, differences in morphology are generally used to separate species. Alternatively, genetic dissimilarity is used to separate morphologically similar species. Many genetic markers, including the maternally inherited mitochondrial cytochrome oxidase I sequence, cannot show interbreeding and therefore species status of groups may remain unresolved. In species of the genera *Dahlica* and *Siederia* (Lepidoptera: Psychidae: Naryciinae) the lack of morphological distinction and unknown interbreeding has led to unclear and unresolved taxonomic status. Mitochondrial DNA sequences suggest five sexual species to occur in Finland. However, their species status remains unconfirmed, due to a lack of knowledge on interbreeding, unclear morphological distinction and the limited variation in mitochondrial DNA. We combine three methods, a cross-mating experiment, an analysis of mitochondrial and nuclear DNA, and a detailed male genital morphological examination, to establish the species status of the five suspected species. All suspected species exhibit intraspecific mating preference, although several interspecific pairs readily produce offspring. The genetic analysis, however, fails to show **hybrids** or introgression, suggesting that both pre- and post-copulation mechanisms isolate the species reproductively. Morphological analysis of the male genitalia confirms that the species have diverged. Our results highlight the need of combining behavioural, morphological and genetic methods to determine species status in challenging taxonomic groups. (c) 2014 The Linnean Society of London, Biological Journal of the Linnean Society, 2014, 112, 584-605.

Sex pheromone of the soybean pod borer, *Leguminivora glycinivorella* (Lepidoptera : Tortricidae): Identification and field evaluation

Van Vang, L; Ishitani, M; Komai, F; Yamamoto, M; Ando, T. 2006

With reference to the data of synthetic standards, GC-EAD and GC-MS analyses of a pheromone gland extract of the soybean pod borer, *Leguminivora glycinivorella* (Lepidoptera: Tortricidae), resulted in three acetates [dodecyl acetate, (8E, 10E)-8, 10-dodecadienyl acetate (E8,E 10-12:OAc), and its (8E,10Z)-isomer] at a ratio of 10: 100: 2.5 as a candidate of the pheromone. While the contents in the glands were low and even the titer of the major pheromonal component was ca. 0.5 ng/female, the positions of the two double bonds were confirmed by a mass spectrum of the adduct with 4-methyl-1, 2,4-triazoline-3,5-dione. In a soybean field, synthetic E8,E10-12:OAc successfully attracted male moths of *L. glycinivorella*, and highly selective attraction was observed for E8,E10-12:OAc among the geometrical isomers of the 8,10-diene. Neither of the two minor components showed a synergistic effect on field attraction by E8,E10-12:OAc, and their roles were unclear. A binary lure of E8,E10-12:OAc and (E)-8-dodecenyl acetate (E8-12:OAc) was necessary to attract the male moths of *Matsumuraesia falcana*, the soybean pod worm, but E8-12:OAc strongly inhibited the attraction of *L. glycinivorella* by E8,E10-12:OAc, indicating that E8-12:OAc secreted by *M. falcana* is one of the most important factors in the reproductive isolation of these two species.

A rearrangement of the Z chromosome topology influences the sex-linked gene display in the European corn borer, *Ostrinia nubilalis*

Kroemer, JA; Coates, BS; Nusawardani, T; Rider, SD; Fraser, LM; Hellmich, RL. 2011

Males are homogametic (ZZ) and females are heterogametic (WZ) with respect to the sex chromosomes in many species of butterflies and moths (insect order Lepidoptera). Genes on the Z chromosome influence traits involved in larval development, environmental adaptation, and reproductive isolation. To facilitate the investigation of these traits across Lepidoptera, we developed 43 degenerate primer pairs to PCR amplify orthologs of 43 *Bombyx mori* Z chromosome-linked genes. Of the 34 orthologs that amplified by PCR in *Ostrinia nubilalis*, 6 co-segregated with the Z chromosome anchor markers *kettin* (*ket*) and *lactate dehydrogenase* (*ldh*), and produced a consensus genetic linkage map of similar to 89 cM in combination with 5 AFLP markers. The *O. nubilalis* and *B. mori* Z chromosomes are comparatively co-linear, although potential gene inversions alter terminal gene orders and a translocation event disrupted synteny at one chromosome end. Compared to *B. mori* orthologs, *O. nubilalis* Z chromosome-linked genes showed conservation of tissue-specific and growth-stage-specific expression, although some genes exhibited species-specific expression across developmental stages or tissues. The *O. nubilalis* Z chromosome linkage map provides new tools for isolating quantitative trait loci (QTL) involved in sex-linked traits that drive speciation and it exposes genome rearrangements as a possible mechanism for differential gene regulation in Lepidoptera.

Pheromone races of *Cydia splendana* (Lepidoptera, Tortricidae) overlap in host plant association and geographic distribution

Bengtsson, M; Boutitie, A; Josvai, J; Toth, M; Andreadis, S; Rauscher, S; Unelius, CR; Witzgall, P. 2014

Identification of the sex pheromone of *Cydia splendana* (Lepidoptera, Tortricidae) by pheromone gland analysis followed by field trapping with synthetic compounds shows the occurrence of two pheromone races. Acorn moth females from Sweden, where oak *Quercus robur* is the only host plant, use a blend of the E,Z and E,E isomers of 8,10-dodecadien-1-yl acetate. In Central and Southern Europe, where *C. splendana* feeds on chestnut *Castanea sativa* and several species of oak, males respond to another isomer blend, E,E and Z,E. The distribution of the two pheromone races of *C. splendana* overlaps in Northern France, where they share oak as plant host. Differences in sex communication signals between these populations of *C. splendana* corroborate the role of specific mate recognition in speciation events.

Comparative transcriptome analysis at the onset of speciation in a mimetic butterfly-The Ithomiini *Melinaea marsaeus*

Piron-Prunier, F; Persyn, E; Legeai, F; McClure, M; Meslin, C; Robin, S; Alves-Carvalho, S; Mohammad, A; Blugeon, C; Jacquin-Joly, E; Montagne, N; Elias, M; Gauthier, J. 2021

Ecological speciation entails divergent selection on specific traits and ultimately on the developmental pathways responsible for these traits. Selection can act on gene sequences but also on regulatory regions responsible for gene expression. Mimetic butterflies are a relevant system for speciation studies because wing colour pattern (WCP) often diverges between closely related taxa and is thought to drive speciation through assortative mating and increased predation on **hybrids**. Here, we generate the first transcriptomic resources for a mimetic butterfly of the tribe Ithomiini, *Melinaea marsaeus*, to examine patterns of differential expression between two subspecies and between tissues that express traits that likely drive reproductive isolation; WCP and chemosensory genes. We sequenced whole transcriptomes of three life stages to cover a large catalogue of transcripts, and we investigated differential expression between subspecies in pupal wing discs and antennae. Eighteen known WCP genes were expressed in wing discs and 115 chemosensory genes were expressed in antennae, with a remarkable diversity of chemosensory protein genes. Many transcripts were differentially expressed between subspecies, including two WCP genes and one odorant receptor. Our results suggest that in *M. marsaeus* the same genes as in other mimetic butterflies are involved in traits causing reproductive isolation, and point at possible candidates for the differences in those traits between subspecies. Differential expression analyses of other developmental stages and body organs and functional studies are needed to confirm and expand these results. Our work provides key resources for comparative genomics in mimetic butterflies, and more generally in Lepidoptera.

Pheromones and Barcoding Delimit Boundaries between Cryptic Species in the Primitive Moth Genus *Eriocrania* (Lepidoptera: Eriocraniidae)

Lassance, JM; Svensson, GP; Kozlov, MV; Francke, W; Lofstedt, C. 2019

Animal classification is primarily based on morphological characters, even though these may not be the first to diverge during speciation. In many cases, closely related taxa are actually difficult to distinguish based on morphological characters alone, especially when there is no substantial niche separation. As a consequence, the diversity of certain groups is likely to be underestimated. Lepidoptera -moths and butterflies- represent the largest group of herbivorous insects. The extensive diversification in the group is generally assumed to have its origin in the spectacular radiation of flowering plants and the resulting abundance of ecological niches. However, speciation can also occur without strong ecological divergence. For example, reproductive isolation can evolve as the result of divergence in mate preference and the associated pheromone communication system. We combined pheromone trapping and genetic analysis to elucidate the evolutionary relationships within a complex of primitive moth species (Lepidoptera: Eriocraniidae). Mitochondrial and nuclear DNA markers provided evidence that *Eriocrania semipurpurella*, as currently defined by morphological characters, includes three cryptic species in Northern and Western Europe. Male moths of these cryptic species, as well as of the closely related *E. sangii*, exhibited relative specificity in terms of their attraction to specific ratios of two major pheromone components, (2S,6Z)-nonen-2-ol and (2R,6Z)-nonen-2-ol. Our data suggest strong assortative mating in these species in the absence of apparent niche separation, indicating that *Eriocrania* moths may represent an example of non-ecological speciation. Finally, our study argues in favour of combining pheromone investigations and DNA barcoding as powerful tools for identifying and delimitating species boundaries.

DIFFERENCES IN SEX-PHEROMONE COMMUNICATION-SYSTEMS OF CLOSELY-RELATED SPECIES - SPODOPTERA LATIFASCIA (WALKER) AND S-DESCOINSI LALANNECASSOU AND SILVAIN (LEPIDOPTERA, NOCTUIDAE)

MONTI, L; LALANNECASSOU, B; LUCAS, P; MALOSSE, C; SILVAIN, JF. 1995

S. latifascia and *S. descoinsi* are closely related species that occur sympatrically over limited areas in French Guiana. We examined allopatric populations, *S. latifascia* originating from Barbados and *S. descoinsi* from French Guiana. Studies on nocturnal activity cycles showed temporal partitioning of female calling behavior, male sexual activity, and mating behavior. *S. descoinsi* were sexually active in the first half of the scotophase whereas *S. latifascia* were sexually active in the second half. Seven compounds (Z9-14: Ac, Z9,E12-14: Ac, Z11-16: Ac, E9,E12-14: Ac, Z9-14: Aid, Z9,E11-14:Ac and Z11-14:Ac) were identified in females of both *S. latifascia* and *S. descoinsi* extracts. Z9-14:Ac was a main pheromone component for the two species. The major difference between the pheromones of *S. latifascia* and *S. descoinsi* was the proportion of Z9,E12-14:Ac in the extracts: 7% for *S. latifascia* and 42% for *S. descoinsi*. The proportion of Z9,E12-14:Ac relative to the sum of Z9-14:Ac and Z9,E12-14:Ac in individual gland extracts was 4 +/- 1% (mean +/- standard deviation) for *S. latifascia* and 44.8 +/- 6% for *S. descoinsi*. Electrophysiological studies showed no major differences between species in the morphology and physiology of the pheromone receptors of males. Receptors were identified for Z9-14:Ac and Z9,E12-14:Ac, but no receptor was found for the other compounds. In the wind tunnel, synthetic blends with Z9-14:Ac and Z9,E12-14:Ac gave the same behavioral responses as conspecific female extracts for the males of the two species. Some cross-attraction was observed with synthetic blends and female extracts. Nevertheless, previous field trapping experiments in French Guiana were species-specific and suggested differences in the attractivity of males. In the laboratory, *S. latifascia* and *S. descoinsi* could **hybridize** in both reciprocal crosses. Female *S. descoinsi* x male *S. latifascia* mating rate was significantly lower than for the reciprocal cross, and 26.7% of female *S. descoinsi* could not separate from male *S. latifascia* after mating. These copulatory problems may involve genital incompatibilities between males and females. Several barriers against interbreeding between *S. latifascia* and *S. descoinsi* seem to combine including differences in nocturnal activity cycles, pheromone differences; and genital barriers. The study of sympatric populations will be necessary to define the role of sex pheromones in the reproductive isolation of *S. latifascia* and *S. descoinsi*.

Mating patterns of the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae) in sympatric and allopatric populations

Muller, K; Thiery, D; Delbac, L; Moreau, J. 2017

Phytophagous insects have been at the heart of investigations of ecological speciation, and it is clear that adaptation to different host plant species can promote host race formation and insect speciation. However, the evolution of host races has typically been studied at the plant species scale, using sympatric populations of insects that are specialized on particular plant species. Because many crop pest species are adapted to various plant varieties selected from a single plant species, it is of interest to establish whether reproductive barriers could evolve at this much smaller geographical scale, between individuals exploiting different plant varieties. To assess this we evaluated premating and postmating prezygotic barriers among sympatric populations of the European grapevine moth *Lobesia botrana* originated from different cultivars of the same plant species (*Vitis vinifera*), and between allopatric populations originated from different geographical sites. We found weak reproductive isolation for sympatric populations of *L. botrana*, but marked reproductive isolation among allopatric populations. In sympatric populations, the only effect was on the latency period prior to mating, which was longer for heterotypic partners that originated from different cultivars than for homotypic partners originated from the same cultivar. In allopatric populations, reproductive isolation was evident in both premating barriers and postmating prezygotic barriers. In summary, we did not find any trend for sympatric host race formation in *L. botrana*, but the occurrence of non-random mating patterns between different allopatric populations suggests the beginning of reproductive isolation, which could lead to the evolution of cryptic species of *L. botrana*. (C) 2016 The Linnean Society of London.

Mating compatibility among populations of codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) from different geographic origins

Taret, G; Sevilla, M; Wornoayporn, V; Islam, A; Ahmad, S; Caceres, C; Robinson, AS; Vreysen, MJB. 2010

The codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) is a serious pest of pome fruit worldwide and the sterile insect technique (SIT) provides an environmentally acceptable approach for its control. As the pest is present in both the southern and northern hemispheres it would be possible for a rearing facility in the northern hemisphere to supply sterile moths to an SIT programme in the southern hemisphere during the northern winter and vice versa. This could greatly improve the economics of moth production and the running costs of rearing facilities. However in order to develop this concept, it is important to assess if populations of codling moth from different geographical regions share mating compatibility. Twelve different laboratory and field populations from both hemispheres were sampled and field cage bisexual mating compatibility tests were carried out between selected combinations. The index of sexual isolation (ISI) and the female and male relative performance index (FRPI and MRPI, respectively) were calculated for each mating combination. In only two of the combinations was there a slight but significant deviation from random mating. There were also some significant differences in mating duration between the homotypic matings and the duration of a particular homotypic mating seemed to depend on the origin of the other population in the cage. It was concluded that there exist no barriers to mating between populations of codling moth from many parts of the world and that it would be feasible for sterile moths to be shipped from one rearing facility to SIT programmes in other parts of the world.

Difference in the time of mating activity between host-associated populations of the rice stem borer, *Chilo suppressalis* (Walker)

Ueno, H; Furukawa, S; Tsuchida, K. 2006

Mating activities of the rice stem borer, *Chilo suppressalis*, were compared between rice- and wateroat-feeding populations, and two potential temporal factors that may act as reproductive barriers were examined. Seasonal data of the number of moths attracted to pheromone traps showed that the peak of emergence at the rice field was approximately 10 days earlier than that at the wateroat vegetation in the first flight season, although there was a broad overlap of emergence at the two locations. Both field observations and a laboratory experiment showed that moths from the rice field started mating earlier than those from the wateroat vegetation. However, whereas the difference was distinctive in the laboratory experiment, mating activity at the wateroat vegetation shifted significantly to an earlier time phase than that observed in the laboratory. Body size data showed that the male moths attracted to the pheromone traps at the wateroat vegetation were significantly larger than those at the rice field, suggesting that the traps at the two locations mainly attracted moths originating from different host plants. However, pheromone-trapped

males at the rice field were significantly larger than those reared from overwintering samples. These results support the idea that males from the wateroat vegetation migrate to the rice field. The differences in seasonal and temporal mating activity and their effects on development of reproductive isolation between host-associated populations are discussed.

Sex pheromone composition and reproductive isolation in two *Busseola* species (Lepidoptera: Noctuidae) in Kenya

Felix, AE; Calatayud, PA; Le Ru, B; Silvain, JF; Frerot, B. 2011

Closely related species provide an interesting and useful model to understand mate communication diversification. Pre-mating isolation is the result of selection processes and is achieved by utilization of different sex pheromones, but also by different ecological and life traits such as geographic distribution, host-plant specialization and allochrony of sexual behavior. Here, we report sex pheromone identifications and pre-mating isolation of two closely related species of stemborers: *Busseola segeta* and the yet undescribed *Busseola* nov. sp., developing on different host-plants and in different geographical locations in Kenya. GC-MS analyses showed that the two species produced the same pheromone components, but in slight different ratios. The respective synthetic pheromone mixtures tested in the field were attractive for males of each species. The low inter-specific variation in the pheromone mixture would allow possible interbreeding in case of accidental introduction of both species in the same environment. Under laboratory conditions, allochrony of the mating period is total and might prevent interbreeding if confirmed in natural conditions. This point would ensure the reproductive isolation between the two species in case of modifications of ecological factors. This study and the chemical structures of the identified pheromone components clearly demonstrate that these *Busseola* species are closely related although they differ in morphology.

Using RNA sequencing to characterize female reproductive genes between Z and E Strains of European Corn Borer moth (*Ostrinia nubilalis*)

Al-Wathiqui, N; Lewis, SM; Dopman, EB. 2014

Background: Reproductive proteins often evolve rapidly and are thought to be subject to strong sexual selection, and thus may play a key role in reproductive isolation and species divergence. However, our knowledge of reproductive proteins has been largely limited to males and model organisms with sequenced genomes. With advances in sequencing technology, Lepidoptera are emerging models for studies of sexual selection and speciation. By profiling the transcriptomes of the bursa copulatrix and bursal gland from females of two incipient species of moth, we characterize reproductive genes expressed in the primary reproductive tissues of female Lepidoptera and identify candidate genes contributing to a one-way gametic incompatibility between Z and E strains of the European corn borer (*Ostrinia nubilalis*). Results: Using RNA sequencing we identified transcripts from similar to 37,000 and similar to 36,000 loci that were expressed in the bursa copulatrix or the bursal gland respectively. Of bursa copulatrix genes, 8% were significantly differentially expressed compared to the female thorax, and those that were up-regulated or specific to the bursa copulatrix showed functional biases toward muscle activity and/or organization. In the bursal gland, 9% of genes were differentially expressed compared to the thorax, with many showing reproduction or gamete production functions. Of up-regulated bursal gland genes, 46% contained a transmembrane region and 16% possessed secretion signal peptides. Divergently expressed genes in the bursa copulatrix were exclusively biased toward protease-like functions and 51 proteases or protease inhibitors were divergently expressed overall. Conclusions: This is the first comprehensive characterization of female reproductive genes in any lepidopteran system. The transcriptome of the bursa copulatrix supports its role as a muscular sac that is the primary site for disruption of the male ejaculate. We find that the bursal gland acts as a reproductive secretory body that might also interact with male ejaculate. In addition, differential expression of proteases between strains supports a potential role for these tissues in contributing to reproductive isolation. Our study provides new insight into how male ejaculate is processed by female Lepidoptera, and paves the way for future work on interactions between post-mating sexual selection and speciation.

The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains

Groot, AT; Marr, M; Heckel, DG; Schofl, G. 2010

2. The two races exhibit habitat isolation through host-plant preference, and two types of behavioural isolation, i.e. differences in sex pheromone composition and timing of mating activity at night. 3. In this paper, we review the selection pressures acting upon these three barriers as well as their genetic bases, to address the question of how divergence of the two strains may have evolved. 4. We also address possible interactions between the three barriers, whether and how they may have evolved in concert, and we view the evolution of these three prezygotic isolation barriers in the light of postzygotic isolation.

Sex pheromones of five olethreutine species (Lepidoptera : Tortricidae) associated with the seedlings and fruits of mangrove plants in the Ryukyu Islands, Japan: Identification and field evaluation

Van Vang, L; Inomata, SI; Kinjo, M; Komai, F; Ando, T. 2005

The sex pheromones of three *Cryptophlebia*, one *Centroxena*, and one *Eucosma* species (Lepidoptera: Olethreutinae) inhabiting mangroves in the Ryukyu Islands, Japan, were studied with coupled gas chromatography/electroantennographic detection, and GC-mass spectrometry (GC-MS). The larvae of each *Cryptophlebia* species are specifically associated with viviparous seedlings from one of three mangrove Rhizophoraceae plants. Whereas three EAG-active alcohol components, (Z)-8-dodecen-1-ol, (E)-8-dodecen-1-ol, and dodecan-1-ol, in a ratio of 100:12:4, were identified from the pheromone gland extract of female of *C. horii* (host: *Bruguiera gymnorhiza*), two other sibling species produced the corresponding acetates, i.e., (Z)-8-dodecenyl acetate, (E)-8-dodecenyl acetate, and dodecyl acetate, in a 100:2:3 ratio from *Cryptophlebia palustris* (host: *Rhizophora stylosa* in Iriomote-jima Island) and in a 100:7:13 ratio from *C. amamiana* (host: *Kandelia candel* in Amami-oshima Island). The double bond positions of the monounsaturated components were confirmed by GC-MS analyses of their adducts with dimethyl disulfide. On the other hand, the larvae of *Centroxena* sp. feed on fruits of *Sonneratia alba*, another mangrove plant in the Sonneratiaceae, and the extract of the female pheromone glands contained (8E, 10E)-dodecadienyl acetate and dodecyl acetate in a ratio of 100:5. The double bond position of the diunsaturated compound was confirmed by GC-MS analysis of its adduct with 4-methyl-1,2,4-triazoline-3,5-dione. (E)-9-Dodecenyl acetate was exclusively identified in the pheromone gland extract of *Eucosma coniogramma* females reared from seedlings of *B. gymnorhiza*. Although the roles of minor components have not been revealed by field tests, synthetic lures baited with the main pheromone component of each species successfully attracted the target males, confirming that the sex pheromone is one of the most important factors for their reproductive isolation.

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

Mikkola, K. 2008

In the Noctuidae, the owlet moths, the internal genitalia, i.e. the aedeagus and vesica (penis) in the males, and the bursa copulatrix in the females, together form a lock-and-key mechanism (LKM). The species-specific structures have their counterparts in the opposite sex. The internal LKM constitutes a specific reproductive isolation mechanism (lock-and-key hypothesis), which seem to be the rule in the ditrysian Lepidoptera, and also occurs in the Carabidae (Coleoptera) and some other insects. In contrast, the external genitalia rarely have species-specific counterparts in the sexes. Several results indicate the presence of LKMs: In the Noctuidae, (1) heterospecific differences in the male vesica may prevent sperm transfer or lead to mechanical failure during copulation, (2) the more complicated the specific genitalia structures, the more aberrations may occur even in conspecific copulations, and (3) in many species pairs and groups, and in one large genus, *Apamea*, the

structures in the opposite sexes show a strictly specific correspondence, but, (4) when there is precopulatory isolation due to differences in pheromone production or perception, the internal genitalia may be identical. Conversely, in the *Colias* butterflies (Pieridae), (5) frequent heterospecific **hybridization** is associated with the similarity of the internal genitalia. The LKMs seem to protect genomes against alien genes, supposedly selected for because of the lower fitness of specimens with an imprecise LKM and/or inferiority of **hybrids**. In the literature, the diversity of the noctuid genitalia has been ascribed to sexual selection, because the females were classified as polyandrous. Most species produce the main part of their eggs monandrously, and remate, if at all, in their old age, and are thus successively monandrous and polyandrous. The allopatric divergence in the structure of the internal genitalia of 39 Holarctic pairs of sister species of Noctuidae is suggested to be due to genetic drift. The insecure function of the female pheromones and external genitalia of males are illustrated with the aid of original photographs.

Identification of the sex pheromone of *Sesamia cretica* Lederer

Avand-Faghih, A; Ferrot, B. 2008

By using solid phase micro-extraction and gas chromatography-mass spectrometry analyses, a sex pheromone blend for the stem borer, *Sesamia cretica* Lederer (Lepidoptera, Noctuidae), was identified as consisting of (Z)-9-tetradecen-1-ol (80%), (Z)-9-tetradecen-1-yl acetate (10%), and (Z)-11-hexadecen-1-ol (10%). The first two components had previously been discovered as attractants for *S. cretica* in field tests, but had not been identified in the female's sex pheromone gland. A field-trapping trial showed that the three-component blend gave the highest catches of male *S. cretica*. This blend, in a sticky trap, was used to monitor a population of *S. cretica* in Iran, allowing the seasonal flight activity of this insect to be compared with that of a sympatric population of *S. nonagrioides*. The role of pheromones in the reproductive isolation of these species is discussed.

Clustering of loci controlling species differences in male chemical bouquets of sympatric *Heliconius* butterflies

Byers, KJRP; Darragh, K; Garza, SF; Almeida, DA; Warren, IA; Rastas, PMA; Merrill, RM; Schulz, S; McMillan, WO; Jiggins, CD. 2021

The degree to which loci promoting reproductive isolation cluster in the genome—that is, the genetic architecture of reproductive isolation—can influence the tempo and mode of speciation. Tight linkage between these loci can facilitate speciation in the face of gene flow. Pheromones play a role in reproductive isolation in many Lepidoptera species, and the role of endogenously produced compounds as secondary metabolites decreases the likelihood of pleiotropy associated with many barrier loci. *Heliconius* butterflies use male sex pheromones to both court females (aphrodisiac wing pheromones) and ward off male courtship (male-transferred antiaphrodisiac genital pheromones), and it is likely that these compounds play a role in reproductive isolation between *Heliconius* species. Using a set of backcross **hybrids** between *H. melpomene* and *H. cydno*, we investigated the genetic architecture of putative male pheromone compound production. We found a set of 40 significant quantitative trait loci (QTL) representing 33 potential pheromone compounds. QTL clustered significantly on two chromosomes, chromosome 8 for genital compounds and chromosome 20 for wing compounds, and chromosome 20 was enriched for potential pheromone biosynthesis genes. There was minimal overlap between pheromone QTL and known QTL for mate choice and color pattern. Nonetheless, we did detect linkage between a QTL for wing androconial area and optix, a color pattern locus known to play a role in reproductive isolation in these species. This tight clustering of putative pheromone loci might contribute to coincident reproductive isolating barriers, facilitating speciation despite ongoing gene flow.

Genetic diversity in *Pareuchaetes insulata* and its implications for biological control of *Chromolaena odorata*

Dube, N; Assefa, Y; Zachariades, C; Olckers, T; Conlong, D. 2014

Chromolaena odorata (L.) King & H.E. Robins. (Asteraceae) threatens several economic and environmental activities in the eastern subtropical regions of South Africa and is a target for biological control. Three populations of *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebididae) originating from Florida (USA), Cuba and Jamaica were released at 30 separate sites in KwaZulu-Natal, South Africa. Subsequent overlapping of two of these populations in the field and the likelihood of interbreeding posed a potential risk of establishment failure. To explore the genetic diversity of the different *P. insulata* populations and test for the existence of pre- and post-zygotic reproductive isolation between them, molecular analyses and cross-mating experiments were conducted. Mitochondrial DNA sequences revealed significant genetic similarity between them. Cross-mating trials between Floridian and Jamaican populations of *P. insulata* revealed no significant pre- and post-zygotic isolation, with no demonstrable differences in measured parameters between the 'parental' populations, the F-1 '**hybrids**' and the backcrosses. These results suggest that *P. insulata* populations released in South Africa are part of the same 'parental' population. Genetic incompatibility is, therefore, relatively unlikely to be the cause of any failure in establishment of *P. insulata* at any of the release sites.

Tissue-specific expression of the pheromone gland-specific fatty acyl reductase gene in *Ostrinia scapularis*

Antony, B; Ishikawa, Y. 2013

Abstract Pheromone gland-specific fatty acyl reductase (pgFAR) is a key enzyme involved in the conversion of fatty acyl precursors to the corresponding alcohols in moth sex pheromone biosynthesis. The gene (pgFAR) encoding for this enzyme is a major contributor to pheromone-based reproductive isolation in moths. Despite the significance of this gene, the tissue-specific expression has not yet been reported. In this study, we amplified pgFAR from *Ostrinia scapularis* Walker (Lepidoptera: Crambidae) 1 day before adult eclosion and in adult female moths that were 0-5 days old. We confirmed that the transcripts were upregulated from 1 day before adult eclosion until the 3rd day after adult emergence; however, the expression level appeared to decrease drastically in female moths that were 4-5 days old. To study the tissue-specific expression of pgFAR, a cDNA encoding pgFAR was cloned, and an RNA probe was designed to localize gene expression in pheromone gland cells using *in situ* **hybridization**. The results unambiguously and specifically confirmed the expression of pgFAR in the modified epidermal cells, which are designated as pheromone-producing cells, located at the dorsal end of the 8th-9th intersegmental membrane. This study is the first report of the tissue-specific localization of pgFAR expression in moths.

Chromosomal evolution in the South American Riodinidae (Lepidoptera: Papilionoidea)

Brown, KS; von Schoultz, B; Saura, AO; Saura, A. 2012

We give the haploid chromosome numbers of 173 species or subspecies of Riodinidae as well as of 17 species or subspecies of neotropical Lycaenidae for comparison. The chromosome numbers of riodinids have thus far been very poorly known. We find that their range of variation extends from $n = 29$ to $n = 2110$ but numbers above $n = 231$ are rare. While lepidopterans in general have stable chromosome numbers, or variation is limited at most a subfamily or genus, the entire family Riodinidae shows variation within genera, tribes and subfamilies with no single modal number. In particular, a stepwise pattern with chromosome numbers that are about even multiples is seen in several unrelated genera. We propose that this variation is attributable to the small population sizes, fragmented populations with little migration, and the behavior of these butterflies. Small and isolated riodinid populations would allow for inbreeding to take place. Newly arisen chromosomal variants could become fixed and contribute to reproductive isolation and speciation. In contrast to the riodinids, the neotropical Lycaenidae (Theclinae and Polyommattinae) conform to the modal $n = 224$ that characterizes the family.

Beyond magic traits: Multimodal mating cues in *Heliconius* butterflies

Merot, C; Frerot, B; Leppik, E; Joron, M. 2015

Species coexistence involves the evolution of reproductive barriers opposing gene flow. *Heliconius* butterflies display colorful patterns affecting mate choice and survival through warning signaling and mimicry. These patterns are called "magic traits" for speciation because divergent natural selection may promote mimicry shifts in pattern whose role as mating cue facilitates reproductive isolation. By contrast, between comimetic species, natural selection promotes pattern convergence. We addressed whether visual convergence interferes with reproductive isolation by testing for sexual isolation between two closely related species with similar patterns, *H. timareta thelxinoe* and *H. melpomene amaryllis*. Experiments with models confirmed visual attraction based on wing phenotype, leading to indiscriminate approach. Nevertheless, mate choice experiments showed assortative mating. Monitoring male behavior toward live females revealed asymmetry in male preference, *H. melpomene* males courting both species equally while *H. timareta* males strongly preferred conspecifics. Experiments with **hybrid** males suggested an important genetic component for such asymmetry. Behavioral observations support a key role for short-distance cues in determining male choice in *H. timareta*. Scents extracts from wings and genitalia revealed interspecific divergence in chemical signatures, and **hybrid** female scent composition was significantly associated with courtship intensity by *H. timareta* males, providing candidate chemical mating cues involved in sexual isolation.

Phenology and population radiation of the nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae) in Hawai'i

Jang, EB; Siderhurst, MS; Conant, P; Siderhurst, LA. 2009

The nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), is an invasive pest with established populations on three Hawai'iian islands. Indigenous to Southeast Asia, *D. pallivitta* caterpillars cause defoliation of ornamental nursery stock and pose a human health hazard due to their urticating hairs that can cause painful skin reactions. Identification of the pheromone component n-butyl (E)-7,9-decadienoate (E7,9-10:COOnBu) from *D. pallivitta* has made it possible to investigate the phenology and population dynamics using baited traps. Male captures in Jackson traps baited with E7,9-10:COOnBu showed a vegetation preference for tall-grass fields and forest/grass interfaces over forest areas. Microlocation preferences were also found for trap height, with over 65% of males being caught in traps suspended at 1 m, compared with the traps at 3 and 5 m. Captures of male moths in traps baited with live females, and direct observations of female calling behavior, showed peak activities 6-7 h after the onset of scotophase. This is a much later communication period than for *D. bradleyi* and *D. trima* and may provide a mechanism by which *D. pallivitta* maintains reproductive isolation in areas where all three species are present. Coastal and inland transects established in eastern Hawai'i measured aspects of population fluctuations and radiation into new areas with relation to elevation and microclimate. Population expansion was measured by comparing moth population means and 80% population boundaries over time. Both population measures showed a higher expansion for the coastal transect. Differences in population expansion may be attributed in part to temperature and elevation, while precipitation does not seem to have a strong effect. Both the behavioral and ecological data collected can be used to optimize deployment of detection/control strategies and to predict population expansion/risk assessment for establishing quarantine protocols for the nettle caterpillar.

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

Scriber, JM; Ording, GJ. 2005

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

A comparison of the developmental and reproductive biology of two soybean pod borers, *Etiella* spp. in Indonesia

Edmonds, RP; Borden, JH; Angerilli, NPD; Rauf, A. 2000

Experiments conducted in West Java, Indonesia investigated the developmental biology and reproductive behavior of two sympatric soybean pod borers, *Etiella zinckenella* Treitschke and *E. hobsoni* Butler (Lepidoptera: Pyralidae). It was determined that: (1) significant interspecific differences occurred between the egg, larval, pupal and total egg-to-adult developmental periods of laboratory raised *E. zinckenella* and *E. hobsoni*; (2) the pre-pupal and total egg-to-adult development periods of female *E. zinckenella* were significantly shorter than for males; (3) the longevity of virgin female *E. zinckenella* was significantly longer than that of virgin males, or virgin male and female *E. hobsoni*; (4) interspecific differences occurred in the female: male sex-ratios of laboratory raised adults; (5) peak mating for both species occurred on the second night after eclosion; (6) interspecific differences occurred in the temporal distribution of calling and mating behaviors; (7) repeated mating was observed for both species at a very low frequency; (8) interspecific mating did not occur; (9) female *E. zinckenella* were significantly more fecund than *E. hobsoni*; (10) the duration in copulo of *E. zinckenella* was significantly longer than that of *E. hobsoni*; and (11) wingtraps baited with virgin females caught only conspecific males, and reduced numbers of males were captured in traps simultaneously baited with virgin females of both species. This study demonstrates distinct biological differences and reproductive isolation between the two *Etiella* spp.

Patterns of genetic variation among geographic and host-plant associated populations of the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae)

Wang, YZ; Li, BY; Hoffmann, AA; Cao, LJ; Gong, YJ; Song, W; Zhu, JY; Wei, SJ. 2017

Background: Populations of herbivorous insects may become genetically differentiated because of local adaptation to different hosts and climates as well as historical processes, and further genetic divergence may occur following the development of reproductive isolation among populations. Here we investigate the population genetic structure of the orchard pest peach fruit moth (PFM) *Carposina sasakii* (Lepidoptera: Carposinidae) in China, which shows distinct biological differences when characterized from different host plants. Genetic diversity and genetic structure were assessed among populations from seven plant hosts and

nine regions using 19 microsatellite loci and a mitochondrial sequence. Results: Strong genetic differentiation was found among geographical populations representing distinct geographical regions, but not in host-associated populations collected from the same area. Mantel tests based on microsatellite loci indicated an association between genetic differentiation and geographical distance, and to a lesser extent environmental differentiation. Approximate Bayesian Computation analyses supported the scenario that PFM likely originated from a southern area and dispersed northwards before the last glacial maximum during the Quaternary. Conclusions: Our analyses suggested a strong impact of geographical barriers and historical events rather than host plants on the genetic structure of the PFM; however, uncharacterized environmental factors and host plants may also play a role. Studies on adaptive shifts in this moth should take into account geographical and historical factors.

Evidence of Sexual Selection in *Neoleucinodes elegantalis* (Lepidoptera: Crambidae): Correlation of Female Moth Genitalia and Solanaceae Host Fruit Size

Diaz-Montilla, AE; Gonzalez, R; Solis, MA; Saldamando-Benjumea, CI. 2015

Neoleucinodes elegantalis (Guenee) (Lepidoptera: Crambidae) is a major pest of fruits in the family Solanaceae in the Western Hemisphere. The objectives of this study were to determine whether life zone or host plant explained morphological variation in females, and if so, if there was evidence of sexual selection driving diversification in this species. We collected larvae feeding on cultivated (*Capsicum annuum* L., *Solanum betaceum* Cavanilles, *Solanum lycopersicum* Lamarck, *Solanum melongena* L., and *Solanum quitoense* Lamarck) and wild species (*Solanum atropurpureum* Schrank, *Solanum acerifolium* Dunal, *Solanum crinitum* Lamarck, and *Solanum hirtum* Vahl) of Solanaceae in Colombia. The genitalia traits of 547 reared females were measured and correlations with host plant fruit size were estimated. Six female genitalia morphological characters, apophysis posterioris, apophysis anterioris, ostium bursae, ductus bursae length, corpus bursae, and the seventh abdominal segment were measured. Principal component analysis and cluster analysis classified individuals based on female morphological similarity and clustered them into four main groups according to host plant: 1) *S. acerifolium*; 2) *S. quitoense*, *S. lycopersicum*, *C. annuum* and *S. hirtum*; 3) *S. atropurpureum*; 4) *S. melongena*, *S. crinitum* and *S. betaceum*. In this unique study, we found that variation in female genitalia size is directly correlated with the size of its host fruit, which suggests a mechanism for reproductive isolation and divergence of the four host races. Ours is one of the first studies that shows female genitalia morphology is correlated with species of host plants and represents a valuable contribution to the study of sexual selection in the evolution of insects.

Sex Pheromones and Reproductive Isolation of Three Species in Genus *Adoxophyes*

Yang, CY; Han, KS; Boo, KS. 2009

We tested differences in female pheromone production and male response in three species of the genus *Adoxophyes* in Korea. Females of all three species produced mixtures of (Z)-9-tetradecenyl acetate (Z9-14:OAc) and (Z)-11-tetradecenyl acetate (Z11-14:OAc) as major components but in quite different ratios. The ratio of Z9-14:OAc and Z11-14:OAc in pheromone gland extracts was estimated to be ca. 100:200 for *Adoxophyes honmai*, 100:25 for *Adoxophyes orana*, and 100:4,000 for *Adoxophyes* sp. Field tests showed that males of each species were preferentially attracted to the two-component blends of Z9-14:OAc and Z11-14:OAc mimicking the blends found in pheromone gland extracts of conspecific females. The effects of minor components identified in gland extracts on trap catches varied with species. Addition of 10-methyldodecyl acetate (10me-12:OAc) or (E)-11-tetradecenyl acetate (E11-14:OAc) to the binary blend of Z9-14:OAc and Z11-14:OAc significantly increased captures of *A. honmai* males, whereas E11-14:OAc exhibited a strongly antagonistic effect on catches of *Adoxophyes* sp. males. Moreover, (Z)-9-tetradecen-1-ol (Z9-14:OH) or (Z)-11-tetradecen-1-ol (Z11-14:OH) added to the binary blends increased attraction of male *A. orana* but not *A. honmai* and *Adoxophyes* sp. males, suggesting that these minor components, in addition to the relative ratios of the two major components, play an important role in reproductive isolation between *Adoxophyes* species in the southern and midwestern Korea where these species occur sympatrically.

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

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

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

Spatio-temporal variation in mating success of female bagworms

Rhainds, M. 2013

The study investigated spatio-temporal variation in the mating success of female bagworms, *Thyridopteryx ephemeraeformis* Haworth (Lepidoptera: Psychidae), across a broad latitudinal range in Indiana, USA, between 2007 and 2009. A series of interconnected equations based on estimates of demographic parameters at different intervals was used to derive the punctual sex ratio and female mating success early and late in the season. Both the mating success of females and the relative abundance of bagworms declined with latitude, which provides indirect support to the mate encounter Allee hypothesis. However, the late emergence of females at northern locations combined with the consistently low mating success of late-emerging females suggests that the impact of latitude on mating probability is indirectly mediated by emergence time. A variable level of protandry was observed each year, and the low ratio of males per female late in the season was associated with low female mating success. The reduced level of activity of males at temperatures below 18 degrees C may also account for the low mating probability of late-emerging females. The weak, inconsistent effect of local variation in sex ratio on female mating success suggests that males commonly disperse from their natal patch, a behavior that may have evolved to reduce inbreeding in local populations of bagworms. Altogether, these results suggest that temporal variation in sex ratio has a greater impact on the mating success of female bagworms than spatial variation in sex ratio.

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

Ohshima, I. 2008

Genetic differentiation in ecological traits plays an important role in the reproductive isolation of phytophagous insects. The present study aims to elucidate the genetic changes involved during the process of host shifts, by combining analyses for (1) host adaptations, (2) pre- and postmating isolation, and (3) phylogeny among populations, using a leaf-mining moth, *Acrocercops transecta*. This species is associated with *Juglans ailanthifolia* and *Lyonia ovalifolia*. Transplantation of the larvae demonstrated that the *Juglans*-associated population completely failed to survive on *Lyonia*, whereas the *Lyonia*-associated population survived on *Juglans* as well as on *Lyonia*. Females of respective host-associated populations oviposited on their natal host plant only. An mtDNA-based phylogeny clearly separated the *Lyonia*-associated population from the *Juglandaceae*-associated population, and indicated that the *Lyonia*-associated population once evolved from the *Juglandaceae*-associated population. These results indicate that the processes of host shifting from juglandaceous species to *Lyonia* involved genetic changes both in larval ability to use host plants and in host preference of females. The derived *Lyonia*-associated population has retained the potential to assimilate the ancestral

host, Juglandaceae. Mating between the two host-associated populations was successful for both directions of crossing, and there were no significant differences in egg hatchability between **hybrids** and control crosses. No adults emerged when the F-1 **hybrid** larvae were maintained on *Lyonia*; however, on *Juglans* the F-1 **hybrid** larvae grew to adulthood as well as in the control, suggesting a lack of genomic incompatibilities between the two host-associated populations. In conclusion, the results showed that the two host-associated populations are host races that are partially reproductively isolated, and that the differences in performance and preference function as strong barriers against gene flow between the host races. (c) 2008 The Linnean Society of London.

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

Deering, MD; Scriber, JM. 2002

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

Heterozygosity and Chain Multivalents during Meiosis Illustrate Ongoing Evolution as a Result of Multiple Holokinetic Chromosome Fusions in the Genus *Melinaea* (Lepidoptera, Nymphalidae)

McClure, M; Dutrillaux, B; Dutrillaux, AM; Lukhtanov, V; Elias, M. 2017

Mitotic and meiotic chromosomes from 2 taxa of the genus *Melinaea*, *M. satevis cydon* and *M. "satevis" tarapotensis* (Lepidoptera: Nymphalidae), and from **hybrids** produced in captivity were obtained using an improved spreading technique and were subsequently analyzed. In one of the taxa, the presence of trivalents and tetravalents at diakinesis/metaphase I is indicative of heterozygosity for multiple chromosome fusions or fissions, which might explain the highly variable number of chromosomes previously reported in this genus. Two large and complex multivalents were observed in the meiotic cells of the **hybrid** males (32 chromosomes) obtained from a cross between *M. "s." tarapotensis* (28 chromosomes) and *M.s. cydon* (40-43 chromosomes). The contribution of the 2 different haploid karyotypes to these complex figures during meiosis is discussed, and a taxonomic revision is proposed. We conclude that chromosome evolution is active and ongoing, that the karyotype of the common ancestor consisted of at least 48 chromosomes, and that evolution by chromosome fusion rather than fission is responsible for this pattern. Complex chromosome evolution in this genus may drive reproductive isolation and speciation, and highlights the difficulties inherent to the systematics of this group. We also show that *Melinaea* chromosomes, classically considered as holocentric, are attached to unique, rather than multiple, spindle fibers. (c) 2018 S. Karger AG, Basel

What shapes the continuum of reproductive isolation? Lessons from *Heliconius* butterflies

Merot, C; Salazar, C; Merrill, RM; Jiggins, CD; Joron, M. 2017

The process by which species evolve can be illuminated by investigating barriers that limit gene flow between taxa. Recent radiations, such as *Heliconius* butterflies, offer the opportunity to compare isolation between pairs of taxa at different stages of ecological, geographical, and phylogenetic divergence. Here, we report a comparative analysis of existing and novel data in order to quantify the strength and direction of isolating barriers within a well-studied clade of *Heliconius*. Our results highlight that increased divergence is associated with the accumulation of stronger and more numerous barriers to gene flow. Wing pattern is both under natural selection for Mullerian mimicry and involved in mate choice, and therefore underlies several isolating barriers. However, pairs which share a similar wing pattern also display strong reproductive isolation mediated by traits other than wing pattern. This suggests that, while wing pattern is a key factor for early stages of divergence, it may become facultative at later stages of divergence. Additional factors including habitat partitioning, **hybrid** sterility, and chemically mediated mate choice are associated with complete speciation. Therefore, although most previous work has emphasized the role of wing pattern, our comparative results highlight that speciation is a multi-dimensional process, whose completion is stabilized by many factors.

REPRODUCTIVE ISOLATING MECHANISMS IN FALL ARMYWORM HOST STRAINS (LEPIDOPTERA, NOCTUIDAE)

PASHLEY, DP; HAMMOND, AM; HARDY, TN. 1992

Four potential reproductive isolating mechanisms were studied in two genetically differentiated host strains of the fall armyworm, *Spodoptera frugiperda* J. E. Smith. The strongest barrier to interstrain mating was temporal partitioning of mating activities throughout the night. The corn strain mated exclusively in the first two-thirds of the night whereas the rice strain mated in the last one-third. Mating preferences assessed by laboratory choice tests also indicated strong assortative mating. Females chose like-strain males 85% of the time. Captures of males responding to caged virgin females in the field provided little evidence of pheromonal differences: almost-equal-to 40% of the males were attracted to females of the opposite strain. Finally, differences in seasonal abundance were observed. The corn strain achieves highest larval densities in spring and midsummer whereas the rice strain is most abundant in late summer and fall. Adult densities assessed with pheromone traps were consistent with larval densities for the rice strain but not for the corn strain. Very few corn strain males were captured despite high larval densities. These studies support the hypothesis that a combination of characteristics function together to inhibit interbreeding and, thus, provide evidence that supports species status of the two fall armyworm strains.

Genetic differentiation without mimicry shift in a pair of hybridizing *Heliconius* species (Lepidoptera: Nymphalidae)

Merot, C; Mavarez, J; Evin, A; Dasmahapatra, KK; Mallet, J; Lamas, G; Joron, M. 2013

Butterflies in the genus *Heliconius* have undergone rapid adaptive radiation for warning patterns and mimicry, and are excellent models to study the mechanisms underlying diversification. In *Heliconius*, mimicry rings typically involve distantly related species, whereas closely related species often join different mimicry rings. Genetic and behavioural studies have now reproductive isolation in many pairs of *Heliconius* taxa is largely mediated by natural and sexual selection on wing colour patterns. However, recent studies have uncovered new cases in which pairs of closely related species are near-perfect mimics of each other. Here, we provide morphometric and genetic evidence for the coexistence of two closely related, **hybridizing** co-mimetic species on the eastern slopes of the Andes, *H. melpomene amaryllis* and *H. timareta* sp. nov., which is described here as *H. timareta thelxinoe*. A joint analysis of multilocus genotyping and geometric morphometrics of wing shape shows a high level of differentiation between the two species, with only limited gene flow and mixing. Some degree of genetic mixing can be detected, but

putative **hybrids** were rare, only one of 175 specimens being a clear **hybrid**. In contrast, we found phenotypic differentiation between populations of *H. timareta* thelxinoe, possibly indicative of strong selection for local mimicry in different communities. In this pair of species, the absence of breakdown of genetic isolation despite near-identical wing patterns implies that factors other than wing patterns keep the two taxa apart, such as chemical or behavioural signals, or ecological adaptation along a strong altitudinal gradient. (c) 2013 The Linnean Society of London, Biological Journal of the Linnean Society, 2013, 109, 830-847.

ROLE OF SEX-PHEROMONE COMPONENTS IN BEHAVIORAL REPRODUCTIVE ISOLATION BETWEEN AUTOGRAPHA-GAMMA (L) AND EITHER TRICHOPLUSIA-NI (HUBNER) OR CHRYSODEIXIS-CHALCITES (ESP) (LEPIDOPTERA, NOCTUIDAE, PLUSIINAE)

MAZOR, M; DUNKELBLUM, E. 1992

The composition of the *Autographa gamma* sex pheromone was reexamined and only (Z)-7-dodecenyl acetate and (Z)-7-dodecenol were identified by capillary GC, GC-MS, and dimethyl disulfide derivatization and subsequent GC-MS analysis. The fatty acid content of the pheromone glands was also studied, and a series of saturated and unsaturated acids was identified. However, most of the related pheromonal compounds were not detected. The male response to the pheromone components was studied in a flight tunnel and compared with the response to calling females. The best synthetic baits evoked a response similar to that observed to the virgin females, but males spent significantly more time at calling females than at the synthetic baits. The preferred synthetic baits consisted of (Z)-7-dodecenyl acetate alone or of a blend with 5% (Z)-7-dodecenol. Increasing the relative amount of the alcohol caused a gradual reduction in male response, particularly in the last steps of the courtship sequence. The addition of the minor sex pheromone components of the sympatric *Plusiinae* species, *Trichoplusia ni* and *Chrysodeixis chalcites*, to the *A. gamma* pheromone was also investigated in the flight tunnel. Some of these components exhibited a significantly antagonistic effect on the *A. gamma* male courtship behavior. The most potent antagonists were (Z)-5-dodecenyl acetate and (Z)-9-tetradecenyl acetate. The response of *A. gamma* and *T. ni* males to conspecific and heterospecific females was also compared in the flight tunnel. Whereas *A. gamma* males were attracted only to their conspecific females, a small percentage of *T. ni* males were also attracted to *A. gamma* females and 11 % performed the whole courtship sequence.

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

Forister, ML. 2005

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

SEX-PHEROMONES OF SPILONOTA-OCELLANA AND SPILONOTA-LARICANA

WITZGALL, P; BENGTSSON, M; BUSER, HR; CHAMBON, PJ; PRIESNER, E; WILDBOLZ, T; ARN, H. 1991

The sex pheromones of *Spilonota ocellana* D. & S. and *Spilonota laricana* Hein. (Lepidoptera: Tortricidae) were identified by chemical analysis and field trapping. Female moths of the two species produce (Z)-8-tetradecenyl acetate, (Z)-8-tetradecen-1-ol and dodecyl acetate in almost the same proportions (98:1:1 and 97:3:1). Males of both species were best attracted to a blend of 10:1 to 1:1 Z8-14Ac:Z8-14OH. This indicates that mating barriers other than sex pheromones exist between sympatric populations.

Reproductive isolation in Arctic species of *Gynaephora* Hubner (Lepidoptera : Lymantriidae)

Morewood, WD. 1998

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

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

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

Reproductive biology of two synchronopatric neotropical species of *Epidendrum* (Orchidaceae)

Sao Leao, LC; de Sa-Haiad, B; Rodarte, ATD; Pimentel, RR; Benevides, CR; de Santiago-Fernandes, LDR; de Lima, HA. 2019

The reproductive system of Orchidaceae is predominantly xenogamous and highly dependent on animal pollen vectors. Nectar is the main floral resource, offered to pollinators in perigonal nectaries, cuniculi or spurs; these structures are often difficult to locate and to evaluate their functionality. The Neotropical *Epidendrum denticulatum* Barb. Rodr. and *Epidendrum orchidiflorum* Salzm. ex. Lindl. bloom synchronously throughout the year in the municipality of Marica (Rio de Janeiro State, SE Brazil). The flowers of *E. denticulatum* open in the morning, have a conspicuous pink colour, a nectar guide, and nuptial and extranuptial nectaries. During the day, they are visited by species of *Hesperiidae* (Lepidoptera), which remove the pollinia. The flowers of *E. orchidiflorum* open at night, are green with nuptial and extranuptial nectaries, and have a strong odour at night, suggesting nocturnal pollination agents, possibly moths. To date, no information is available on the reproductive biology and pollination mechanisms for *E. orchidiflorum*, nor is micromorphological, anatomical or histochemical data related to the presence and functionality of secretory structures associated with pollination in either species. Also, no studies have examined the occurrence of mechanisms that hinder or prevent interspecific pollination. Here, through reproductive, morphological, structural and histochemical analyses, we evaluated the floral biology and reproductive

systems of these synchronopatric *Epidendrum* species. Both species have extranuptial nectaries, a functional cuniculus, and osmophores. Hand-pollination experiments revealed that the species are self-and intercompatible, although individuals with intermediate morphology were not identified. Pre-pollination barriers related to the floral biology of the species seem to be sufficient to prevent **hybridization**, but other biological aspects certainly contribute to the genetic integrity of the populations, such as the low rates of visits to flowers, low percentage of fruit and seed set, low seedling recruitment, and high investment in vegetative propagation.

Differences in timing of the emergence of the overwintering generation between rice and water-oats populations of the striped stem borer moth, *Chilo suppressalis* (Lepidoptera: Crambidae)

Matsukura, K; Hoshizaki, S; Ishikawa, Y; Tatsuki, S. 2009

The striped stem borer moth, *Chilo suppressalis*, consists of two host-associated populations: a rice-feeding population and a water-oats-feeding population. We investigated the seasonal occurrence of each population using sex pheromone traps in paddy fields and adjacent water-oats vegetation. Trapped males were individually classified into their respective populations by morphometric analyses of genitalia. Although the first flight of the water-oats population was long, with 1 to 3 poorly resolved peaks during April to June, that of the rice population was much shorter, with a sharp peak in early June. This result supports previous observations that adults of the overwintering generation of the water-oats population occur about two months earlier than those of the rice population; however, it is uncertain how this difference enhances reproductive isolation between the two populations because the occurrence overlaps.

Evidence for short-range sonic communication in lymantriine moths

Rowland, E; Schaefer, PW; Belton, P; Gries, G. 2011

Sexual communication of nun moth, *Lymantria monacha* (L), pink gypsy moth, *Lymantria mathura* Moore, and fumida tussock moth, *Lymantria fumida* Butler (all Lepidoptera: Noctuidae: Lymantriinae), is known to be mediated by pheromones. We now show that males are attracted by the sounds of conspecific females over short distances and that wing fanning male and female *L. monacha*, *L. mathura* and *L. fumida* produce species- and sex-specific wing beat and associated click sounds that could contribute to reproductive isolation. Evidence for short-range communication in these lymantriines includes (i) scanning electron micrographs revealing metathoracic tympanate ears, (ii) laser interferometry showing particular sensitivity of tympana tuned to frequency components of sound signals from conspecifics, and (iii) phonotaxis of male *L. monacha* and *L. fumida* to speakers playing back sound signals from conspecific females. We conclude that tympanate ears of these moths have evolved in response not only to bat predation, but also for short-range mate finding and possibly recognition. (C) 2010 Elsevier Ltd. All rights reserved.

TEMPORAL REPRODUCTIVE ISOLATION BETWEEN 2 SPECIES OF PLUME MOTHS (LEPIDOPTERA, PTEROPHORIDAE)

HAYNES, KF; BIRCH, MC. 1986

REPRODUCTIVE ISOLATION IN CLEARWING MOTHS (LEPIDOPTERA, SESIIDAE) - A TROPICAL TEMPERATE COMPARISON

GREENFIELD, MD. 1983

Incipient sympatric speciation via host race formation in *Phengaris arion* (Lepidoptera: Lycaenidae)

Bereczki, J; Poliska, S; Varadi, A; Toth, JP. 2020

The plausibility of sympatric speciation is still debated despite increasing evidence, such as host races in insects. This speciation process may be occurring in the case of the two phenological forms of the obligatorily myrmecophilous *Phengaris arion*. The main goal of our research was to study the nature and causes of difference between these forms focusing primarily on the incipient speciation via host races. Molecular analyses based on highly variable microsatellites together with *Wolbachia* screening, male genitalia morphometrics and host ant studies were carried out on four syntopic sample pairs. Our results show that the two phenological forms of *P. arion* may meet the criteria for host plant races. They coexist in sympatry in certain parts of the species range which is allowed by the adaptation to the distinct phenology of the host plants. Negative selection acts against the intermediate individuals which are on the wing in the inappropriate time frame. Thus, disruptive selection affects and produces bimodal distributions of phenotypes. However, the phenology of food plants is not entirely distinct and fluctuates year by year. Therefore, the two forms can exchange genes occasionally depending on the length of the time slot when they can meet with each other. Consequently, the reproductive isolation could not be completed and the existence of the two *arion* forms may represent only an incipient stage of sympatric speciation. It is also clear that *Wolbachia* is likely not a driver of sympatric speciation in this case.

Influence of host plant phenology on *Mitoura nelsoni* (Lepidoptera : Lycaenidae)

Forister, ML. 2005

The influence of host plant phenology on the univoltine specialist lepidopteran herbivore *Mitoura nelsoni* Boisduval was investigated using incense cedar, *Calocedrus decurrens* Torrey. The hypothesis that new spring growth is an optimal resource for *M. nelsoni* was tested by rearing larvae on plants collected along an elevational gradient at two times in the spring (both before and during the typical flight period of *M. nelsoni*). The oviposition preferences of females were assayed with the same plants. *M. nelsoni* pupae grew to consistently greater pupal weights when reared on incense cedar branches in the earliest phenological stages (largely consisting of branches taken from trees that had not initiated new growth), although females avoided ovipositing on prenew growth branches. Trees in the earliest phenological stages, which resulted in the highest larval performance, were collected before the typical flight season of *M. nelsoni*. The phenology of *M. nelsoni* does not seem to be synchronized to host conditions that are optimal for larval development. These results are discussed within the context of host-associated speciation in the genus *Mitoura* and temporal isolation that may be an important component of reproductive isolation between *M. nelsoni* and a closely related species in northern California.

Plant volatiles mediate attraction to host and non-host plant in apple fruit moth, *Argyresthia conjugella*

Bengtsson, M; Jaastad, G; Knudsen, G; Kobro, S; Backman, AC; Pettersson, E; Witzgall, P. 2006

Plant volatiles mediate host finding in insect herbivores and lead to host fidelity and habitat-specific mating, generating premating reproductive isolation and facilitating sympatric divergence. The apple fruit moth, *Argyresthia conjugella* Zeller (Lepidoptera: Argyresthiidae), is a particularly suitable species to study the cues

and behavioural mechanisms leading to colonization of a new host: it recurrently oviposits on the non-host plant, apple *Malus domestica* Borkh. (Rosaceae), where the larvae cannot complete their development. The larval host of the apple fruit moth (Lepidoptera, Argyroresthiidae), is rowan *Sorbus aucuparia* L. (Rosaceae). Fruit setting in rowan, however, fluctuates strongly over large areas in Scandinavia. Every 2-4 years, when too few rowanberries are available for egg laying in forests, apple fruit moth females oviposit instead on apple in nearby orchards, but not on other fruits, such as pear or plum. This poses the question of which cues mediate attraction to rowan and apple, and how apple fruit moth discriminates rowan from apple. Chemical analysis and antennal recordings showed that 11 out of 15 rowan volatiles eliciting an antennal response in *A. conjugella* females co-occur in rowan and apple headspace, in a different proportion. In the field, *A. conjugella* was attracted to several of these plant volatiles, especially to 2-phenyl ethanol, methyl salicylate, and decanal. Addition of anethole to 2-phenyl ethanol had a strong synergistic effect, the 1 : 1 blend is a powerful attractant for *A. conjugella* males and females. These results confirm that volatiles common to both plants may account for a host switch in *A. conjugella* from rowan to apple. Some of the most attractive compounds, including 2-phenyl ethanol, anethole, and decanal, which have been found in several apple cultivars, were not present in the headspace of the apple cultivar, Aroma, which is also susceptible to attack by *A. conjugella*. This supports the idea that the odour signal from apple is suboptimal for attraction of *A. conjugella*, but is nonetheless sufficient for attraction, during times when rowan is not available for egg laying.

Polymorphic microsatellite loci for the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera : Crambidae)

Ishiguro, N; Tsuchida, K. 2006

Four microsatellite loci were isolated and characterized from the rice stem borer moth *Chilo suppressalis*, which is an important insect pest of rice plants in Japan. These loci were not in linkage disequilibrium; however, in two of four loci, the observed heterozygosities were significantly less than expected, possibly due to the effect of null alleles. Although caution should be taken in analysis, these loci were sufficiently polymorphic to be used for population genetic analysis of this species.

Divergence in larval diapause induction between the rice and water-oat populations of the striped stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae)

Zhou, Y; Sun, D; Quan, WL; Ding, N; Liu, W; Ma, WH; Wang, XP. 2018

Differences in diapause traits can result in the seasonal reproductive isolation of host plant-associated insect populations and thereby facilitate the population divergence. The striped stem borer, *Chilo suppressalis*, has two host plant-associated populations: rice population and water-oat population. Several studies have found evidence that seasonal reproductive isolation between these populations is at least partially due to interpopulation differences in diapause. However, there still lack unambiguous evidence comparing characteristics of diapause induction for both populations. We compared the photoperiodic response and the age of peak photoperiod sensitivity of these populations and used RNA-Seq to compare the molecular response of diapause induction between populations. The photoperiodic response of the two populations differed at 25 degrees C; the critical night length of larvae from the rice population was 11 h and 20 min, whereas no obvious critical night length was in those from the water-oat population. In rice population, larvae were most sensitive to photoperiod at 9-12 days of age, whereas in water-oat population, larvae were the most sensitive to photoperiod at 9-10 days of age. The RNA-Seq results indicated that there were several differences in the molecular response of diapause induction and small overlap in differentially expressed genes (DEGs) between populations. Furthermore, GO analysis indicated that both rice and water-oat population's DEGs were significantly enriched in heme and iron binding. Besides, water-oat population's DEGs were significantly enriched in metabolizing nutrients but rice population's DEGs do not. Thus, our results described differences in diapause induction between rice and water-oat populations of *C. suppressalis* which could affect the timing of diapause and thereby contribute to the seasonal reproductive isolation of these host plant-associated populations. In conclusion, this work suggests that difference in diapause induction could promote the population divergence in insects associated with different host plants.

Genital variation in a dimorphic moth *Selenia tetralunaria* (Lepidoptera, Geometridae)

Mutanen, M; Kaitala, A. 2006

Insect genitals vary greatly among species and provide a key tool for species-level taxonomy. Insects differing in the genitalia are often treated as discrete, reproductively isolated species. This principle dates back to the lock-and-key hypothesis, which states that genitalia vary between species in order to provide a mechanical reproductive isolation system. Thus, the hypothesis assumes low within-species variability in genital traits. However, recent studies suggest that sexual selection may be responsible for the evolution of insect genitalia. We studied allometry and genital size and shape variation in a dimorphic moth *Selenia tetralunaria*. We found that the genitalia showed negative allometry in relation to body size as reported in many insect and spider species. This allometry was stronger in internal genital structures than it was in external genitalia. We also found that there was more variation in internal compared with external genitalia. Finally, we found that the shape of genital structures differed between morphs in all three examined areas. *S. tetralunaria* is among the first reported cases of genitally dimorphic insect species. Considerable variation in internal genitalia and especially the presence of genital shape differences between morphs were not consistent with the predictions of the lock-and-key hypothesis. (c) 2006 The Linnean Society of London, Biological Journal of the Linnean Society, 2006, 87, 297-307.

Genetic differences between rice and water-oat feeders in the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera : Crambidae)

Ishiguro, N; Yoshida, K; Tsuchida, K. 2006

The rice stem borer, *Chilo suppressalis* (Walker) is an important lepidopteran pest of rice plants in Japan, and feeds on both rice (*Oryza sativa*) and water-oats (*Zizania latifolia*). We evaluated the difference in mating time of the two feeders under both laboratory and field conditions. The male moth of the water-oat feeders (WF) began to mate 7 h after the beginning of the scotophase, about 4 h later than the peak of mating of the rice feeders (RF). In accordance with this result, the body size of males trapped using synthetic sex pheromones differed between the two trap periods (sunset to 1:00 am vs. 1:00 am to sunrise) in the field, suggesting that the rice feeders were trapped earlier than the water-oat feeders. However, there was no unequivocal evidence showing limited gene flow between the two feeders at the level of allozyme polymorphisms. These results suggest that as the two feeders of *C. suppressalis* have recently diverged, the allozyme loci have not yet diverged to fixation.

Mate recognition and reproductive isolation in the sibling species *Spodoptera littoralis* and *Spodoptera litura*

Saveer, AM; Becher, PG; Birgersson, G; Hansson, BS; Witzgall, P; Bengtsson, M. 2014

Mate recognition is crucial for reproductive isolation and for maintaining species integrity. Chemosensory-mediated sexual communication with pheromones is an essential component of mate recognition in moths. Confronted with sex pheromone stimuli released from conspecific and closely related heterospecific females, which partially overlap in chemical composition, male moths are under strong selection to recognize compatible mates. Here, we investigated the role of pheromone signals in premating communication in the sibling species *Spodoptera littoralis* and *S. litura* (Lepidoptera, Noctuidae). Further, we measured the reproductive consequence of conspecific vs. heterospecific matings. Both species use Z9,E11-14:Ac as the major pheromone compound, and the 11-component blend found in pheromone glands of *S. littoralis* comprises the compounds found in *S. litura*. Accordingly, *S. littoralis* and *S. litura* males readily responded to conspecific and heterospecific calling females in no-choice behavioral tests. In contrast, in a dual-choice test, *S. littoralis* males choose conspecific calling females, whereas *S. litura* males did not discriminate between conspecific and heterospecific females. In *S. littoralis* females, heterospecific matings had a negative fitness effect as compared

to conspecific matings. Female longevity, egg-laying and hatching of larvae were significantly reduced by matings with heterospecific males. Reciprocal crossings, between *S. litura* females and *S. littoralis* males, were prevented by genital morphology, which is consistent with reduced heterospecific attraction of *S. littoralis* males in a dual-choice assay. On the other hand, matings between *S. littoralis* females and *S. litura* males, under a no-choice situation, show that interspecific matings occur in zones of geographical overlap and corroborate the idea that mate quality, in these closely related species, is a continuous and not a categorical trait.

Reproductive isolation and differentiation between five populations of *Cotesia plutellae* (Hymenoptera : Braconidae), parasitoid of *Plutella xylostella* (Lepidoptera : Plutellidae)

Rincon, C; Bordat, D; Lohr, B; Dupas, S. 2006

Many taxa used in biological control may be composed of several population units reproductively isolated to various degrees. Yet, the introductions of non-compatible populations may result in a depression of intended biological control. *Cotesia plutellae* has been widely used as a biological control agent of the diamondback moth, *Plutella xylostella*. However, not all introductions and augmentations were successful. With the aim to develop markers of compatibility for this species, we studied the reproductive compatibility between five populations (South Africa, Benin, Martinique, Reunion, and Taiwan) and analyzed its statistical relationship with three morphological characters (antennae and body length and antennae/body ratio), one molecular character (sequence of a cytochrome b fragment) and the *Wolbachia* infection status. This study shows that *C. plutellae* populations formed two interbreeding groups. The antenna/body length ratio of females, the ratio between male and female body length, one non-silent base pair substitution observed in cytochrome b sequences, and *Wolbachia* infection status differentiate these groups and were all good predictors of reproductive compatibility. Our results suggest also that genetic factors independent from *Wolbachia* are needed to fully explain reproductive isolation between *C. plutellae* populations. We conclude that *C. plutellae* is a single species composed of at least two partially incompatible population aggregates. Diagnostic markers were proposed for the prediction of the level of reproductive compatibility between populations which can be used for further biocontrol introductions. (c) 2005 Elsevier Inc. All rights reserved.

Morphometric differences between rice and water-oats population of the striped stem borer moth, *Chilo suppressalis* (Lepidoptera : Crambidae)

Matsukura, K; Hoshizaki, S; Ishikawa, Y; Tatsuki, S. 2006

The striped stem borer moth, *Chilo suppressalis*, has two major host plants; rice, *Oryza sativa*, and the water-oats, *Zizania latifolia*. It has been suggested that there is reproductive isolation between the populations feeding on rice (rice population) and water-oats (water-oats population). We compared the morphological characteristics of the two populations using multi variate analyses based on eight genital and ten non-genital traits in male adults. Body size differed between the two populations as shown previously. However the results of a principal component analysis (PCA) based on genital traits showed a proportional difference between the two populations, but not on the non-genital traits. This shows the existence of a morphological difference in the genitalia of male adults between the populations. We also conducted a discriminant function analysis (DFA) based on seven genital and five non-genital traits. The discriminant function obtained could classify the two populations with 94.3% accuracy. Another discriminant function using only four genital traits also could distinguish the two populations with 88.6% accuracy. DFA would be of use in the practical forecasting of occurrence.

Odorant receptor phylogeny confirms conserved channels for sex pheromone and host plant signals in tortricid moths

Gonzalez, F; Borrero-Echeverry, F; Josvai, JK; Strandh, M; Unelius, CR; Toth, M; Witzgall, P; Bengtsson, M; Walker, WB. 2020

The search for mates and food is mediated by volatile chemicals. Insects sense food odorants and sex pheromones through odorant receptors (ORs) and pheromone receptors (PRs), which are expressed in olfactory sensory neurons. Molecular phylogenetics of ORs, informed by behavioral and functional data, generates sound hypotheses for the identification of semiochemicals driving olfactory behavior. Studying orthologous receptors and their ligands across taxa affords insights into the role of chemical communication in reproductive isolation and phylogenetic divergence. The female sex pheromone of green budworm moth *Hedya nubiferana* (Lepidoptera, Tortricidae) is a blend of two unsaturated acetates, only a blend of both elicits male attraction. Females produce in addition codlemone, which is the sex pheromone of another tortricid, codling moth *Cydia pomonella*. Codlemone also attracts green budworm moth males. Concomitantly, green budworm and codling moth males are attracted to the host plant volatile pear ester. A congruent behavioral response to the same pheromone and plant volatile in two tortricid species suggests co-occurrence of dedicated olfactory channels. In codling moth, one PR is tuned to both compounds, the sex pheromone codlemone and the plant volatile pear ester. Our phylogenetic analysis finds that green budworm moth expresses an orthologous PR gene. Shared ancestry, and high levels of amino acid identity and sequence similarity, in codling and green budworm moth PRs offer an explanation for parallel attraction of both species to the same compounds. A conserved olfactory channel for a sex pheromone and a host plant volatile substantiates the alliance of social and habitat signals in insect chemical communication. Field attraction assays confirm that in silico investigations of ORs afford powerful predictions for an efficient identification of behavior-modifying semiochemicals, for an improved understanding of the mechanisms of host plant attraction in insect herbivores and for the further development of sustainable insect control.

GEOGRAPHIC AND HOST-ASSOCIATED DIFFERENTIATION IN THE FRUITTREE LEAFROLLER (LEPIDOPTERA, TORTRICIDAE)

GOYER, RA; PAINE, TD; PASHLEY, DP; LENHARD, GJ; MEEKER, JR; HANLON, CC. 1995

The fruittree leafroller, *Archips argyrospila* (Walker), has recently reached outbreak densities on a newly recorded host plant, baldcypress, *Taxodium distichum* (L.) Richard. We conducted a genetic analysis of the cypress population and one feeding on oaks in Louisiana. To obtain a geographic perspective on differentiation within the fruittree leafroller, we compared the Louisiana cypress population with a California oak population genetically and conducted cross-rearing studies on oak and cypress. A citrus population from California was also included for a host-associated perspective. Genetic data suggest the existence of three distinct taxa. One taxon feeds on oaks and citrus in California, one feeds on oaks in Louisiana, and a third feeds on baldcypress in Louisiana. Developmental cross-rearing studies indicate the cypress population is virtually unable to survive on Louisiana oaks, further supporting the existence of host-associated differentiation. Pheromone attraction studies conducted in Louisiana forests with California citrus and Louisiana cypress virgin females indicate California citrus females are not attractive to male fruittree leafrollers in cypress swamps. Differences in pheromonal response suggest evolutionary divergence in mating behavior on a geographic scale. Further studies are required to determine the degree of reproductive isolation between host associated and geographic populations, but genetic, developmental, and pheromonal studies suggest sibling species or host races, or both, exist.

Inheritance and evolution of male response to sex pheromone in *Trichoplusia ni* (Lepidoptera : Noctuidae)

Evenden, ML; Spohn, BG; Moore, AJ; Preziosi, RF; Haynes, KF. 2002

The evolution of chemical communication in moths has implications for speciation because mate attraction and reproductive isolation are achieved primarily by unique blends of female-produced sex pheromones and the specificity of male responses to these signals. For most organisms, the genetics of speciation remains an open empirical question, but both major gene effects and additive genetic variation are likely to play roles in the diversification of chemical communication that accompanies speciation. In the cabbage looper, *Trichoplusia ni* (Hubner), a single gene mutation results in females that produce a drastically different signal. We conducted four wind-tunnel experiments to examine the potential role of additive genetic variation in the evolution of male *T. ni* response to this novel pheromone

blend. The heritability of male response to both normal and mutant pheromone signals was tested in two experiments using a father-son regression design. Normal male response to the mutant blend showed a heritability of 0.25. The heritability of normal male response to the normal blend and the mutant male responses to both normal and mutant blends were at or near 0. In a third experiment, we imposed selection on normal male lines for increased response to the mutant pheromone blend. After three generations of selection, normal male response had increased by 24% as compared to only 5% in control lines, confirming the heritability value determined earlier. A fourth experiment showed that the change in the selected lines was due to an improved male response to both the normal and mutant signals.

Spodoptera frugiperda (Lepidoptera: Noctuidae) host-plant variants: two host strains or two distinct species?

Dumas, P; Legeai, F; Lemaitre, C; Scaon, E; Orsucci, M; Labadie, K; Gimenez, S; Clamens, AL; Henri, H; Vavre, F; Aury, JM; Fournier, P; Kergoat, GJ; d'Alencon, E. 2015

The moth *Spodoptera frugiperda* is a well-known pest of crops throughout the Americas, which consists of two strains adapted to different host-plants: the first feeds preferentially on corn, cotton and sorghum whereas the second is more associated with rice and several pasture grasses. Though morphologically indistinguishable, they exhibit differences in their mating behavior, pheromone compositions, and show development variability according to the host-plant. Though the latter suggest that both strains are different species, this issue is still highly controversial because **hybrids** naturally occur in the wild, not to mention the discrepancies among published results concerning mating success between the two strains. In order to clarify the status of the two host-plant strains of *S. frugiperda*, we analyze features that possibly reflect the level of post-zygotic isolation: (1) first generation (F1) **hybrid** lethality and sterility; (2) patterns of meiotic segregation of **hybrids** in reciprocal second generation (F2), as compared to the meiosis of the two parental strains. We found a significant reduction of mating success in F1 in one direction of the cross and a high level of microsatellite markers showing transmission ratio distortion in the F2 progeny. Our results support the existence of post-zygotic reproductive isolation between the two laboratory strains and are in accordance with the marked level of genetic differentiation that was recovered between individuals of the two strains collected from the field. Altogether these results provide additional evidence in favor of a sibling species status for the two strains.

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

Hillier, NK; Vickers, NJ. 2011

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

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

Ohshima, I; Yoshizawa, K. 2010

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

CHEMICALLY MEDIATED REPRODUCTIVE ISOLATION BETWEEN CABBAGE-LOOPER AND SOYBEAN LOOPER MOTHS (LEPIDOPTERA, NOCTUIDAE)

LEPPLA, NC. 1983

Specialized androconial scales conceal species-specific semiochemicals of sympatric sulphur butterflies (Lepidoptera: Pieridae: Coliadinae)

Nobre, CEB; Lucas, LAD; Padilha, RJR; Navarro, DMDF; Alves, LC; Maia, ACD. NA

Chemical cues play an important role in short-range communication of butterflies, remarkably in sexual attraction and mate choice. Differentiated scale patches on the wings of male butterflies, the androconia, are involved in the emission of pheromones. Here, we describe the androconial morphology of six sympatric species of Neotropical sulphur butterflies belonging to two genera of the *Colias*-clade (Pieridae) based on SEM imaging. Gas chromatography-mass spectrometry analyses were used to access the chemical compositions of androconial secretions, which were comparatively investigated to determine species-specific trends and to verify if they yield a phylogenetic signal. The androconial patches from all species are differentiated from the non-androconial male wing surface and exhibit morphological features that may act in both preventing the volatilization of secretions and facilitating the release of semiochemicals, such as high density and length of scales and large perforations in the upper lamellae. A total of 55 compounds were exclusive to the androconia, and unique chemical profiles are present in each butterfly species, verified through multivariate analysis. The majority of androconial compounds were autapomorphic for each species and only four were dominant in more than one species. Cluster analyses placed the two species of *Anteos* in a single clade, but otherwise evidenced low similarities in the androconial secretion compositions among species, and a moderate correlation between genetic distances and chemical dissimilarities was obtained. Our findings suggest that androconial substances are involved in mating-oriented strategies and might be associated with the evolutionary history of the reproductive isolation of sulphurs.

Occurrence of Interspecific Mating between Two Species of *Danaus* & *IT* Kluk, 1780 (Lepidoptera: Nymphalidae) in Nature

Baidya, S; Basu, DN; Roy, S; Roy, AB. 2018

Mating across species occurs rarely in nature, which contends prevalent idea of biological species concept. Throughout species range, mating pattern varies and reproductive barriers are also not fixed among different species. In this study, two instances of interspecific mating between two widely common Nymphalid Tiger butterflies (*Danaus kluk*, 1780) in Indian region are reported. Observations imply lack of absolute reproductive barriers, where possible interplay exists among prezygotic and postzygotic isolating factors compensating each other in order to restrain interspecific **hybridization**.

Experimental evidence of host race formation in *Mitoura* butterflies (Lepidoptera: Lycaenidae)

Downey, MH; Nice, CC. 2011

A population of herbivorous insects that shifts to a novel host can experience selection pressures that result in adaptation to the new resource. Host race formation, considered an early stage of the speciation process, may result. The current study investigates host shifts and variation in traits potentially involved in the evolution of reproductive isolation among populations of the juniper hairstreak butterfly, *Mitoura gryneus*. *Mitoura* are closely associated with their host trees (Cupressaceae) and exhibit host plant fidelity: in addition to larval development and oviposition, host trees support male leks and mating. Female oviposition preference for the natal host, and differential fitness of larvae when reared on natal versus alternate hosts, are indications that specialization and local adaptation to the natal host plant are occurring. Populations with single host plant associations (*Juniperus ashei*, *J. pinchotii* and *J. virginiana*) as well as populations with multiple hosts (both *J. ashei* and *J. pinchotii*) were examined. Concordance between female preference and larval performance was found for *J. ashei*-associated populations. Population-level variation in the patterns of female preference and larval performance, both within and among host associations, may reflect differences in the timing and direction of colonization of hosts. For a single nominal species that otherwise exhibits no morphological or phenological differences, the experimental assessment of specialization and host fidelity in *M. gryneus* provides strong support for the hypothesis of ongoing host race formation in these butterflies.

Hairstreak butterflies (Lepidoptera, Lycaenidae) and evolution of their male secondary sexual organs

Martins, ARP; Duarte, M; Robbins, RK. 2019

Hairstreak butterflies in the Atlides Section of the Eumaeini are biologically notable for a diverse array of male secondary sexual organs. A "species recognition" hypothesis postulates that females use these organs to choose between conspecific and non-conspecific males, thereby promoting reproductive isolation. Alternately, a "sexual selection" hypothesis posits that females use these organs to choose among conspecific males. These hypotheses need not be mutually exclusive but make different predictions about the evolutionary gain and loss of male secondary sexual organs. We analysed most of the Atlides Section (Theclinae, Eumaeini) phylogenetically. Sister lineages were sympatric at 22 of 37 nodes. Nine evolutionary gains occurred in lineages that were sympatric with their phylogenetic sister, and one occurred in a lineage that was allopatric/parapatric with its sister. By contrast, seven of ten evolutionary losses occurred in lineages that were allopatric/parapatric with their sisters. These results are significantly different from those predicted by a sexual selection hypothesis. We conclude that male secondary sexual organs in the Atlides Section function primarily for species recognition and thereby promote sympatric diversification. (c) The Willi Hennig Society 2018.

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

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

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

Female mate choice determines reproductive isolation between sympatric butterflies

Friberg, M; Vongvanich, N; Borg-Karlson, AK; Kemp, DJ; Merilaita, S; Wiklund, C. 2008

Animal courtship rituals are important for species recognition, and a variety of cues might be utilized to recognize conspecific mates. In this paper, we investigate different species-recognition mechanisms between two sympatric butterfly sister species: the wood white (*Leptidea sinapis*) and Real's wood white (*Leptidea reali*). We show that males of both species frequently court heterospecific females both under laboratory and field conditions. The long-lasting elaborate courtships impose energetic costs, since the second courtship of males that were introduced to two subsequent conspecific females lasted on average only one fourth as long as the first courtship. In this paper, we demonstrate that premating reproductive isolation is dependent on female unwillingness to accept heterospecific mates. We studied female and male courtship behavior, chemical signaling, and the morphology of the sexually dimorphic antennae, one of the few male traits visible for females during courtship. We found no differences in ultraviolet (UV) reflectance and only small differences in longer wavelengths and brightness, significant between-species differences, but strongly overlapping distributions of male *L. sinapis* and *L. reali* antennal morphology and chemical signals and minor differences in courtship behavior. The lack of clear-cut between-species differences further explains the lack of male species recognition, and the overall similarity might have caused the long-lasting elaborate courtships, if females need prolonged male courtships to distinguish between con- and heterospecific suitors.

Sex attractant for *Izatha peroneanella* (Walker) (Lepidoptera: Oecophoridae sensu lato), a lichen tuft moth

Twidle, AM; Clare, GK; Stanbury, ME; El-Sayed, AM. 2014

The first sex attractant for the genus *Izatha* is reported as (Z)-7-tetradecen-1-yl acetate in *I. peroneanella*. A trapping trial for tortricid moths in New Zealand forests resulted in the serendipitous capture of 310 male lichen tuft moths, *I. peroneanella*, to this compound which is presumed to be part of the sex pheromone. The inhibitory response from the addition of saturated tetradecyl acetate suggests that this compound may play a role in the reproductive isolation of *I. peroneanella* from related species.

Calling Behavior, Copulation Time, and Reproductive Compatibility of Corn-Strain Fall Armyworm (Lepidoptera: Noctuidae) From Populations in Mexico

Cruz-Esteban, S; Rojas, JC; Malo, EA. 2017

The calling behavior, mating time, and the reproductive compatibility of virgin adults of fall armyworms, *Spodoptera frugiperda* (J. E. Smith), were studied in this work. Larvae were collected on maize (*Zea mays* L.) from six states located on the Pacific coast (Chiapas, Michoacan, and Sinaloa), on the Gulf of Mexico (Veracruz and Yucatan), and in central Mexico (Morelos). Before the experiments, insects were reared under laboratory conditions for one generation. We recorded the age at which females called for the first time, the onset time of calling, the duration of calling, the onset time of copulation, and the duration of copulation. The calling rhythms of the six populations were dissimilar. Females from all populations began to call in the second or third scoto-phase. The time for onset of calling and the duration of calling were significantly different among the *S. frugiperda* populations studied. *Spodoptera frugiperda* pairs from Sinaloa, Veracruz, Yucatan, and Morelos started to copulate earlier than the pairs from Chiapas and Michoacan. Pairs from Veracruz and Yucatan copulated longer than those from Michoacan, Morelos, Chiapas, and Sinaloa. Our crossing experiment using females and males from the six populations showed that individuals from different populations could copulate and produce fertile offspring. Thus, although the *S. frugiperda* populations showed variability in the timing of reproduction, the populations were not reproductively incompatible, which indicated that geographic distance has not led to reproductive isolation in corn-strain populations of *S. frugiperda* in Mexico.

Reproductive isolation between two populations of *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) from different host plant species and regions in Argentina

Fogliata, SV; Vera, A; Gastaminza, G; Cuenya, MI; Zucchi, MI; Willink, E; Castagnaro, AP; Murua, MG. 2016

The sugarcane borer, *Diatraea saccharalis* (F.), has a widespread distribution throughout the Western Hemisphere and is a pest of many crop plants including sugarcane, corn, sorghum and rice. The use of *Bacillus thuringiensis* (Bt) corn has been the primary tool for managing this species in corn fields. Sugarcane borer control has been recently threatened by observations of susceptibility and/or resistance to certain varieties of Bt corn and the protein used in many newer varieties. This has led to increased interest in understanding sugarcane borer genetic diversity and gene flow within and among its populations and the consequent exchange of alleles between geographically distant populations. The objective of this study was to examine reproductive compatibility between host-associated geographic populations of *D. saccharalis* in Argentina and to determine whether this pest represents a complex of host-associated cryptic species rather than a wide ranging generalist species. Intra and inter-population crosses revealed that *D. saccharalis* populations from the northwestern and Pampas regions presented evidence of prezygotic and postzygotic incompatibility. Such a result is likely to be the product of an interruption of gene flow produced by either geographic or host plant associated isolation, suggesting that Tucuman (northwestern) and Buenos Aires (Pampas) populations of *D. saccharalis* are a distinct genotype and possibly an incipient species.

SEX-PHEROMONES AND REPRODUCTIVE ISOLATION IN 4 EUROPEAN SMALL ERMINE MOTHS (LEPIDOPTERA-YPONOMEUTIDAE LATR)

LOFSTEDT, C; VANDERPER, JNC. 1985

Differentiation in putative male sex pheromone components across and within populations of the African butterfly *Bicyclus anynana* as a potential driver of reproductive isolation

Bacquet, PMB; de Jong, MA; Brattstrom, O; Wang, HL; Molleman, F; Heuskin, S; Lognay, G; Lofstedt, C; Brakefield, PM; Vanderpoorten, A; Nieberding, CM. 2016

Sexual traits are often the most divergent characters among closely related species, suggesting an important role of sexual traits in speciation. However, to prove this, we need to show that sexual trait differences accumulate before or during the speciation process, rather than being a consequence of it. Here, we contrast patterns of divergence among putative male sex pheromone (pMSP) composition and the genetic structure inferred from variation in the mitochondrial cytochrome oxidase 1 and nuclear CAD loci in the African butterfly *Bicyclus anynana* (Butler, 1879) to determine whether the evolution of "pheromonal dialects" occurs before or after the differentiation process. We observed differences in abundance of some shared pMSP components as well as differences in the composition of the pMSP among *B. anynana* populations. In addition, *B. anynana* individuals from Kenya displayed differences in the pMSP composition within a single population that appeared not associated with genetic differences. These differences in pMSP composition both between and within *B. anynana* populations were as large as those found between different *Bicyclus* species. Our results suggest that "pheromonal dialects" evolved within and among populations of *B. anynana* and may therefore act as precursors of an ongoing speciation process.

Reproductive isolation and patterns of genetic differentiation in a cryptic butterfly species complex

Dinca, V; Wiklund, C; Lukhtanov, VA; Kodandaramaiah, U; Noren, K; Dapporto, L; Wahlberg, N; Vila, R; Friberg, M. 2013

Molecular studies of natural populations are often designed to detect and categorize hidden layers of cryptic diversity, and an emerging pattern suggests that cryptic species are more common and more widely distributed than previously thought. However, these studies are often decoupled from ecological and behavioural studies of species divergence. Thus, the mechanisms by which the cryptic diversity is distributed and maintained across large spatial scales are often unknown. In 1988, it was discovered that the common Eurasian Wood White butterfly consisted of two species (*Leptidea sinapis* and *Leptidea reali*), and the pair became an emerging model for the study of speciation and chromosomal evolution. In 2011, the existence of a third cryptic species (*Leptidea juvernica*) was proposed. This unexpected discovery raises questions about the mechanisms preventing gene flow and about the potential existence of additional species hidden in the complex. Here, we compare patterns of genetic divergence across western Eurasia in an extensive data set of mitochondrial and nuclear DNA sequences with behavioural data on inter- and intraspecific reproductive isolation in courtship experiments. We show that three species exist in accordance with both the phylogenetic and biological species concepts and that additional hidden diversity is unlikely to occur in Europe. The *Leptidea* species are now the best studied cryptic complex of butterflies in Europe and a promising model system for understanding the formation of cryptic species and the roles of local processes, colonization patterns and heterospecific interactions for ecological and evolutionary divergence.

Sex pheromone of *Ostrinia* sp newly found on the leopard plant *Farfugium japonicum*

Tabata, J; Huang, Y; Ohno, S; Yoshiyasu, Y; Sugie, H; Tatsuki, S; Ishikawa, Y. 2008

Recently, larvae of *Ostrinia* were found feeding on the leopard plant *Farfugium japonicum* (Asteraceae), previously unrecorded as a host plant of this genus. The adult moths that developed from these borers were morphologically similar to, but distinct from, *Ostrinia zaguliaevi*, a monophagous species specialized for feeding on another Asteraceae plant, the butterbur *Petasites japonicus*. Although the taxonomical status of the moth feeding on *F. japonicum* is to be determined, distinct morphological differences in the adults strongly suggest this to be a new species (hereafter referred to as *O. sp.*). To gain an insight into the reproductive isolation between *O. sp.* and other members of the genus *Ostrinia*, the female sex pheromone and the males' response to it were investigated using samples collected from *F. japonicum*. (Z)-9-tetradecenyl acetate (Z9-14:OAc), (Z)-11-tetradecenyl acetate (Z11-14:OAc), (E)-11-tetradecenyl acetate (E11-14:OAc), tetradecyl acetate, and (Z)-11-hexadecenyl acetate were identified as candidates for sex pheromone components by analyses using gas chromatographs coupled to a mass spectrometer (GC-MS) and electroantennographic detector (GC-EAD). A series of bioassays of male responses in a wind-tunnel and a field cage indicated that the former three compounds

are essential for attracting males, and the latter two have no synergistic effect on the attraction. We therefore concluded that Z9-14:OAc, Z11-14:OAc and E11-14:OAc are the sex pheromone components of *O. sp.* Although the same three compounds are used as the sex pheromone components of *O. zaguliaevi* and another congener, *Ostrinia zealis*, the blend proportions differed greatly among the three (Z9-14:OAc/Z11-14:OAc/E11-14:OAc = 18/76/6 in *O. sp.*, 45/50/5 in *O. zaguliaevi* and 70/6/24 in *O. zealis*). Differences in sex pheromones could contribute to the reproductive isolation between *O. sp.* and the other two *Ostrinia* species if males of each species exhibit a narrow window of response to their own blend ratio.

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

Ohshima, I. 2010

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

Canas-Hoyos, N; Marquez, EJ; Saldamando-Benjumea, CI. 2014

Spodoptera frugiperda is an important pest throughout the Western Hemisphere, particularly in corn, cotton, rice, and sorghum, among other crops. This insect has evolved two strains named the corn and the rice strains, as they have been their most frequently used hosts. However, the corn strain can also be found in cotton and the rice strain in pasture grasses and corn. Both strains have been identified by using different molecular markers, including allozymes, polymerase chain reaction-restriction fragment-length polymorphism, amplified fragment-length polymorphism, and FR tandem repeat unit, among others. So far, all reports have claimed no morphological differences between the strains. However, none of these studies have used geometric morphometrics, as we performed in this work. We applied wing morphometrics to analyze wing size and shape in 159 individuals of the corn and the rice strains reared under laboratory conditions since 2009 and already genotyped. Our results demonstrated that wing morphometrics is a promising tool to differentiate both strains, as they were statistically significant in wing shape. We also found that this species presents sexual dimorphism in the rice strain in wing shape and that wing size differs between males of both strains. Further studies will require the use of this new tool in field populations, as their identification is relevant, given that they exhibit reproductive isolation and differences in susceptibility to chemical and biological controls, suggesting they are in the process of speciation via host plant association.

The pheromone of the eastern tent caterpillar, *Malacosoma americanum* (F) (Lepidoptera, Lasiocampidae)

Kochansky, J; Hill, A; Neal, JW; Bentz, JA; Roelofs, W. 1996

The pheromone system of the eastern tent caterpillar, *M. americanum*, has been identified as a mixture of (E,Z)-5,7-dodecadial and the corresponding alcohol. Field data on the attractiveness of the aldehyde alone were not consistent, but mixtures of aldehyde and alcohol in varying proportions were attractive to males. Addition of small amounts of E,Z acetate to E,Z aldehyde had no effect on male response, but larger amounts reduced trap catch. Traps baited with Z,E, E,E, or Z,Z aldehydes were not more attractive than blank traps. Pherocon 1C traps fortified with extra adhesive and baited with lures consisting of 500 µg (E,Z)-5,7-dodecadial with either 250 or 100 µg of the corresponding alcohol trapped as many as 100 males/trap/night with means of 15-20. Lures prepared from purified (94% E,Z) aldehyde and alcohol were more attractive than those prepared from unpurified (58% E,Z) materials.

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

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

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

Species specificity and intraspecific variation in the chemical profiles of *Heliconius* butterflies across a large geographic range

Darragh, K; Montejo-Kovacevich, G; Kozak, KM; Morrison, CR; Figueiredo, CME; Ready, JS; Salazar, C; Linares, M; Byers, KJRP; Merrill, RM; McMillan, WO; Schulz, S; Jiggins, CD. 2020

In many animals, mate choice is important for the maintenance of reproductive isolation between species. Traits important for mate choice and behavioral isolation are predicted to be under strong stabilizing selection within species; however, such traits can also exhibit variation at the population level driven by neutral and adaptive evolutionary processes. Here, we describe patterns of divergence among androconial and genital chemical profiles at inter- and intraspecific levels in mimetic *Heliconius* butterflies. Most variation in chemical bouquets was found between species, but there were also quantitative differences at the population level. We found a strong correlation between interspecific chemical and genetic divergence, but this correlation varied in intraspecific comparisons. We identified "indicator" compounds characteristic of particular species that included compounds already known to elicit a behavioral response, suggesting an approach for identification of candidate compounds for future behavioral studies in novel systems. Overall, the strong signal of species identity suggests a role for these compounds in species recognition, but with additional potentially neutral variation at the population level.

EFFECT OF TEMPORAL FACTORS ON REPRODUCTIVE ISOLATION BETWEEN CHORISTONEURA-OCCIDENTALIS AND CHORISTONEURA-RETINIANA (LEPIDOPTERA, TORTRICIDAE)

LIEBHOLD, AM; VOLNEY, WJA. 1984

GENETIC-EVIDENCE FOR REPRODUCTIVE ISOLATION BETWEEN HYBRIDIZING LIMENITIS BUTTERFLIES (LEPIDOPTERA, NYMPHALIDAE) IN SOUTHWESTERN NEW-MEXICO

PORTER, AH. 1989

Specificity of communication channels in four limacodid moths: *Darna bradleyi*, *Darna trima*, *Setothosea asigna*, and *Setora nitens* (Lepidoptera : Limacodidae)

Sasaerila, Y; Gries, G; Gries, R; Boo, TC. 2000

Darna bradleyi Holloway, *D. trima* Moore, *Setothosea asigna* van Eecke and *Setora nitens* Walker are sympatric and coseasonal limacodid moths in plantations of oil palm, *Elaeis guineensis* Jacq. (Arecales: Palmae), in Borneo, southeast Asia. We tested the hypothesis that these four species maintain reproductive isolation through specificity in diel periods of communication, microlocation for communication and/or communication signal (pheromone). Studying diel periodicity of calling behavior by female moths and response by male moths to traps baited with virgin females or synthetic pheromone, we determined that sexual communication of *D. bradleyi* and *D. trima* took place from similar to 17:30 to 18:45 hr and that of *S. asigna* and *S. nitens* from similar to 18:45 to 20:00 hr and from similar to 18:30 to 19:30 hr, respectively. Over 80% of male *S. asigna* and *S. nitens* were captured in pheromone-baited traps suspended >5 m high, whereas male *D. bradleyi* and *D. trima* were captured mostly in traps < 5 m high. Synthetic pheromone baits attracted male moths in a species-specific manner. Moreover, baits containing both *S. asigna* and *S. nitens* pheromones failed to attract any male moths, indicating that female *S. asigna* and *S. nitens*, with overlapping communication periods, use bifunctional pheromone components that attract conspecific males while repelling heterospecifics. Similarly, addition of *D. bradleyi* pheromone to *S. asigna* or *S. nitens* pheromone reduced attraction of male *S. asigna* and *S. nitens*. The failure of *D. bradleyi* and *D. trima*, which overlap in time and microlocation for communication, to evolve bifunctional pheromones may be attributed to the recent occurrence of sympatry between *D. bradleyi* and *D. trima* in Borneo, apparently too recent for bifunctional pheromones to have evolved. We conclude that *D. bradleyi*, *D. trima*, *S. asigna* and *S. nitens* utilize any or all of diel periodicity, intra and interspecific effects of communication signal and/or microlocation for signaling, allowing these limacodids to co-inhabit the same habitat and remain reproductively isolated.

Variability in Pheromone Communication Among Different Haplotype Populations of *Busseola fusca*

Felix, AE; Genestier, G; Malosse, C; Calatayud, PA; Le Ru, B; Silvain, JF; Frerot, B. 2009

The relationship between pheromone composition and mitochondrial haplotype clades was investigated by coupling DNA analyses with pheromone identification and male mate searching behavior among different geographic populations of *Busseola fusca*. The within-population variations in pheromone blend were as great as those observed between geographic populations, suggesting that the female sex pheromone blend was not the basis of reproductive isolation between the geographic clades. Furthermore, while data from wind tunnel experiments demonstrated that most of the tested males were sensitive to small variations in pheromone mixture, there was considerable within-population variability in the observed response. The study identified a new pheromone component, (Z)-11-hexadecen-1-yl acetate, which when added to the currently used three-component synthetic blend resulted in significantly higher traps catches. The new recommended blend for monitoring flight phenology and for timing control measures for optimal efficacy of *B. fusca* is (Z)-11-tetradecen-1-yl acetate (62%), (E)-11-tetradecen-1-yl acetate (15%), (Z)-9-tetradecen-1-yl acetate (13%), and (Z)-11-hexadecen-1-yl acetate (10%).

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

Scriber, JM. 2002

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

Analyses of lepidopteran sex pheromones by mass spectrometry

Ando, T; Yamakawa, R. 2011

Lepidoptera, including about 150,000 species in the world, comprise the second largest insect group, and sex pheromones have been identified from virgin female moths of more than 600 species. The chemical structures are simple, but diverse, because species-specific pheromones play an important role in the reproductive isolation of each species. The pheromone content in each female is quite low, and gas chromatography coupled to mass spectrometry (GC-MS) is most frequently utilized to reveal the chemical structure. Almost all pheromone components are straight-chain compounds and are classified into two major groups [i.e. unsaturated C-10-C-18 fatty alcohols and their derivatives (Type I) and C-17-C-23 polyenyl hydrocarbons and their epoxides (Type II)]. In addition to the unbranched compounds, some species secrete methyl-branched compounds (e.g., 2-ketones). For the identification of these compounds, determining the positions of the double bond, the epoxy ring, and the methyl group is an important key step. Copious spectral information measured by electron-impact ionization (70 eV) has been accumulated for these compounds. This review therefore deals with their spectral characteristics, namely, diagnostic ions, to apply them to pheromone studies on new target insects. (C) 2011 Elsevier Ltd. All rights reserved.

INHERITANCE OF ALLOZYMES IN YPONOMEUTA (LEPIDOPTERA) .2. INTERSPECIFIC CROSSES WITHIN THE PADELLUS-COMPLEX AND REPRODUCTIVE ISOLATION

MENKEN, SBJ. 1980

Male sex pheromone components in *Heliconius* butterflies released by the androconia affect female choice

Darragh, K; Vanjari, S; Mann, F; Gonzalez-Rojas, MF; Morrison, CR; Salazar, C; Pardo-Diaz, C; Merrill, RM; McMillan, WO; Schulz, S; Jiggins, CD. 2017

Sex-specific pheromones are known to play an important role in butterfly courtship, and may influence both individual reproductive success and reproductive isolation between species. Extensive ecological, behavioural and genetic studies of *Heliconius* butterflies have made a substantial contribution to our understanding of speciation. Male pheromones, although long suspected to play an important role, have received relatively little attention in this genus. Here, we combine morphological, chemical and behavioural analyses of male pheromones in the Neotropical butterfly *Heliconius melpomene*. First, we identify putative androconia that are specialized brush-like scales that lie within the shiny grey region of the male hindwing. We then describe putative male sex pheromone compounds, which are largely confined to the androconial region of the hindwing of mature males, but are absent in immature males and females. Finally, behavioural choice experiments reveal that females of *H. melpomene*, *H. erato* and *H. timareta* strongly discriminate against conspecific males which have their androconial region experimentally blocked. As well as demonstrating the importance of chemical signalling for female mate choice in *Heliconius* butterflies, the results describe structures involved in release of the pheromone and a list of potential male sex pheromone compounds.

Species and mate recognition in two sympatric Grayling butterflies: *Hipparchia fagi* and *H. hermione genava* (Lepidoptera)

Pinzari, M; Sbordoni, V. 2013

The main aim of this study was to investigate and compare the patterns of male courtship behaviour in *Hipparchia fagi* (Woodland Grayling) and *H. hermione genava* (Rock Grayling) in order to determine if premating barriers are involved in the reproductive isolation between the two sympatric and syntopic species. Although they show a similar pattern of sexual behaviour (perching, flight pursuit, courtship), there were significant quantitative inter-specific differences in the step Bowing. During this step, the male bashes and strokes the female antennae between his forewings on the androconial scales revealing the importance of scent stimuli in mate communication. Moreover, different signals, spanning several sensory modalities (visual, chemical, tactile stimuli) and spatio-temporal scales (during the pair flight and the courtship step), might serve as premating barriers between the two species. In particular, both males and females of *H. fagi* (a woodland habitat specialist) seem to take less time in recognising their conspecific partner, while *H. hermione* males need to spend more time in courting (and identifying) the right females.

Mating time of the rice-feeding and water-oat-feeding strains of the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae).

Konno, Y; Tsukuba, K; Tanaka, F. 1996

The Rice Stem Borer, *Chilo suppressalis* (WALKER), is a serious pest of rice plants in Asia. In Japan, host plants of are mainly rice plants (*Oryza sativa*) and the water-oat (*Zizania latifolia*). Our previous study indicated significant differences in insecticide susceptibility and esterase isozyme patterns between the rice-feeding and water-oat-feeding strains. In the present study, the mating time of the two strains was investigated in the laboratory. The mating time was significantly different. The peak of mating time of the rice-feeder was 5 h earlier than that of the water-oat-feeder, suggesting that the two strains are reproductively isolated.

Functional consequences of sequence variation in the pheromone biosynthetic gene *pgFAR* for *Ostrinia* moths

Lassance, JM; Lienard, MA; Antony, B; Qian, SG; Fujii, T; Tabata, J; Ishikawa, Y; Lofstedt, C. 2013

Pheromones are central to the mating systems of a wide range of organisms, and reproductive isolation between closely related species is often achieved by subtle differences in pheromone composition. In insects and moths in particular, the use of structurally similar components in different blend ratios is usually sufficient to impede gene flow between taxa. To date, the genetic changes associated with variation and divergence in pheromone signals remain largely unknown. Using the emerging model system *Ostrinia*, we show the functional consequences of mutations in the protein-coding region of the pheromone biosynthetic fatty-acyl reductase gene *pgFAR*. Heterologous expression confirmed that *pgFAR* orthologs encode enzymes exhibiting different substrate specificities that are the direct consequences of extensive nonsynonymous substitutions. When taking natural ratios of pheromone precursors into account, our data reveal that *pgFAR* substrate preference provides a good explanation of how species-specific ratios of pheromone components are obtained among *Ostrinia* species. Moreover, our data indicate that positive selection may have promoted the observed accumulation of nonsynonymous amino acid substitutions. Site-directed mutagenesis experiments substantiate the idea that amino acid polymorphisms underlie subtle or drastic changes in *pgFAR* substrate preference. Altogether, this study identifies the reduction step as a potential source of variation in pheromone signals in the moth genus *Ostrinia* and suggests that selection acting on particular mutations provides a mechanism allowing pheromone reductases to evolve new functional properties that may contribute to variation in the composition of pheromone signals.

Biological differences between five populations of fall armyworm (Lepidoptera : Noctuidae) collected from corn in Mexico

Lopez-Edwards, M; Hernandez-Mendoza, JL; Pescador-Rubio, A; Molina-Ochoa, J; Lezama-Gutierrez, R; Hamm, JJ; Wiseman, BR. 1999

Biological characterizations of five fall armyworm populations, *Spodoptera frugiperda* (J. E. Smith) (FAW) collected from corn, *Zea mays* L., in Mexico, were reared and evaluated under laboratory conditions. The period from larvae to pupal stage, pupal weights, and survival rates were determined. The reproductive compatibility of adults, and the neonatal susceptibility to Endosulfan, Carbofuran and *Bacillus thuringiensis* (Bt) were also evaluated. Populations from Aguascalientes, Colima, Nuevo Leon, Sinaloa, and Yucatan were reared on corn at 25 degrees C. The Colima population reared on corn leaves required the least number of days to reach the pupal stage (13.04 D). Significant differences between the pupal weights of the different populations were found, ranging from 0.215 to 0.156 g. Survival rates varied from 80 to 45%, the Colima and Sinaloa populations had the highest survival. The Aguascalientes, Nuevo Leon and Yucatan populations were reproductively compatible as they produced progeny when paired. However, no progeny were obtained when the Colima and Sinaloa populations were paired with any other populations. The Aguascalientes, Nuevo Leon and Sinaloa populations tested for susceptibility to *B. thuringiensis* resulted in LC50 values, from 0.001 to 0.045 mg/ml. The Aguascalientes and Yucatan populations showed similar susceptibility to Carbofuran and Endosulfan insecticide with an LC50 ranging from 0.033 to 0.188 mg/ml, and 0.023 to 0.054 mg/ml, respectively. The Nuevo Leon population was the least susceptible. Results suggest that two corn FAW strains may have developed reproductive isolation due to geographic isolation. One strain formed by the Yucatan, Aguascalientes and Nuevo Leon populations, which are distributed along the Coastal Gulf and the geographic center of Mexico, and the other corn strain is formed by the Colima and Sinaloa populations found along the Mexican Pacific Coast, as the two strains produce no progeny when paired.

Evidence of olfactory antagonistic imposition as a facilitator of evolutionary shifts in pheromone blend usage in *Ostrinia* spp. (Lepidoptera : Crambidae)

Domingue, MJ; Musto, CJ; Linn, CE; Roelofs, WL; Baker, TC. 2007

Olfactory receptor neuron (ORN) response was measured to assess why some males ("rare males") of the Asian corn borer (ACB), *Ostrinia furnacalis*, have a broad behavioral response to fly upwind to both the ACB and the European corn borer (ECB), *Ostrinia nubilalis*, pheromone blends. We performed single-sensillum electrophysiological recordings on ACB males that had been behaviorally assessed for upwind flight response to the ACB blend [60:40 (Z)-12-tetradecenyl acetate (Z12-14:OAc) to (E)-12-tetradecenyl acetate (E12-14:OAc)], as well as to ECB (Z-strain) and ECB (E-strain) blends [3:97 and 99:1 (Z)-11-tetradecenyl acetate (Z11-14:OAc) to (E)-11-tetradecenyl acetate (E11-14:OAc)]. Sensilla from all types of males had large- and small-spike-sized ORNs responding strongly to Z12- or E12-14:OAc, but weakly to Z11- and E11-14:OAc. In the majority of males ("normal males") that flew upwind only to the ACB blend, Z11-14:OAc elicited responses in an intermediate spike-sized ORN associated with behavioral antagonism that is mainly tuned to (Z)-9-tetradecenyl acetate (Z9-14:OAc). In the rare-type ACB males that flew to both the ACB and ECB pheromone blends, Z11-14:OAc did not stimulate this ORN. Increased responsiveness to ancestral pheromone components by ORNs associated with behavioral antagonism could be instrumental in reproductive character displacement, or in reinforcement and reproductive isolation during speciation by helping to increase assortative mating between males and females in derived populations that use novel sex pheromone blends. (C) 2007 Elsevier Ltd. All rights reserved.

Molecular dissection of nuptial gifts in divergent strains of *Ostrinia* moths

Al-Wathiqui, N; Lewis, SM; Dopman, EB. 2018

Seminal fluid proteins (SFPs) produced in the male accessory glands and ejaculatory duct are subject to strong sexual selection, often evolve rapidly and therefore may play a key role in reproductive isolation and species formation. However, little is known about reproductive proteins for species in which males transfer ejaculate to females using a spermatophore package. By combining RNA sequencing and proteomics, we characterize putative SFPs, identify proteins transferred in the male spermatophore and identify candidate genes contributing to a one-way gametic incompatibility between Z and E strains of the European corn borer moth *Ostrinia nubilalis*. We find that the accessory glands and ejaculatory duct secrete over 200 highly expressed gene products, including peptidases, peptidase regulators and odourant-binding proteins. A comparison between *Ostrinia* strains reveals that accessory gland and ejaculatory duct sequences with hormone degradation and peptidase activity are among the most extremely differentially expressed. However, most spermatophore peptides lack reproductive tissue bias or canonical secretory signal motifs and approximately one-quarter may be produced elsewhere before being sequestered by the male accessory glands during spermatophore production. In addition, most potential gene candidates for postmating reproductive isolation do not meet standard criteria for predicted SFPs and almost three-quarters are novel, suggesting that both postmating sexual interactions and gametic isolation likely involve molecular products beyond traditionally recognized SFPs.

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

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

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

Tracking pyrethroid resistance in the polyphagous bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), in the shifting landscape of a cotton-growing area

Brevault, T; Achaleke, J; Sougnabe, SP; Vaissayre, M. 2008

In cotton-growing areas of Central Africa, timing of host crops and pest management practices in annual rainfed cropping systems result in a shifting mosaic of habitats that influence the dynamics and resistance of *Helicoverpa armigera* (Hubner) populations oil spatial scales, both within and across seasons. From 2002 to 2006, regional and local resistance was monitored among cotton fields and among the major host plants of the bollworm. From 2002, pyrethroid resistance increased within and across cotton-growing seasons to reach a worrying situation at the end of the 2005 growing season. Cotton crops played a fundamental role in the increase in seasonal resistance, even if the intensive use of insecticides on local tomato crops strongly concentrated resistance alleles in residual populations throughout the off-season. Due to the relative stability of resistance in *H. armigera* populations despite a long off-season, we believe that after the dispersal of the moths southwards at the end of the growing season, reverse migration mainly accounts for the reconstitution of Populations at the onset of the following growing season. In addition, local resistance monitoring in 2005 and 2006 showed that it was possible to control the increase in resistance by temporarily stopping the use of pyrethroids during the period of peak infestation of cotton by *H. armigera*. On the other hand, the similar resistance frequency of populations sampled from sprayed and unsprayed synchronous hosts confirmed the absence of reproductive isolation between adults. As a result, diversity in cropping systems should be encouraged by planting alternative host plants to provide a mosaic of habitats, which in return would provide insecticide-free refuges. The implications for insecticide resistance management in annual cropping systems are discussed.

A comparison of the larval overwintering biology of the striped stem borer, *Chilo suppressalis* (Lepidoptera: Crambidae), in rice and water-oat fields

Ding, N; Dalin, P; Zhu, QH; Ma, WH; Zhu, F; Wang, XP. 2013

It has been suggested that the striped stem borer, *Chilo suppressalis* (Walker), has developed distinct populations inhabiting rice *Oryza sativa* (Linnaeus) and water-oats *Zizania latifolia* (Turcz). In this study, we compared several biological traits between overwintering larvae from rice and water-oat fields during the winter of 2010. The parasitism rate was significantly higher in the larvae that overwintered in the rice field than in those that overwintered in the water-oat field. The average body weight of overwintering larvae and the weight of 3-day-old pupae, as well as the number of eggs carried by adult females, were all significantly greater in the

samples from the water-oat field. The diapause intensity, defined as the number of days required until pupation by overwintering larvae transferred to 25 A degrees C and L:D 16:8 h, decreased progressively from January to March but decreased more rapidly in the water-oat field. Our results demonstrate differences in the overwintering biology of *C. suppressalis* larvae from rice and water-oat fields. These differences may contribute to the differentiation and reproductive isolation between host-plant populations in this species.

How protandry and protogyny affect female mating failure: a spatial population model

Larsen, E; Calabrese, JM; Rhinds, M; Fagan, WF. 2013

Population growth and persistence depend on the collective ability of individuals to find mates in both space and time. When individuals are reproductively mature for only a portion of a population's breeding season, reproductive asynchrony can cause mating failure and a temporal Allee effect, which is exacerbated by spatial constraints in isolated populations. However, the effect of phenological variation by sex (protandry, protogyny) in spatiotemporal mate finding is not well understood. Here, we examine the interacting roles of sex-specific and population-wide individual asynchrony on female matelessness in spatially isolated populations. By incorporating sex-specific phenology into a two-sex reaction-diffusion system, we explore female matelessness as a function of phenology, movement behavior, and patch size. Although individual asynchrony may lead to female mating failure in small and isolated populations, we find that moderate protandry reduces female mating failure across a variety of scenarios. We go on to examine model behavior for a case study based on the bagworm, *Thyridopteryx ephemeraeformis* Haworth (Lepidoptera: Psychidae), where many populations exhibit pronounced protogyny. Overall, we find a consistent benefit of moderate protandry, which may mitigate female matelessness for many populations.

Genomic introgression from a distant congener in the Levant fritillary butterfly, *Melitaea acentria*

Pazhenkova, EA; Lukhtanov, VA. 2021

Introgressive **hybridization** is more common in nature than previously thought, and its role and creative power in evolution is hotly discussed but not completely understood. Introgression occurs more frequently in sympatry between recently diverged taxa, or when the speciation process has not yet been completed. However, there are relatively few documented cases of **hybridization** that erodes reproductive barriers between distantly related species. Here, we use whole genome and mitochondrial data to examine how introgression from a distant congener affects pattern of genetic differentiation in the Levant fritillary butterfly *Melitaea acentria*. We show that this local taxon has evolved as a peripatric geographic isolate of the widespread *Melitaea perseia*, and that there has been significant unidirectional gene flow from the sympatric, nonclosely related *Melitaea didyma* to *M. acentria*. We found direct evidence of ongoing sporadic **hybridization** between *M. didyma* and *M. acentria*, which are separated by at least 5 million years of independent evolution. Elevated differentiation and lower level of introgression on the sex Z chromosome compared to autosomes suggest that the Z chromosome has accumulated loci acting as intrinsic postzygotic barriers. Our results show that introgression from *M. didyma* has been an additional source of nucleotide diversity in the *M. acentria* population, providing material for drift and selection.

Pheromone communication channels in tortricid moths: lower specificity of alcohol vs. acetate geometric isomer blends

Witzgall, P; Trematerra, P; Liblikas, I; Bengtsson, M; Unelius, CR. 2010

Discrimination of conspecific and heterospecific signals is a key element in the evolution of specific mate recognition systems. Lepidopteran pheromone signals are typically composed of several compounds that synergize attraction of conspecific and inhibit attraction of heterospecific males. Blends convey specificity, but not their single components, that are typically shared by several species. Many sex pheromones are blends of geometric or positional isomers of straight-chain acetates, while species-specific blends of analogous alcohols have not been described. We have, therefore, studied the attraction of tortricid moths to the geometric isomers (E,E)-, (E,Z)-, (Z,E)- and (Z,Z)-8,10-dodecadien-1-ol. Only one species responding to these alcohols seemed to be attracted to a blend of two isomers, while most species are attracted to only one alcohol isomer. Lack of a pronounced synergist or antagonist effect of the other geometric isomers explains the lack of specific attraction to isomer blends and reduces accordingly the number of specific communication signals composed of these alcohols. In comparison, many more species respond to the analogous (E,E)-, (E,Z)-, (Z,E)- and (Z,Z)-8,10-dodecadienyl acetates and their binary blends. The acetate isomers all play a behavioural role, either as attractants, attraction synergists or antagonists, and thus promote specific communication with acetate blends. Male moths seem to discriminate the acetate isomers with greater precision than the analogous alcohols. It is proposed that discrimination is facilitated by steric differences between the four acetate isomers, as compared to the more uniform steric properties of the alcohols.

Chemically mediated species recognition in two sympatric Grayling butterflies: *Hipparchia fagi* and *Hipparchia hermione* (Lepidoptera: Nymphalidae, Satyrinae)

Pinzari, M; Santonico, M; Pennazza, G; Martinelli, E; Capuano, R; Paolesse, R; Di Rao, M; D'Amico, A; Cesaroni, D; Sbordoni, V; Di Natale, C. 2018

Pheromones are known to play an important role in butterfly courtship and may influence both individual reproductive success and reproductive isolation between species. Recent studies have focused on courtship in *Hipparchia* butterflies (Nymphalidae: Satyrinae) emphasizing morphological and behavioural traits, as well as genetic differences. Behavioural observations suggested a role for chemical cues in mate and species recognition, where the androconial scales on the forewings of these species may be involved in chemical communication between individuals. Chemical-mediated signals have received relatively little attention in this genus. Here, we report the results of a three-year investigation of the volatile organic compounds (VOCs) released by *Hipparchia fagi* and *H. hermione* in order to identify differences in VOCs between these species where they live in syntopy. Our study was carried out using an array of cross-selective sensors known as an "Electronic Nose" (EN) that operates by converting chemical patterns into patterns of sensor signals. While the identity of volatile compounds remained unknown, sensor signals can be compared to identify similar or dissimilar chemical patterns. Based on the EN signals, our results showed that: 1) the two sexes have a similar VOCs pattern in *H. fagi*, while they significantly diverge in *H. hermione*; 2) VOCs patterns were different between females of the two species, while those of males were not.

Population genetic structure and genetic diversity of the threatened White Mountain arctic butterfly (*Oeneis melissa semidea*)

Gradish, AE; Keyghobadi, N; Otis, GW. 2015

The White Mountain arctic butterfly [WMA; *Oeneis melissa semidea* (Say)] is endemic to the alpine zone of Mts. Washington and Jefferson, New Hampshire, USA, and because of its small and declining population size, it is considered threatened. White Mountain arctic adults occur only within four alpine meadows, and it has been suggested that dispersal, and hence gene flow, may be restricted among these meadow subpopulations. Furthermore, although the WMA likely is biennial (i.e., requires 2 years for development) like all other species of *Oeneis*, adults emerge annually. Thus the WMA population may be further structured into two allochronic cohorts, reproductively isolated by their asynchronous adult emergence in either even- or odd-numbered years. We assessed the spatial (among meadows) and temporal (between even- and odd-year cohorts) genetic structure and diversity of the WMA using mtDNA and AFLP markers generated from non-lethally sampled wing and leg tissue. We found no evidence for restricted gene flow among meadows. AFLPs indicated weak differentiation between alternate year cohorts; however, it remains unclear whether this resulted from allochronic reproductive isolation or genetic drift. Despite the WMA's small population size and isolation, levels of AFLP genetic diversity were generally high. Rather than focusing on factors related to population connectivity and adult dispersal, our results suggest that management efforts for the WMA should instead focus explicitly on factors affecting recruitment and mortality.

Reproductive barriers between two sympatric beetle species specialized on different host plants

Xue, HJ; Magalhaes, S; Li, WZ; Yang, XK. 2009

Knowledge on interspecific pre- and post-zygotic isolation mechanisms provides insights into speciation patterns. Using crosses (F-1 and backcrosses) of two closely related flea beetles species, *Altica fragariae* and *A. viridicyanea*, specialized on different hosts in sympatry, we measured: (a) the type of reproductive isolation and (b) the inheritance mode of preference and host-specific performance, using a joint-scaling test. Each species preferred almost exclusively its host plant, creating strong prezygotic isolation between them, and suggesting that speciation may occur at least partly in sympatry. Reproductive isolation was intrinsic between females of *A. fragariae* and either *A. viridicyanea* or F-1 males, whereas the other crosses showed ecologically dependent reproductive isolation, suggesting ecological speciation. The genetic basis of preference and performance was at least partially independent, and several loci coded for preference, which limits the possibility of sympatric speciation. Hence, both ecological and intrinsic factors may contribute to speciation between these species.

Taxonomy as a hypothesis: testing the status of the Bermuda buckeye butterfly *Junonia coenia bergi* (Lepidoptera: Nymphalidae)

Peters, MJ; Marcus, JM. NA

Species determination and definition in eukaryotes have traditionally been based on morphology, with little focus on genetic differentiation. Molecular methods allow for the independent assessment of morphology-based taxonomic hypotheses. Three criteria used to define a full species for taxonomic purposes are morphological distinction, formation of a monophyletic lineage, and reproductive isolation. *Junonia* butterflies (Nymphalidae) are becoming an important experimental model system, but the taxonomy of many New World *Junonia* species is unclear. One of these species is *J. coenia*, which contains the subspecies *J. coenia coenia*, *J. coenia grisea* and *J. coenia bergi*. Previous studies suggest that *J. coenia grisea* may meet the criteria for full species status. Therefore, we evaluated the geographically isolated and rarely studied Bermuda buckeye butterfly *J. coenia bergi* to determine if it was similarly distinct. Physical examination of specimens and phylogenetic and population genetic analyses of mitochondrial cytochrome c oxidase subunit I, nuclear wingless, and complete mitochondrial genome sequences suggest that while *J. coenia bergi* is smaller in body size than many *Junonia* and has distinctive ventral hindwing colouration, it does not form a monophyletic lineage and shows indications of continued gene flow with North American mainland *J. coenia coenia* populations. Thus, *J. coenia bergi* does not meet the criteria for full species designation, but geographic isolation, morphological distinctiveness, and cultural importance suggest that it remain recognized as a subspecies of *J. coenia*. Similar analyses will be useful for addressing further taxonomic questions in *Junonia* and other taxa, especially where morphology-based taxonomic determinations are ambiguous.

Evidence for low-level hybridization between two allochronic populations of the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae)

Burban, C; Gautier, M; Leblais, R; Landes, J; Santos, H; Paiva, MR; Branco, M; Kerdelhue, C. 2016

Divergence between populations sharing the same habitat can be initiated by different reproductive times, leading to allochronic differentiation. A spatially localized allochronic summer population (SP) of the pine processionary moth *Thaumetopoea pityocampa*, recently discovered in Portugal, occurs in sympatry with the local winter population (WP). We examined the level of genetic differentiation between the two populations and estimated the current gene flow within the spatial framework of their co-occurrence. Mitochondrial data indicated that the two sympatric populations were genetically closer than other WP populations. Conversely, microsatellite genotyping uncovered greater differentiation between the two sympatric populations than between allopatric ones. While male trapping confirmed that reproduction of SP and WP occurred at distinct times, clustering approaches demonstrated the presence of a few LateSP individuals emerging within the WP flight period, although genetically identified as SP. We also identified rare recent hybridization events apparently occurring mainly in the margins of the current SP range. The ongoing gene flow detected between the ancestral and the emerging allochronic populations revealed an incomplete reproductive isolation, which must therefore be taken into account and integrated with studies focussed on ecological drivers, so that a complete understanding of the ongoing speciation process might be achieved. (c) 2016 The Linnean Society of London, Biological Journal of the Linnean Society, 2016, 119, 311-328.

The spatial genetic differentiation of the legume pod borer, *Maruca vitrata* F. (Lepidoptera: Crambidae) populations in West Africa

Agunbiade, TA; Coates, BS; Kim, KS; Forgacs, D; Margam, VM; Murdock, LL; Ba, MN; Binso-Dabire, CL; Baoua, I; Ishiyaku, MF; Tamo, M; Pittendrigh, BR. 2012

The legume pod borer, *Maruca vitrata*, is an endemic insect pest that causes significant yield loss to the cowpea crop in West Africa. The application of population genetic tools is important in the management of insect pests but such data on *M. vitrata* is lacking. We applied a set of six microsatellite markers to assess the population structure of *M. vitrata* collected at five sites from Burkina Faso, Niger and Nigeria. Observed polymorphisms ranged from one (marker 3393) to eight (marker 32008) alleles per locus. Observed and expected heterozygosities ranged from 0.0 to 0.8 and 0.0 to 0.6, respectively. Three of the loci in samples from Nigeria and Burkina Faso deviated significantly from Hardy-Weinberg Equilibrium (HWE), whereas no loci deviated significantly in samples from Niger. Analysis of molecular variance (AMOVA) indicated that 67.3% level of the genetic variation was within individuals compared to 17.3% among populations. A global estimate of $F_{ST} = 0.1$ (ENA corrected $F_{ST} = 0.1$) was significant ($P \leq 0.05$) and corroborated by pairwise F_{ST} values that were significant among all possible comparisons. A significant correlation was predicted between genetic divergence and geographic distance between subpopulations ($R^2 = 0.6$, $P = 0.04$), and cluster analysis by the program STRUCTURE predicted that co-ancestry of genotypes were indicative of three distinct populations. The spatial genetic variance among *M. vitrata* in West Africa may be due to limited gene flow, south-north seasonal movement pattern or other reproductive barriers. This information is important for the cultural, chemical and biological control strategies for managing *M. vitrata*.

Two's company, three's a crowd: new insights on spruce budworm species boundaries using genotyping-by-sequencing in an integrative species assessment (Lepidoptera: Tortricidae)

Brunet, BMT; Blackburn, GS; Muirhead, K; Lumley, LM; Boyle, B; Levesque, RC; Cusson, M; Sperling, FAH. 2017

Species delimitation requires an assessment of varied traits that can contribute to reproductive isolation, as well as of the permanence of evolutionary differentiation among closely related lineages. Integrative taxonomy, including the combination of genome-wide molecular data with ecological data, offers an effective approach to this issue. We use genotyping-by-sequencing together with a review of ecological divergence to assess the traditionally recognized species status of three closely related members of the spruce budworm species complex, *Choristoneura fumiferana* (Clemens), *C. occidentalis* Freeman (= *C. freemani* Razowski) and *C. biennis* Freeman, each of which is a major defoliator of conifer forests. We sampled a broad region of overlap between these three taxa in Alberta and British Columbia (Canada) where potential for gene flow provides a strong test of the durability of divergence among lineages. A total of 2218 single nucleotide polymorphisms (SNPs) were assayed, and patterns of differentiation were evaluated under the biological, ecological, genotypic cluster and phylogenetic species concepts. *Choristoneura fumiferana* was genetically distinct with substantial barriers to genetic exchange with *C. occidentalis* and *C. biennis*. Conversely, divergence between *C. occidentalis* and *C. biennis* was limited to a small subset of outlier loci and was within the range observed within any one of the taxa. Considering both population genetic and ecological patterns of divergence, *C. fumiferana* should continue to be recognized as a distinct species, and *C. biennis* (syn.n.) should be treated as a subspecies (*C. occidentalis biennis* Freeman, 1967) of *C. occidentalis*, thereby automatically establishing the nominate name *C. occidentalis occidentalis* Freeman, 1967 for univoltine populations of this species.

Multiple factors contribute to reproductive isolation between two co-existing *Habenaria* species (Orchidaceae)

Zhang, WL; Gao, JY. 2017

Reproductive isolation is a key feature that forms barriers to gene flow between distinct plants. In orchids, prezygotic reproductive isolation has been considered to be strong, because their associations with highly specific pollinators. In this study, the reproductive ecology and reproductive isolation of two sympatric *Habenaria* species, *H. davidii* and *H. fordii*, was investigated by floral phenology and morphology, hand-pollination experiments and visitor observation in southwest China. The two species were dependent on insects for pollination and completely self-compatible. A number of factors have been identified to limit gene flow between the two species and achieved full reproductive isolation. Ecogeographic isolation was a weak barrier. *H. fordii* and *H. davidii* had completely overlapped flowering periods, and floral morphology plays an important role in floral isolation. The two species shared the same hawkmoth pollinator, *Cechenena lineosa*, but the pollinaria of the two orchids were attached on different body parts of pollinators. Prezygotic isolation was not complete, but the interspecific pollination treatments of each species resulted in no seed sets, indicating that unlike many other orchid species, in which the postzygotic reproductive isolation is very weak or complete absence, the post-zygotic isolation strongly acted in the stage of seed production between two species. The results illustrate the reproductive isolation between two species involves multiple plant life-history stages and a variety of reproductive barriers can contribute to overall isolation.

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

Chew, FS; Watt, WB. 2006

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

Allochronic Separation versus Mate Choice: Nonrandom Patterns of Mating between Fall Armyworm Host Strains

Schoff, G; Dill, A; Heckel, DG; Groot, AT. 2011

Assortative mating may result from intrinsic individual mating preferences or from assortment traits not requiring expression of preferences. Assortment traits are phenotypes expressed in both sexes that enhance the probability of encountering individuals possessing similar trait values. In the noctuid moth *Spodoptera frugiperda*, it has been suggested that nonrandom mating between two host strains is caused by a temporal assortment trait—that is, differential timing of calling and copulation during the night. By experimental manipulation of this trait in controlled mate-choice experiments, we investigated whether mating by same-strain individuals is enhanced mainly by the allochronic shift of mating activity or is also affected by time-independent intrinsic mating preferences. The observed patterns suggest that nonrandom mating between the two host strains in the laboratory is shaped by an interaction of both effects that is dominated by mating preferences during the first encounter night. This interaction changes over time as the preferences become weaker on subsequent nights. Males were less restricted than females with regard to both the time shift in mating activity and mate preferences. Although the nature of the mate-preference mechanism remains elusive, its restriction to females suggests that male-produced close-range pheromones emitted during courtship play a role.

The Genetic Basis of Pheromone Evolution in Moths

Groot, AT; Dekker, T; Heckel, DG. 2016

Moth sexual pheromones are widely studied as a fine-tuned system of intra-specific sexual communication that reinforces interspecific reproductive isolation. However, their evolution poses a dilemma: How can the female pheromone and male preference simultaneously change to create a new pattern of species-specific attraction? Solving this puzzle requires us to identify the genes underlying intraspecific variation in signals and responses and to understand the evolutionary mechanisms responsible for their interspecific divergence. Candidate gene approaches and functional analyses have yielded insights into large families of biosynthetic enzymes and pheromone receptors, although the factors controlling their expression remain largely unexplored. Intra- and interspecific crosses have provided tantalizing evidence of regulatory genes, although, to date, mapping resolution has been insufficient to identify them. Recent advances in high-throughput genome and transcriptome sequencing, together with established techniques, have great potential to help scientists identify the specific genetic changes underlying divergence and resolve the mystery of how moth sexual communication systems evolve.

Host specificity and reproductive success of yucca moths (*Tegeticula* spp. Lepidoptera: Prodoxidae) mirror patterns of gene flow between host plant varieties of the Joshua tree (*Yucca brevifolia*: Agavaceae)

Smith, CI; Drummond, CS; Godsoe, W; Yoder, JB; Pellmyr, O. 2009

Coevolution between flowering plants and their pollinators is thought to have generated much of the diversity of life on Earth, but the population processes that may have produced these macroevolutionary patterns remain unclear. Mathematical models of coevolution in obligate pollination mutualisms suggest that phenotype matching between plants and their pollinators can generate reproductive isolation. Here, we test this hypothesis using a natural experiment that examines the role of natural selection on phenotype matching between yuccas and yucca moths (*Tegeticula* spp.) in mediating reproductive isolation between two varieties of Joshua tree (*Yucca brevifolia* var. *brevifolia* and *Y. brevifolia* var. *jaegeriana*). Using passive monitoring techniques, DNA barcoding, microsatellite DNA genotyping, and sibship reconstruction, we track host specificity and the fitness consequences of host choice in a zone of sympatry. We show that the two moth species differ in their degree of host specificity and that oviposition on a foreign host plant results in the production of fewer offspring. This difference in host specificity between the two moth species mirrors patterns of chloroplast introgression from west to east between host varieties, suggesting that natural selection acting on pollinator phenotypes mediates gene flow and reproductive isolation between Joshua-tree varieties.

ROLE OF FEMALE-PRODUCED SEX-PHEROMONE IN BEHAVIORAL REPRODUCTIVE ISOLATION BETWEEN *TRICHOPLUSIA-NI* (HUBNER) AND *PSEUDOPPLUSIA-INCLUDENS* (WALKER) (LEPIDOPTERA, NOCTUIDAE, PLUSIINAE)

Reproductive Isolation between Japanese and Myanmar Populations of *Cotesia vestalis* (=plutellae) (Hymenoptera: Braconidae), a Larval Parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae)

Htwe, AN; Takagi, M; Takasu, K. 2009

Cotesia vestalis (Hymenoptera: Braconidae) has been widely used in many parts of the world as a major biocontrol agent, of the diamondback moth, *Plutella xylostella*. The rate of parasitism and the rate of control exerted on the pests are highly variable geographically as well as not. all introduction and augmentations were successful. Populations from different geographic regions often display various Levels of reproductive incompatibility because they have been geographically isolated, experienced different selection pressures and/or adapted to various local environments. Experiment were conducted to compare reproductive compatibility between two geographic populations of *C. vestalis* one from Fukuoka, Japan (Temperate population) and the other from Mandalay, Myanmar (Tropical population). Results indicated that there was partial incompatibility between the two populations.

POST-MATING REPRODUCTIVE ISOLATION BETWEEN FRUIT-FEEDING AND PINACEAE-FEEDING TYPES OF THE YELLOW PEACH MOTH, *CONOGETHES-PUNCTIFERALIS* (GUENEE) (LEPIDOPTERA, PYRALIDAE)

HONDA, H. 1986

Testing Classical Species Properties with Contemporary Data: How "Bad Species" in the Brassy Ringlets (*Erebia tyndarus* complex, Lepidoptera) Turned Good

Gratton, P; Trucchi, E; Trasatti, A; Riccarducci, G; Marta, S; Allegrucci, G; Cesaroni, D; Sbordoni, V. 2016

All species concepts are rooted in reproductive, and ultimately genealogical, relations. Genetic data are thus the most important source of information for species delimitation. Current ease of access to genomic data and recent computational advances are blooming a plethora of coalescent-based species delimitation methods. Despite their utility as objective approaches to identify species boundaries, coalescent-based methods (1) rely on simplified demographic models that may fail to capture some attributes of biological species, (2) do not make explicit use of the geographic information contained in the data, and (3) are often computationally intensive. In this article, we present a case of species delimitation in the *Erebia tyndarus* species complex, a taxon regarded as a classic example of problematic taxonomic resolution. Our approach to species delimitation used genomic data to test predictions rooted in the biological species concept and in the criterion of coexistence in sympatry. We (1) obtained restriction-site associated DNA(RAD) sequencing data from a carefully designed sample, (2) applied two genotype clustering algorithms to identify genetic clusters, and (3) performed within clusters and between-clusters analyses of isolation by distance as a test for intrinsic reproductive barriers. Comparison of our results with those from a Bayes factor delimitation coalescent-based analysis, showed that coalescent-based approaches may lead to overconfident splitting of allopatric populations, and indicated that incorrect species delimitation is likely to be inferred when an incomplete geographic sample is analyzed. While we acknowledge the theoretical justification and practical usefulness of coalescent-based species delimitation methods, our results stress that, even in the phylogenomic era, the toolkit for species delimitation should not dismiss more traditional, biologically grounded, approaches coupling genomic data with geographic information.

Cryptic *Plutella* species show deep divergence despite the capacity to hybridize

Perry, KD; Baker, GJ; Powis, KJ; Kent, JK; Ward, CM; Baxter, SW. 2018

Background: Understanding genomic and phenotypic diversity among cryptic pest taxa has important implications for the management of pests and diseases. The diamondback moth, *Plutella xylostella* L., has been intensively studied due to its ability to evolve insecticide resistance and status as the world's most destructive pest of brassicaceous crops. The surprise discovery of a cryptic species endemic to Australia, *Plutella australiana* Landry & Hebert, raised questions regarding the distribution, ecological traits and pest status of the two species, the capacity for gene flow and whether specific management was required. Here, we collected *Plutella* from wild and cultivated brassicaceous plants from 75 locations throughout Australia and screened 1447 individuals to identify mtDNA lineages and *Wolbachia* infections. We genotyped genome-wide SNP markers using RADseq in coexisting populations of each species. In addition, we assessed reproductive compatibility in crossing experiments and insecticide susceptibility phenotypes using bioassays. Results: The two *Plutella* species coexisted on wild brassicas and canola crops, but only 10% of *Plutella* individuals were *P. australiana*. This species was not found on commercial Brassica vegetable crops, which are routinely sprayed with insecticides. Bioassays found that *P. australiana* was 19-306 fold more susceptible to four commonly-used insecticides than *P. xylostella*. Laboratory crosses revealed that reproductive isolation was incomplete but directionally asymmetric between the species. However, genome-wide nuclear SNPs revealed striking differences in genetic diversity and strong population structure between coexisting wild populations of each species. Nuclear diversity was 1.5-fold higher in *P. australiana*, yet both species showed limited variation in mtDNA. Infection with a single *Wolbachia* subgroup B strain was fixed in *P. australiana*, suggesting that a selective sweep contributed to low mtDNA diversity, while a subgroup A strain infected just 1.5% of *P. xylostella*. Conclusions: Despite sympatric distributions and the capacity to **hybridize**, strong genomic and phenotypic divergence exists between these *Plutella* species that is consistent with contrasting colonization histories and reproductive isolation after secondary contact. Although *P. australiana* is a potential pest of brassicaceous crops, it is of secondary importance to *P. xylostella*.

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

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

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

Pre- and postzygotic isolation and Haldane rule effects in reciprocal crosses of *Danaus erippus* and *Danaus plexippus* (Lepidoptera : Danainae), supported by

differentiation of cuticular hydrocarbons, establish their status as separate species

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

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

Allopatric divergence and secondary contact without genetic admixture for *Arichanna perimelaina* (Lepidoptera: Geometridae), an alpine moth endemic to the Hengduan Mountains

Li, XX; Jiang, N; Cheng, R; Xue, DY; Qu, YH; Han, HX. 2017

Mountain systems, especially at high altitudes, are an excellent model for determining the mechanisms underlying high species diversity and endemism. Herein, we elucidate the evolutionary history of the alpine moth *Arichanna perimelaina* (Wehrli), which is endemic to the Hengduan Mountains (HM) region in southwest China, based on three mitochondrial genes and two nuclear genes. Our results revealed six deeply divergent clades that corresponded to populations in different mountain systems in the HM region. Bayesian divergence time estimations suggested a mid to late Pleistocene genetic divergence. The results also showed that the Mt Yulong (YL) region was a refugium and valley corridors established by glaciation during the Pleistocene allowed populations on the separate mountains to migrate. The reproductive isolation among the different clades on contact zone in the YL region may be associated with the asynchronous mating rhythms and/or the divergent mate recognition caused by the ecological source of divergent selection. Allopatric divergence associated with complex topographies and climatic oscillations, regional dispersal via valley corridors and the suitable refugium of the YL region shaped the genetic divergence and distribution pattern of *A. perimelaina* in the HM region. These findings highlight the essential role of complex terrain and climatic fluctuations in shaping the unique phylogeographic history of a narrow alpine moth, and provide insights into the mechanisms underlying high species richness and endemism in the HM region.

REPRODUCTIVE ISOLATION BETWEEN SPECIES OF THE GENUS OSTRINIA (LEPIDOPTERA, PYRAUSTIDAE) AS A CRITERION OF THEIR DIFFERENTIATION .2. SEARCH FOR POST-COPULATIVE MECHANISMS

FROLOV, AN. 1982

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

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

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

Multiple sources of reproductive isolation in a bimodal butterfly hybrid zone

Munoz, AG; Salazar, C; Castano, J; Jiggins, CD; Linares, M. 2010

An important evolutionary question concerns whether one or many barriers are involved in the early stages of speciation. We examine pre- and post-zygotic reproductive barriers between two species of butterflies (*Heliconius erato chesteronii* and *H. e. venus*) separated by a bimodal **hybrid** zone in the Cauca Valley, Colombia. We show that there is both strong pre- and post-mating reproductive isolation, together leading to a 98% reduction in gene flow between the species. Pre-mating isolation plays a primary role, contributing strongly to this isolation (87%), similar to previous examples in *Heliconius*. Post-mating isolation was also strong, with absence of Haldane's rule, but an asymmetric reduction in fertility (< 11%) in inter-specific crosses depending on maternal genotype. In summary, this is one of the first examples of post-zygotic reproductive isolation playing a significant role in early stages of parapatric speciation in *Heliconius* and demonstrates the importance of multiple barriers to gene flow in the speciation process.

REPRODUCTIVE ISOLATION BETWEEN SPECIES OF THE GENUS OSTRINIA (LEPIDOPTERA, PYRAUSTIDAE) AS A CRITERION OF THEIR DIFFERENTIATION .1. ANALYSIS OF PRE-COPULATION BARRIERS

FROLOV, AN. 1982

Floral odour and reproductive isolation in two species of *Silene*

Waelti, MO; Muhlemann, JK; Widmer, A; Schiestl, FP. 2008

Mechanisms preventing interspecific pollination are important in closely related plant species, in particular when post-zygotic barriers are weak or absent. We

investigated the role of floral odour in reproductive isolation between the two closely related species *Silene latifolia* and *S. dioica*. First, we tested whether floral odour composition and emission differed between the species. We found significant odour differences, but contrary to expectations, both species showed a rhythmic emission of the same compounds between day and night. Second, in a field experiment, odour of the two species was made more similar by applying phenylacetaldehyde to flowers. This manipulation led to higher pollen-analogue transfer between species, revealing that floral odour differences are important for maintaining reproductive isolation. We conclude that differences in single key compounds can reduce pollen transfer across species boundaries by pollinators and demonstrate that odour differences are an important component of premating floral isolation between closely related plant species.

CHEMICAL CHARACTERIZATION AND SPECIES SPECIFICITY OF SEX-PHEROMONES OF PLUSIINAE MOTHS IN ISRAEL

DUNKELBLUM, E; MAZOR, M. 1993

The female sex pheromones of four sympatric Plusiinae species, *Autographa gamma*, *Chrysodeixis chalcites*, *Cornutiplusia circumflexa*, and *Trichoplusia ni* were investigated in Israel. The research concentrated on the behavioral reproductive isolation regulated by their sex pheromones. The chemical composition of the four sex pheromones was determined by analysis of pheromone gland extracts, and in addition entrained volatiles released from virgin female *C. chalcites* and *T. ni* were also analyzed. The pheromones of these latter two species were found to contain several components, among which Z7-12:Ac was the main one. The pheromone glands of *A. gamma* and *C. circumflexa* were found to contain relatively small amounts of material and only Z7-12:Ac and Z7-12:OH could be positively identified. These two pheromone components were present in the two species in roughly inverse ratios. Whereas *A. gamma* utilizes Z7-12:Ac as the main or sole pheromone component, *C. circumflexa* requires both Z7-12:OH and Z7-12:Ac, with the alcohol being predominant. This is the first reported Plusiinae species using Z7-12:OH as the main pheromone component. Behavioral studies in the wind tunnel and field tests indicate that females of each of the four Plusiinae species utilizes a species-specific blend of chemicals, consisting of attractive and antagonistic components, to attract conspecific males. Such a complementary strategy provides improved behavioral reproductive isolation among the sympatric species. For example, Z5-12:Ac, which is a specific component of the *T. ni* sex pheromone, may be redundant as regards the attraction of *T. ni* males. However, addition of small amounts of this compound to pheromone blends of *A. gamma* and *C. chalcites* resulted in complete inhibition of the males' flight behavior and trap catch; thus contributing significantly to the specificity of the *T. ni* sex pheromone. The relatively high proportion of Z7-12:OH in the sex pheromone of *C. circumflexa*, essential to the latter, is highly inhibitory to the three other Plusiinae species. The only case of cross-attraction occurred when *T. ni* males were attracted to a certain extent to *A. gamma* lures and females.

Divergence in *Heliconius* flight behaviour is associated with local adaptation to different forest structures

Dell'Aglio, DD; Mena, S; Mauxion, R; McMillan, WO; Montgomery, SH. 2022

Microhabitat choice plays a major role in shaping local patterns of biodiversity. In butterflies, stratification in flight height has an important role in maintaining community diversity. Despite its presumed importance, the role of behavioural shifts in early stages of speciation in response to differences in habitat structure is yet to be established. Here, we investigated variation in flight height behaviour in two closely related *Heliconius* species, *H. erato cyrbia* and *H. himera*, which produce viable hybrids but are isolated across an environmental gradient, spanning lowland wet forest to high-altitude scrub forest. Speciation in this pair is associated with strong assortative mating, but ecological isolation and local adaptation are also considered essential for complete reproductive isolation. We quantified differences in flight height and forest structure across the environmental gradient and tested the importance of resource distribution in explaining flight behaviour. We then used common garden experiments to test whether differences in flight height reflect individual responses to resource distribution or genetically determined shifts in foraging behaviour. We found that the two species fly at different heights in the wild, and demonstrated that this can be explained by differences in the vertical distribution of plant resources. In both the wild and captivity, *H. himera* choose to fly lower and feed at lower positions, closely mirroring differences in resource availability in the wild. Given expectations that foraging efficiency contributes to survival and reproductive success, we suggest that foraging behaviour may reflect local adaptation to divergent forest structures. Our results highlight the potential role of habitat-dependent divergence in behaviour during the early stages of speciation.

Time-shifted reproductive behaviours among fall armyworm (*Noctuidae: Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance

Schofl, G; Heckel, DG; Groot, AT. 2009

The noctuid moth *Spodoptera frugiperda* consists of two strains associated with different larval host plants (most notably corn and rice). These strains exhibit differential temporal patterns of female calling and copulation during scotophase, with the corn strain more active earlier in the night. We investigated strain-specific constraints in reproductive timing, mating interactions between the two strains, and the mode of inheritance of timing of female calling, male calling, copulation and oviposition. We observed an allochronic shift of all reproductive behaviours by approximately 3 h and a parallel shift of nonreproductive locomotor activity, suggesting involvement of the circadian clock. The corn strain was more variable in the timing of calling and copulation than the rice strain. Rice strain females were more restricted in the timing of copulation than rice strain males, while such differences between the sexes were not apparent in the corn strain. There were significant interactions between the strains affecting onset times of copulation and male calling. The four investigated reproductive traits differed in their modes of inheritance: timing of female and male calling exhibited strong maternal effects, timing of copulation was controlled by a combination of maternal effects and corn strain dominant autosomal factors, and timing of oviposition was inherited in a corn strain dominant fashion. We conclude that the allochronic separation of reproduction between fall armyworm strains is asymmetric, less pronounced than previously thought, and under complex genetic control.

From Russia with lobe: genetic differentiation in trilobed uncus *Ostrinia* spp. follows food plant, not hairy legs

Frolov, AN; Audiot, P; Bourguet, D; Kononchuk, AG; Malysh, JM; Ponsard, S; Streiff, R; Tokarev, YS. 2012

Trilobed uncus taxa of the genus *Ostrinia* (Lepidoptera, Crambidae) illustrate the complex relationship, at early stages of speciation, between reproductive isolation and differentiation in morphology, resource use and genetic variation. On the basis of behaviour and ecology, we recently hypothesized that individuals with small mid-tibiae belong to two distinct species depending on host plant-*O. nubilalis* and *O. scapularis* sensu Frolov et al. (2007) feeding on maize and on a number of dicotyledons, respectively. Individuals with small, medium or massive mid-tibiae would all belong to *O. scapularis* as long as they feed on these dicotyledons. This contrasts with previous taxonomy, which distinguished three species by male mid-tibia morphology, regardless of host plant. Here, we test our hypothesis by examining the genetic structure of *Ostrinia* populations from regions with mid-tibia polymorphism Western Russia and Kazakhstan and comparing it with that of French populations where only small mid-tibiae occur. Results support two predictions: (1) maize- and dicotyledon-collected populations are genetically differentiated from each other like in France, and (2) dicotyledon-collected populations show no genetic evidence of consisting of more than one species. Between-species differentiation was unrelated to geographic distance, despite significant isolation by distance within species. The distinction between two and only two species differing by host plant thus holds at continental scale. Interestingly, one microsatellite locus contributed 10 times more than the others to differentiation between both taxa. This deserves further investigation, as it might reveal a linkage between this outlier and loci involved in host-plant adaptation and/or reproductive isolation. *Heredity* (2012) 108, 147-156; doi:10.1038/hdy.2011.58; published online 20 July 2011

INTERSPECIFIC SEX ATTRACTION IN *EARIAS-VITTELLA* AND *EARIAS-INSULANA*

Interspecific attraction between *Earias vittella* and *Earias insulana* was studied by conducting sex pheromone bioassays and by making visual observations on the behaviour or individual interspecific pairs. Olfactometer bioassays using virgin "calling" females revealed interspecific attraction amongst about 10% males. In bioassays using female sex pheromone, *E. insulana* did not attract any *E. vittella* males, but the *E. vittella* female sex pheromone attracted 8.7% interspecific males. Electroantennogram recordings revealed detection of interspecific sex pheromones by antennae of males of both the species. When kept at a close range and allowed free access to interspecific females, more than 50% of *E. insulana* males courted and copulated with *E. vittella* females, but incidence of such courting and copulation was negligible in the reciprocal cross. In both the cases, there was no interspecific insemination. These studies suggested that dissimilarities in: (a) sex pheromone components or their ratio, (b) courtship behaviour and (c) sperm transfer mechanism play a role in the reproductive isolation of these two species.

MECHANISMS OF REPRODUCTIVE ISOLATION BETWEEN THE FRUIT-FEEDING AND THE PINACEAE-FEEDING TYPES OF THE YELLOW PEACH MOTH, *DICHOCROCIS-PUNCTIFERALIS* GUENEE (LEPIDOPTERA, PYRALIDAE)

KONNO, Y; HONDA, H; MATSUMOTO, Y. 1981

Two regional strains of a phoretic egg parasitoid, *Telenomus euproctidis* (Hymenoptera: Scelionidae), that use different sex pheromones of two allopatric tussock moth species as kairomones

Arakaki, N; Wakamura, S; Yasuda, T; Yamagishi, K. 1997

The egg parasitoid, *Telenomus euproctidis* Wilcox (Hymenoptera: Scelionidae), is phoretic on females of two allopatrically distributed tussock moths, *Euproctis pseudoconspersa* (Strand) and *Euproctis taiwana* (Shiraki) (Lepidoptera: Lymantriidae). Crossing experiments between the two regional parasitoid strains indicated no evidence for their reproductive isolation. More wasps were found on the locally occurring host, *E. pseudoconspersa*, than on *E. taiwana*, when virgin females of the two moth species were exposed concurrently in the field for 24 hr in Ibaraki Japan. In Ibaraki, many wasps were caught in traps baited with the synthetic sex pheromone of *E. pseudoconspersa*, 10, 14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu), but none with that of *E. taiwana*, (Z)-16-methyl-9-heptadecenyl isobutyrate (16Me-Z9-17:iBu) or blank traps. In Okinawa, Japan, more wasps were found on *E. taiwana* than on *E. pseudoconspersa*, and many wasps were caught in traps baited with 16Me-Z9-17:iBu, but only a few with 10Me14Me-15:iBu, and none with blank traps. These results suggest that local wasp strains discriminate between the two sex pheromones, and they strongly prefer the sex pheromone of the moth occurring at their location.

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

Gourbiere, S; Mallet, J. 2010

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

Postmating transcriptional changes in the female reproductive tract of the European corn borer moth

Al-Wathiqui, N; Dopman, EB; Lewis, SM. 2016

Mating triggers a cascade of physiological and behavioural responses in females that persist after copulation. In insects, seminal fluid proteins contained within male ejaculates are known to initiate some responses, but our understanding of how females mediate these reactions remains limited. Few studies have examined postmating transcriptional changes within ejaculate-receiving organs within females or how these changes might depend on the identity of the male. Furthermore, whereas males of many insects transfer packaged ejaculates, transcriptional dynamics have mainly been examined in dipterans, in which males transfer a free ejaculate. To identify genes that may be important in mediating female physiological responses in a spermatophore-producing species, we sequenced the transcriptomes of the ejaculate-receiving organs and examined postmating gene expression within and between pheromone strains of the European corn borer (ECB) moth, *Ostrinia nubilalis*. After within-strain mating, significant differential expression of 978 transcripts occurred in the female bursa or its associated bursal gland, including peptidases, transmembrane transporters, and hormone processing genes; such genes may potentially play a role in postmating male-female interactions. We also identified 14 transcripts from the bursal gland that were differentially expressed after females mated with cross-strain males, representing candidates for previously observed postmating reproductive isolation between ECB strains.

GENE GENEALOGIES REVEAL DIFFERENTIATION AT SEX PHEROMONE OLFACTORY RECEPTOR LOCI IN PHEROMONE STRAINS OF THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS*

Lassance, JM; Bogdanowicz, SM; Wanner, KW; Lofstedt, C; Harrison, RG. 2011

Males of the E and Z strains of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae) are attracted to different blends of the same pheromone components. The difference in male behavioral response is controlled by the sex-linked locus *Resp*. The two types of males have identical neuroanatomy but their physiological specificity is reversed, suggesting that variation at the periphery results in behavioral change. Differences in the olfactory receptors (ORs) could explain the strain-specific antennal response and blend preference. Gene genealogies can provide insights into the processes involved in speciation and allow delineation of genome regions that contribute to reproductive barriers. We used intronic DNA sequences from five OR-encoding genes to investigate whether they exhibit fixed differences between strains and therefore might contribute to reproductive isolation. Although two genealogies revealed shared polymorphism, molecular polymorphism at three genes revealed nearly fixed differences between strains. These three OR genes map to the sex chromosome, but our data indicate that the distance between *Resp* and the ORs is > 20 cM, making it unlikely that variation in pheromone-sensitive OR genes is directly responsible for the difference in behavioral response. However, differences in male antennal response may have their origin in the selection of strain-specific alleles.

Allelic variation in a fatty-acyl reductase gene causes divergence in moth sex pheromones

Lassance, JM; Groot, AT; Lienard, MA; Antony, B; Borgwardt, C; Andersson, F; Hedenstrom, E; Heckel, DG; Lofstedt, C. 2010

Pheromone-based behaviours are crucial in animals from insects to mammals(1,2), and reproductive isolation is often based on pheromone differences(1-4). However, the genetic mechanisms by which pheromone signals change during the evolution of new species are largely unknown(4). In the sexual communication system of moths (Insecta: Lepidoptera), females emit a species-specific pheromone blend that attracts males over long distances(1,2,4). The European corn borer, *Ostrinia nubilalis*, consists of two sex pheromone races, Z and E, that use different ratios of the cis and trans isomers of acetate pheromone components(5). This subtle difference leads to strong reproductive isolation in the field between the two races(6,7), which could represent a first step in speciation. Female sex pheromone production and male behavioural response are under the control of different major genes(8,9), but the identity of these genes is unknown. Here we show that allelic variation in a fatty-acyl reductase gene essential for pheromone biosynthesis accounts for the phenotypic variation in female pheromone production, leading to race-specific signals. Both the cis and trans isomers of the pheromone precursors are produced by both races, but the precursors are differentially reduced to yield opposite ratios in the final pheromone blend as a result of the substrate specificity of the enzymes encoded by the Z and E alleles. This is the first functional characterization of a gene contributing to intraspecific behavioural reproductive isolation in moths, highlighting the importance of evolutionary diversification in a lepidopteran-specific family of reductases. Accumulation of substitutions in the coding region of a single biosynthetic enzyme can produce pheromone differences resulting in reproductive isolation, with speciation as a potential end result.

Detecting Deep Divergence in Seventeen Populations of Tea Geometrid (*Ectropis obliqua* Prout) in China by COI mtDNA and Cross-Breeding

Zhang, GH; Yuan, ZJ; Zhang, CX; Yin, KS; Tang, MJ; Guo, HW; Fu, JY; Xiao, Q. 2014

The tea geometrid (*Ectropis obliqua* Prout, Lepidoptera: Geometridae) is a dominant chewing insect endemic in most tea-growing areas in China. Recently some *E. obliqua* populations have been found to be resistant to the nucleopolyhedrovirus (EoNPV), a host-specific virus that has so far been found only in *E. obliqua*. Although the resistant populations are morphologically indistinguishable from susceptible populations, we conducted a nationwide collection and examined the genetic divergence in the COI region of the mtDNA in *E. obliqua*. Phylogenetic analyses of mtDNA in 17 populations revealed two divergent clades with genetic distance greater than 3.7% between clades and less than 0.7% within clades. Therefore, we suggest that *E. obliqua* falls into two distinct groups. Further inheritance analyses using reciprocal single-pair mating showed an abnormal F-1 generation with an unbalanced sex ratio and the inability to produce fertile eggs (or any eggs) through F1 self-crossing. These data revealed a potential cryptic species complex with deep divergence and reproductive isolation within *E. obliqua*. Uneven distribution of the groups suggests a possible geographic effect on the divergence. Future investigations will be conducted to examine whether EoNPV selection or other factors prompted the evolution of resistance.

Comparison of male antennal morphology and sensilla physiology for sex pheromone olfactory sensing between sibling moth species: *Ectropis grisea* and *Ectropis obliqua* (Geometridae)

Liu, J; Li, ZQ; Luo, ZX; Cai, XM; Bian, L; Xin, ZJ; Chen, ZM. 2019

Ectropis grisea and *Ectropis obliqua* (Lepidoptera: Geometridae) are sibling pest species that co-occur on tea plants. The sex pheromone components of both species contain (Z,Z)-3,6,9-octadecatriene and (Z,Z)-3,9-cis-6,7-epoxy-octadecadiene. *E. obliqua* has (Z,Z)-3,9-cis-6,7-epoxy-nonadecadiene as an additional sex pheromone component, which ensures reproductive segregation between the two species. To ascertain the detection mechanism of olfactory organs for sex pheromone components of *E. grisea* and *E. obliqua*, we applied scanning electron microscopy and single sensillum recording to compare antennal morphology and sensillum physiology in the two species. There was no apparent morphological difference between the antennae of the two species. Both species responded similarly to all three sex pheromone components, including, *E. obliqua* specific component. The distribution patterns of antennal sensilla trichodea differed between the two species. Sex pheromone olfactory sensing in these sibling species appears to be determined by the density of different types of olfactory sensing neurons. Dose-dependent responses of sensilla trichodea type 1 to (Z,Z)-3,9-cis-6,7-epoxy-octadecadiene, the most abundant component, showed an "all or none" pattern and the other two components showed sigmoidal dose-response curves with a half threshold of 10^{-4} (dilution equal to the concentration of $10 \mu\text{g}/\mu\text{l}$). These results suggest that the major sex pheromone component functions as an on-off controller while secondary components function as modulators during olfactory transmission to the primary olfactory center.

Sex pheromones of two Melittini species, *Macroscelia japona* and *M. longipes*: Identification and field attraction

Naka, H; Inomata, SI; Matsuoka, K; Yamamoto, M; Sugie, H; Tsuchida, K; Arita, Y; Ando, T. 2007

Two Melittini species, *Macroscelia japona* and *M. longipes* (Lepidoptera: Sesiidae), are native to Japan, but occupy different localities as their host plants seldom grow together. The contents of the sex pheromone gland of adult females of both species, obtained after rearing larvae collected from the field, were investigated by gas chromatograph-electroantennogram detection (GC-EAD) and gas chromatograph-mass spectrometry (GC-MS) analyses. Two GC-EAD-active components were found in a crude extract of *M. japona* female pheromone gland, and identified as (2E,13Z)-2,13-octadecadien-1-ol (E2,Z13-18:OH) and (2E,13Z)-2,13-octadecadienal (E2,Z13-18:Ald). The average ratio of these two components was about 1:10. In the field, *M. japona* males were attracted to traps baited with E2,Z13-18:Ald alone, but the strongest attraction was observed with a 1:100 mixture of E2,Z13-18:OH and E2,Z13-18:Ald. The same two components were found in extracts of *M. longipes* females, but in a markedly different ratio. Male *M. longipes* were attracted most strongly to lures containing a 20:1 mixture of E2,Z13-18:OH and E2,Z13-18:Ald, although some males were also attracted to lures with E2,Z13-18:OH alone. Although the two species do not generally occur in sympatry, our data indicate that, in the event of overlap, cross attraction of the two species is unlikely.

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

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

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

Identification of sex pheromones of *Anadevidia peponis* and *Macdunnoughia confusa* and field tests of their role in reproductive isolation of closely related Plusiinae moths

Inomata, SI; Komoda, M; Watanabe, H; Nomura, M; Ando, T. 2000

Anadevidia peponis and Macdunnoughia confusa are defoliators of plants in the family Cucurbitaceae and Compositae, respectively, in Japan. GC-MS analyses of crude pheromone gland extracts treated with or without dimethyl disulfide indicated that females of A. peponis produced six monoene acetates and two monoene alcohols and that M. confusa females produced five monoene acetates. These components include (Z)-7-dodecenyl acetate as a major common constituent and three other acetates as minor common constituents. The minor constituents are quite different in blend composition. In addition, with (Z)-7-dodecenyl acetate, an indispensable component for male attraction is (Z)-5-decenyl acetate for A. peponis and (Z)-9-tetradecenyl acetate is essential for M. confusa. Field tests with synthetic lures showed synergistic effects of some other minor components and male attraction of three additional Plusiinae species, Macdunnoughia purissima, Ctenoplusia albostrata, and Chrysodeixis eriosoma, suggesting their reproductive isolation is based in part on pheromonal communication.

Alfred Russel Wallace and the Darwinian Species Concept: His Paper on the Swallowtail Butterflies (Papilionidae) of 1865

Mallet, J. 2009

Soon after his return from the Malay Archipelago, Alfred Russel Wallace published one of his most significant papers. The paper used butterflies of the family Papilionidae as a model system for testing evolutionary hypotheses, and included a revision of the Papilionidae of the region, as well as the description of some 20 new species. Wallace argued that the Papilionidae were the most advanced butterflies, against some of his colleagues such as Bates and Trimen who had claimed that the Nymphalidae were more advanced because of their possession of vestigial forelegs. In a very important section, Wallace laid out what is perhaps the clearest Darwinist definition of the differences between species, geographic subspecies, and local 'varieties.' He also discussed the relationship of these taxonomic categories to what is now termed 'reproductive isolation.' While accepting reproductive isolation as a cause of species, he rejected it as a definition. Instead, species were recognized as forms that overlap spatially and lack intermediates. However, this morphological distinctness argument breaks down for discrete polymorphisms, and Wallace clearly emphasised the conspecificity of non-mimetic males and female Batesian mimetic morphs in Papilio polytes, and also in P. memnon, on the grounds of reproductive continuity. Finally, Wallace detailed how natural selection explains various forms of parallel evolution, including Mimicry.

Pupal melanization is associated with higher fitness in Spodoptera exigua

Liu, SS; Wang, M; Li, XC. 2015

Melanism has long been thought to be a habitat adaptation with a fitness cost. Here we reported a homozygous melanic strain (SEM) of Spodoptera exigua (Hubner) (Insecta: Lepidoptera: Noctuidae) established with black pupae spontaneously occurring within a typical laboratory population (SEW). The melanization is expressed globally, and only in the pupal stage. After pupation, the melanic SEM pupae gradually accumulate melanin to become completely black within 6 hours, whereas the wildtype SEW pupae gradually turn yellow-brown. The melanic SEM strain exhibits faster development in all life stages, heavier pupa weight, more mating time, higher fecundity, and accordingly, higher net reproductive rate and population trend index. While no reproductive isolation was observed between the SEM and SEW strains, the mating times per female of the reciprocal crosses and the SEM intracrosses were significantly higher than those of the SEW intracrosses. This represents a rare case of melanization that has fitness gains, rather than costs. Analysis of the life-history traits of this case and 14 previously reported cases of insect melanism indicate that none of melanization origin, stage, space and variation type determining whether melanism will cause fitness gain or cost.

Interpretation of the biological species concept from interspecific hybridization of two Helicoverpa species

Wang, CZ. 2007

The biological species concept defines species in terms of interbreeding. Interbreeding between species is prevented by reproductive isolation mechanisms. Based on our results of interspecific **hybridization** between Helicoverpa armigera and Helicoverpa assulta, reproductive isolation mechanisms of the two species are analyzed. A combination of prezygotic factors (absent sex attraction and physical incompatibility of the genitalia) and postzygotic factors (female absence and partial sterility in F-1 **hybrids**) causes reproductive isolation of the two species. In addition, the role of interspecific **hybridization** in speciation is discussed.

The Parasitic Wasp, Cotesia congregata (Say), Consists of Two Incipient Species Isolated by Asymmetric Reproductive Incompatibility and Hybrid Inability to Overcome Host Defenses

Bredlau, JP; Kuhar, D; Gundersen-Rindal, DE; Kester, KM. 2019

Parasitic wasps are highly diverse and play a major role in suppression of herbivorous insect pest populations. Several previously identified species of parasitic wasps have been found to be complexes of cryptic species resulting from adaptations to specific hosts or host foodplants. Cotesia congregata (Say) (Hymenoptera: Braconidae), which has long served as a model system for host-parasitoid interactions, can be used for investigating the process of diversification among sympatric populations that differ in host and host foodplant usage. Two incipient species of C. congregata have been identified in the USA mid-Atlantic region, "MsT wasps" originate from Manduca sexta (L.) (Lepidoptera: Sphingidae) on tobacco and "CcC wasps" originate from Ceratonia catalpae (Boisduval) (Lepidoptera: Sphingidae) on catalpa. Both wasp sources can develop in either host species. **Hybrids** resulting from MsT male xCcC female crosses are fertile, whereas **hybrids** from CcC male xMsT female crosses are typically sterile. In this study, we compared relative expression in vivo of seven C. congregata bracovirus (CcBV) genes among MsT and CcC parental and **hybrid** crosses. Also, we established **hybrid** crosses between MsT and CcC wasps and four additional host foodplant sources of C. congregata. Patterns of relative expression in vivo of MsT and CcC CcBV genes differed; a few were not expressed in hosts parasitized by CcC wasps. Overall, relative expression of CcBV genes from MsT and CcC wasps did not differ with respect to the host species parasitized. Low or absent expression of CcBV genes was found in hosts parasitized by sterile **hybrids**. For the most part, the other four host-foodplant wasp sources were reproductively compatible with either MsT or CcC wasps and **hybrid** crosses with the alternative wasp source were asymmetrically sterile. Crosses involving CcC males or MsT females produced sterile **hybrids** that lacked mature ovaries. Cumulatively, results indicate that C. congregata is composed of two sympatric incipient species that can utilize multiple host species rather than several host-associated races or cryptic species.

Female sex pheromone of Ostrinia orientalis - throwing a light on the relationship between O-orientalis and the European corn borer, O-nubilalis

Fu, X; Tabata, J; Huang, Y; Takanashi, T; Ohno, S; Honda, H; Tatsuki, S; Ishikawa, Y. 2004

The sex pheromone of Ostrinia orientalis (Lepidoptera: Crambidae) was analyzed by gas chromatography-electroantennographic detection (GC-EAD), GC-mass spectrometry and a series of bioassays. Three EAD-active compounds were detected in the female sex pheromone gland extract, and identified as tetradecyl acetate (14:OAc), (Z)-11-tetradecenyl acetate (Z11-14:OAc) and (E)-11-tetradecenyl acetate (E11-14:OAc). The titers (ratio) of 14:OAc, Z11-14:OAc and E11-14:OAc in 3-day-old virgin females were 0.49 ng (10), 4.86 ng (98) and 0.10 ng (2), respectively. In a wind-tunnel bioassay, the 98:2 blend of Z11- and E11-14:OAc, but not Z11-14:OAc alone, elicited the same male behavioral responses as virgin females and crude gland extracts. 14:OAc was inactive by itself, and did not show any synergistic effect on the binary blend. Field trapping experiments also confirmed the attractiveness of the binary blend to O. orientalis males. Based on these results, we concluded that the sex pheromone of O. orientalis is a 98:2 mixture of Z11-14:OAc and E11-14:OAc. This sex pheromone is very similar to that of the Z-type European corn borer, O. nubilalis. The present finding raises the question of whether O. orientalis, which is indistinguishable from O. nubilalis based on external morphology, is a biologically distinct species independent from O. nubilalis.

Proteome profiling reveals tissue-specific protein expression in male and female accessory glands of the silkworm, *Bombyx mori*

Dong, ZM; Wang, XH; Zhang, Y; Zhang, LP; Chen, QM; Zhang, XL; Zhao, P; Xia, QY. 2016

Male accessory gland (MAG) and female accessory gland (FAG) of the reproductive system are, respectively, responsible for producing seminal proteins and adhesive proteins during copulation and ovulation. Seminal proteins are ejaculated to female along with sperms, whereas adhesive proteins are excreted along with eggs. Proteins from the male and female reproductive organs are usually indicative of rapid adaptive evolution. Understanding the reproductive isolation and species divergence requires identifying reproduction-related proteins from many different species. Here, we present our proteomic analyses of male and female accessory glands of the silkworm, *Bombyx mori*. Using LC/MS-MS, we identified 2133 MAG proteins and 1872 FAG proteins. In total, 652 proteins were significant more abundant in the MAG than in the FAG, including growth factors, odorant-binding proteins, enzymes, and proteins of unknown function. Growth factors and odorant-binding proteins are potential signaling molecules, whereas most of proteins of unknown function were found to be Lepidoptera-specific proteins with high evolutionary rates. Microarray experiments and semi-quantitative RT-PCR validated that MAG-specific proteins were expressed exclusively in male moths. Totally, 192 proteins were considered as FAG-specific proteins, including protease inhibitors, enzymes, and other proteins. Protease inhibitors were found to be the most abundant FAG-specific proteins, which may protect eggs from infection by inhibiting pathogen-derived proteases. These results provide comprehensive insights into copulation and oviposition. Moreover, the newly identified Lepidoptera-specific MAG proteins provide useful data for future research on the evolution of reproductive proteins in insects.

Identification of polyenic hydrocarbons from the northern winter moth, *Operophtera fagata*, and development of a species specific lure for pheromone traps

Szocs, G; Toth, M; Karpati, Z; Zhu, JW; Lofstedt, C; Plass, E; Francke, W. 2004

In order to elucidate the composition of the female sex pheromone of the northern (beech) winter moth, *Operophtera fagata* Scharf. (Lepidoptera: Geometridae), ovipositor extracts of unmated, calling females were analysed by gas chromatography with simultaneous electroantennographic and flame ionization detection (GC-EAD/FID). Male antennal responses indicated three active components, two of which had distinct matching peaks in the FID trace. Using coupled gas chromatography-mass spectrometry (GC-MS), these two compounds were identified as (9Z)-nonadecene (9Z-19:Hy), and (6Z,9Z)-nonadecadiene (6Z9Z-19:Hy), respectively. The third component, present in very small amounts only, was identified as (1,3Z,6Z,9Z)-nonadecatetraene (1,3Z6Z9Z-19:Hy), known as the sex pheromone of the common winter moth, *O. brumata*. Field tests revealed that traps baited with 6Z9Z-19:Hy and 1,3Z6Z9Z-19:Hy caught large numbers of male *O. fagata*. Both compounds were found to be essential for attraction of *O. fagata*. In addition, the diene prevented captures of co-occurring *O. brumata*. In contrast, 9Z-19:Hy neither influenced the attractiveness of the two-component mixture towards *O. fagata* nor contributed to bait specificity. A binary mixture of 6Z9Z-19:Hy and 1,3Z6Z9Z-19:Hy in a ratio of 10:1, applied to pieces of rubber tubing, constituted a highly attractive and species-specific bait for *O. fagata*, which can be used for monitoring of the flight of this defoliator pest of deciduous forests.

Electrophysiological and behavioral activities of sex pheromone and structurally related compounds in lightbrown apple moth, *Epiphyas postvittana*

Roh, GH; Park, KC. 2022

Species-specific pheromone communication in moths is often achieved by the precise control of the production of a multi-component sex pheromone blend in females and selective perception of pheromone compounds in males. Reproductive isolation mediated by sex pheromone can be enhanced by the sensitive detection of structurally related non-pheromone components that are not used as pheromone in the same species but used as pheromone components in similar species. Here, we identified several unsaturated aliphatic acetates inhibiting the attraction of male moths to conspecific female sex pheromone in the lightbrown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), through electroantennogram (EAG) and field trapping studies. In EAG screening with 46 pheromone and structurally related compounds, eleven compounds exhibited significant male-specific EAG responses at 1 μ g dose. The EAG-active compounds were mainly mono- or di-unsaturated 14-carbon acetates. In subsequent field trapping tests to evaluate the behavioral activities of the EAG-active compounds on male attraction to the binary blend (E11-14:Ac + E9E11-14:Ac) of female sex pheromone of *E. postvittana*, each of nine compounds (E9-12:Ac, Z9-12:Ac, E9-14:Ac, Z9-14:Ac, Z10-14:Ac, Z11-14:Ac, Z12-14:Ac, Z9E11-14:Ac and Z9E12-14:Ac) displayed clear inhibition of male moths to the sex pheromone blend in a dose-dependent manner. Our findings provide useful information in understanding the pheromone communication system of *E. postvittana* and related species.

3-Acetoxy-fatty acid isoprenyl esters from androconia of the ithomiine butterfly *Ithomia salapia*

Mann, F; Szczerbowski, D; de Silva, L; McClure, M; Elias, M; Schulz, S. 2020

Male ithomiine butterflies (Nymphalidae: Danainae) have hairpencils on the forewings (i.e., androconia) that disseminate semiochemicals during courtship. While most ithomiines are known to contain derivatives of pyrrolizidine alkaloids, dihydropyrrolizines, or gamma-lactones in these androconia, here we report on a new class of fatty acid esters identified in two subspecies, *Ithomia salapia* aquinia and *I. s. derasa*. The major components were identified as isoprenyl (3-methyl-3-butenyl) (Z)-3-acetoxy-11-octadecenoate, isoprenyl (Z)-3-acetoxy-13-octadecenoate (12) and isoprenyl 3-acetoxyoctadecanoate (11) by GC/MS and GC/IR analyses, microderivatizations, and synthesis of representative compounds. The absolute configuration of 12 was determined to be R. The two subspecies differed not only in the composition of the ester bouquet, but also in the composition of more volatile androconial constituents. While some individuals of *I. s. aquinia* contained ithomiolide A (3), a pyrrolizidine alkaloid derived gamma-lactone, *I. s. derasa* carried the sesquiterpene alpha-elemol (8) in the androconia. These differences might be important for the reproductive isolation of the two subspecies, in line with previously reported low gene exchange between the two species in regions where they co-occur. Furthermore, the occurrence of positional isomers of unsaturated fatty acid derivatives indicates activity of two different desaturases within these butterflies, Delta 9 and Delta 11, which has not been reported before in male Lepidoptera.

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

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

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

selection from these data alone. Our statistical approach provides a useful general method for estimating mate choice in the wild.

Molecular and functional characterization of a candidate sex pheromone receptor OR1 in *Spodoptera litura*

Zhang, QH; Wu, ZN; Zhou, JJ; Du, YJ. 2017

Olfaction is primarily mediated by highly specified olfactory receptors (ORs). Here, we cloned and identified an olfactory receptor, named SlituOR1 (Genbank no. JN835269), from *Spodoptera litura* and found evidence that it is a candidate pheromone receptor. It exhibited male-biased expression in the antennae, where it was localized at the base of sensilla trichoidea, the antennal sensilla mainly responsive to pheromones in moths. Conserved orthologues of this receptor, found among known pheromone receptors within the Lepidoptera, and SlituOR1 were placed among a clade of candidate pheromone receptors in a phylogeny tree of insect OR gene sequences. SlituOR1 showed differential expression in *S. litura* populations attracted to traps baited with different ratios of the two sex pheromone components (9Z, 11E)-tetradecadienyl acetate (Z9E11-14: OAc) and (9Z, 12E)-tetradecadienyl acetate (Z9E12-14: OAc). Knocking down of SlituOR1 by RNA interference reduced the electroantennogram (EAG) response to Z9E11-14: OAc, and this result is consistent with the field trapping experiment. We infer that variation in transcription levels of olfactory receptors may modulate sex pheromone perception in male moths and could provide some of the flexibility required to maintain the functionality of communication with females when a population is adapting to a new niche and reproductive isolation becomes an advantage.

The fate of W chromosomes in hybrids between wild silkmoths, *Samia cynthia* ssp.: no role in sex determination and reproduction

Yoshido, A; Marec, F; Sahara, K. 2016

Moths and butterflies (Lepidoptera) have sex chromosome systems with female heterogamety (WZ/ZZ or derived variants). The maternally inherited W chromosome is known to determine female sex in the silkworm, *Bombyx mori*. However, little is known about the role of W chromosome in other lepidopteran species. Here we describe two forms of the W chromosome, W and neo-W, that are transmitted to both sexes in offspring of **hybrids** from reciprocal crosses between subspecies of wild silkmoths, *Samia cynthia*. We performed crosses between *S. c. pryeri* (2n= 28, WZ/ZZ) and *S. c. walkeri* (2n = 26, neo-Wneo-Z/neo-Zneo-Z) and examined fitness and sex chromosome constitution in their **hybrids**. The F1 **hybrids** of both reciprocal crosses had reduced fertility. Fluorescence in situ **hybridization** revealed not only the expected sex chromosome constitutions in the backcross and F2 **hybrids** of both sexes but also females without the W (or neo-W) chromosome and males carrying the W (or neo-W) chromosome. Furthermore, crosses between the F2 **hybrids** revealed no association between the presence or absence of W (or neo-W) chromosome and variations in the hatchability of their eggs. Our results clearly suggest that the W (or neo-W) chromosome of *S. cynthia* ssp. plays no role in sex determination and reproduction, and thus does not contribute to the formation of reproductive barriers between different subspecies.

Sex pheromones of *Thysanoplusia intermixta* and *T-orichalcea*: Identification and field tests

Ando, T; Inomata, S; Shimada, R; Nomura, M; Uehara, S; Pu, GQ. 1998

Thysanoplusia intermixta, which inhabits the temperate zone in Japan, is a defoliator of plants in the family Compositae, Apiaceae, and others. By GC-MS analysis, (Z)-7-dodecenyl acetate, (Z)-7-dodecen-1-ol, (5E, 7Z)-5,7-dodecadienyl acetate and (5E,7Z)-5,7-dodecadien-1-ol were identified from a pheromone gland extract of the virgin females in a ratio of 18:3:100: 13. A lure baited only with the major diene acetate could attract male moths of *T. intermixta* in the field. Other minor components had a synergistic effect on the attraction of the diene acetate. The same four components were included in a very different ratio of 100:6:11:1 in the gland extract of *Thysanoplusia orichalcea*, a closely related subtropical species. The monoene and diene acetates are essential for field attraction, and a 100:11 mixture is an optimum lure for *T. orichalcea* males. However, this mixture, including the diene acetate as a minor component, captured very few *T. intermixta* males, suggesting the possibility that these two *Thysanoplusia* species are reproductively isolated from each other by pheromonal communication.

Sex pheromone components of pitch pine looper, *Lambdina pellucidaria*

Maier, CT; Gries, R; Gries, G. 1998

Two methylated hydrocarbons, 7-methylheptadecane (7) and 7,11-dimethylheptadecane (7,11), are sex pheromone components of female pitch pine looper (PPL), *Lambdina pellucidaria*. Compounds extracted from the pheromone glands of female moths were identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) and coupled GC-mass spectrometry (GC-MS) in selected ion monitoring mode. In field-trapping experiments, 7 and 7,11 in combination, but not singly, attracted numerous male moths. 5,11-Dimethylheptadecane (5,11) was detected by GC-EAD in female PPL pheromone gland extract, but did not significantly increase attraction of PPL males to 7 plus 7,11. Although 7 was > 10 times more abundant than 7,11 in pheromone gland extracts, traps baited with synthetic 7 plus 7,11 at a blend ratio of 1:1, rather than 1:0.1 or 1:0.01, captured the most PPL males. The chemical communication of PPL and spring hemlock looper (SHL), *Lambdina athasaria*, is strikingly similar. Both species employ 7 plus 7,11 as sex pheromone. Restriction of SHL to forests with eastern hemlock or balsam fir and PPL to forests with pitch or other hard pines contributes to their reproductive isolation. PPL and SHL may also use different optical isomers of enantiomeric 7 and stereoisomeric 7,11 to maintain specificity of their chemical communication.

Sex Pheromones of Three Citrus Leafrollers, *Archips atrolucens*, *Adoxophyes privatana*, and *Homona* sp., Inhabiting the Mekong Delta of Vietnam

Vang, LV; Thuy, HN; Chau, NQK; Son, PK; Yan, Q; Yamamoto, M; Jinbo, U; Ando, T. 2013

Archips atrolucens, *Adoxophyes privatana*, and *Homona* sp. are serious defoliators of citrus trees in the Mekong Delta of Vietnam. In order to establish a sustainable pest-management program for the three species, their female-produced sex pheromones were investigated by GC-EAD and GC-MS analyses, and the following multi-component pheromones were identified: (Z)-11-tetradecenyl acetate (Z11-14:OAc), (E)-11-tetradecenyl acetate (E11-14:OAc), and tetradecyl acetate (14:OAc) in a ratio of 64:32:4 for *A. atrolucens*; Z11-14:OAc and (Z)-9-tetradecenyl acetate (Z9-14:OAc) in a ratio of 92:8 for *A. privatana*; and Z11-14:OAc and (Z)-9-dodecenyl acetate (Z9-12:OAc) in a ratio of 96:4 for *Homona* sp. Each lure baited with synthetic components as a mimic of the natural pheromone attracted males of the target species specifically, indicating that each monounsaturated minor component plays a significant role for mating communication and reproductive isolation of the three species inhabiting the same citrus orchards. In an extract of the pheromone glands of *A. atrolucens* females, the content of 14:OAc was very low, but a synergistic effect was observed clearly when the saturated compound was mixed at the same level as the E11-14:OAc. The synthetic lures will provide useful tools for monitoring flights of adults of the three species.

Identification and Differential Expression of a Candidate Sex Pheromone Receptor in Natural Populations of *Spodoptera litura*

Lin, XD; Zhang, QH; Wu, ZN; Du, YJ. 2015

Olfaction is primarily mediated by highly specific olfactory receptors (ORs), a subfamily of which are the pheromone receptors that play a key role in sexual

communication and can contribute to reproductive isolation. Here we cloned and identified an olfactory receptor, SlituOR3 (Genbank NO. JN835270), from *Spodoptera litura*, to be the candidate pheromone receptor. It exhibited male-biased expression in the antennae, where they were localized at the base of sensilla trichoidea. Conserved orthologues of these receptors were found amongst known pheromone receptors within the Lepidoptera, and SlituOR3 were placed amongst a clade of candidate pheromone receptors in a phylogeny tree of insect ORs. SlituOR3 is required for the EAG responses to both Z9E11-14:OAc and Z9E12-14:OAc. SlituOR3 showed differential expression in *S. litura* populations attracted to traps baited with a series of sex pheromone blends composed of different ratios of (9Z, 11E)-tetradecadienyl acetate (Z9E11-14:OAc) and (9Z, 12E)-tetradecadienyl acetate (Z9E12-14:OAc). The changes in the expression level of SlitOR3 and antennal responses after SlitOR3 silencing suggested that SlitOR3 is required for the sex pheromone signaling. We infer that variation in transcription levels of olfactory receptors may modulate sex pheromone perception in male moths and could affect both of pest control and monitoring efficiency by pheromone application after long time mass trapping with one particular ratio of blend in the field.

Pheromone components and diel periodicity of pheromonal communication in *Lymantria fumida*

Schaefer, PW; Gries, G; Gries, R; Holden, D. 1999

Extracts of pheromone glands from female *Lymantria fumida* were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) and by coupled GC-mass spectrometry (MS). The two compounds that elicited responses from male *L. fumida* antennae were identified as cis-7,8-epoxy-2-methyloctadecane (disparlure) and 2-methyl-Z7-octadecene (2me-Z7-18Hy). Field experiments in northern Japan demonstrated that synthetic (7R,8S)-cis-7,8-epoxy-2-methyloctadecane [(+)-disparlure] and 2me-Z7-18Hy are synergistic sex pheromone components of *L. fumida*. (7S,8R)-cis-7,8-Epoxy-2-methyloctadecane [(-)-disparlure] had no behavioral effect on male *L. fumida*. Traps baited with (+)-disparlure and 2me-Z7-18Hy captured male *L. fumida* between 21:00 and 24:00 hr, whereas traps baited with (+)-monachalure [(7R,8S)-cis-7,8-epoxy-octadecane], (+)-disparlure and 2me-Z7-18Hy attracted males of the nun moth, *L. monacha* L., between 02:00 and 04:00 hr. Both temporal separation of pheromonal communication and specificity of pheromone blends seem to contribute to the reproductive isolation of sympatric and coseasonal *L. fumida* and *L. monacha*.

Consequences of reproductive barriers for genealogical discordance in the European corn borer

Dopman, EB; Perez, L; Bogdanowicz, SM; Harrison, RG. 2005

Speciation involves the origin of trait differences that limit or prevent gene exchange and ultimately results in daughter populations that form monophyletic or exclusive genetic groups. However, for recently diverged populations or species between which reproductive isolation is often incomplete, gene genealogies will be discordant, and most regions of the genome will display nonexclusive genealogical patterns. In these situations, genome regions for which one or both species are exclusive groups may mark the footprint of recent selective sweeps. Alternatively, such regions may include or be closely linked to "speciation genes," genes involved in reproductive isolation. Therefore, comparisons of gene genealogies allow inferences about the genetic architectures of both reproductive isolation and adaptation. Contrasting genealogical relationships in sexually isolated pheromone strains of the European corn borer moth (*Ostrinia nubilalis*) demonstrate the relevance of this approach. Genealogies for five gene regions are discordant, and only one molecular marker, the sex-linked gene *Tpi*, has evidence for pheromone strain exclusivity. *Tpi* maps to a position on the sex chromosome that is indistinguishable from a major factor (*Pdd*) affecting differences in postdiapause development time. The major factor (*Resp*) determining male behavioral response to pheromone is also sex-linked, but maps 20-30 cM away. Exclusivity at *Tpi* may be a consequence of these linkage relationships because evidence from phenotypic variation in natural populations implicates both *Pdd* and *Resp* as candidates for genes involved in recent sweeps and/or reproductive isolation between strains.

Chemical signals act as the main reproductive barrier between sister and mimetic *Heliconius* butterflies

Gonzalez-Rojas, MF; Darragh, K; Robles, J; Linares, M; Schulz, S; McMillan, WO; Jiggins, CD; Pardo-Diaz, C; Salazar, C. 2020

Colour pattern is the main trait that drives mate recognition between *Heliconius* species that are phylogenetically close. However, when this cue is compromised such as in cases of mimetic, sympatric and closely related species, alternative mating signals must evolve to ensure reproductive isolation and species integrity. The closely related species *Heliconius melpomene malleti* and *H. timareta florencea* occur in the same geographical region, and despite being co-mimics, they display strong reproductive isolation. In order to test which cues differ between species, and potentially contribute to reproductive isolation, we quantified differences in the wing phenotype and the male chemical profile. As expected, the wing colour pattern was indistinguishable between the two species, while the chemical profile of the androconial and genital males' extracts showed marked differences. We then conducted behavioural experiments to study the importance of these signals in mate recognition by females. In agreement with our previous results, we found that chemical blends and not wing colour pattern drive the preference of females for conspecific males. Also, experiments with **hybrid** males and females suggested an important genetic component for both chemical production and preference. Altogether, these results suggest that chemicals are the major reproductive barrier opposing gene flow between these two sister and co-mimic species.

The period gene and allochronic reproductive isolation in *Bactrocera cucurbitae*

Miyatake, T; Matsumoto, A; Matsuyama, T; Ueda, HR; Toyosato, T; Tanimura, T. 2002

Clock genes that pleiotropically control circadian rhythm and the time of mating may cause allochronic reproductive isolation in the melon fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Flies with a shorter circadian period (ca. 22 h of locomotor activity rhythm) mated 5 h earlier in the day than those with a longer circadian period (ca. 30 h). Mate-choice tests demonstrated significant pre-mating isolation between populations with short and long circadian periods. Pre-mating isolation did not occur when the mating time was synchronized between the two populations by photoperiodic controls, indicating that reproductive isolation is due to variations in the time of mating and not any unidentified ethological difference between the two populations. We cloned the period (*per*) gene of *B. cucurbitae* that is homologous to the *per* gene in *Drosophila*. The relative level of *per* mRNA in the melon fly exhibited a robust daily fluctuation under light : dark conditions. The fluctuation of *per* expression under dark : dark conditions is closely correlated to the locomotor rhythm in *B. cucurbitae*. These results suggest that clock genes can cause reproductive isolation via the pleiotropic effect as a change of mating time.

Ecological speciation in mimetic butterflies

Jiggins, CD. 2008

There has been a recent revival of interest in the role of ecology in speciation. The wing patterns of *Heliconius* butterflies are signals to predators as well as mates, and can cause strong reproductive isolation between populations. Reproductive isolation has been studied in some detail between the sympatric species *Heliconius melpomene* and *Heliconius cydno*, and in reviewing this work I show that habitat isolation and color pattern preference are by far the most important factors causing speciation. The surprising observation that genes for mate preference and color pattern are genetically associated implies divergence in sympatry or resulting from sexual selection. Color pattern is therefore an example of an ecological trait that contributes to speciation through pleiotropic effects on mate choice, although phylogenetic evidence shows that it is only one of many factors responsible for speciation in mimetic butterflies.

Introgression between divergent corn borer species in a region of sympatry: Implications on the evolution and adaptation of pest arthropods

Wang, YZ; Kim, KS; Guo, WC; Li, QY; Zhang, YY; Wang, ZY; Coates, BS. 2017

The Asian corn borer, *Ostrinia furnacalis*, and European corn borer, *O. nubilalis* (Lepidoptera: Crambidae), cause damage to cultivated maize in spatially distinct geographies and have evolved divergent hydrocarbons as the basis of sexual communication. The Yili area of Xinjiang Uyghur Autonomous Region in China represents the only known region where *O. furnacalis* has invaded a native *O. nubilalis* range, and these two corn borer species have made secondary contact. Genetic differentiation was estimated between *Ostrinia* larvae collected from maize plants at 11 locations in Xinjiang and genotyped using high-throughput SNP and microsatellite markers. Maternal lineages were assessed by direct sequencing of mitochondrial cytochrome c oxidase subunit I and II haplotypes, and a high degree of genotypic diversity was demonstrated between lineages based on SNP genotypes. Furthermore, historical introgression was predicted among SNP genotypes only at sympatric locations in the Yili area, whereas in Xinjiang populations only *O. furnacalis* haplotypes were detected and no analogous introgressed genotypes were predicted. Our detection of putative **hybrids** and historical evidence of introgression defines Yili area as a **hybrid** zone between the species in normal ecological interactions and furthermore, might indicate that adaptive traits could spread even between seemingly divergent species through horizontal transmission. Results of this study indicate there may be a continuum in the degree of reproductive isolation between *Ostrinia* species and that the elegance of distinct and complete speciation based on modifications to the pheromone communication might need to be reconsidered.

Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae)

Huber, FK; Kaiser, R; Sauter, W; Schiestl, FP. 2005

We investigated scent composition and pollinator attraction in two closely related orchids, *Gymnadenia conopsea* (L.) R.Br. s.l. and *Gymnadenia odoratissima* (L.) Rich. in four populations during the day and night. We collected pollinators of both species using hand nets and sampled floral odour by headspace sorption. We analysed the samples by gas chromatography with mass spectrometry to identify compounds and with electroantennographic detection to identify compounds with physiological activity in pollinators. In order to evaluate the attractiveness of the physiologically active compounds, we carried out trapping experiments in the field with single active odour substances and mixtures thereof. By collecting insects from flowers, we caught eight pollinators of *G. conopsea*, which were members of four Lepidoptera families, and 37 pollinators of *G. odoratissima*, from five Lepidopteran families. There was no overlap in pollinator species caught from the two orchids using nets. In the scent analyses, we identified 45 volatiles in *G. conopsea* of which three (benzyl acetate, eugenol, benzyl benzoate) were physiologically active. In *G. odoratissima*, 44 volatiles were identified, of which seven were physiologically active (benzaldehyde, phenylacetaldehyde, benzyl acetate, 1-phenyl-2,3-butanedione, phenylethyl acetate, eugenol, and one unknown compound). In field bioassays using a mixture of the active *G. odoratissima* compounds and phenylacetaldehyde alone we caught a total of 25 moths, some of which carried *Gymnadenia* pollinia. A blend of the active *G. conopsea* volatiles placed in the *G. odoratissima* population did not attract any pollinators. The two orchids emitted different odour bouquets during the day and night, but *G. odoratissima* showed greater temporal differences in odour composition, with phenylacetaldehyde showing a significant increase during the night. The species differed considerably in floral odour emission and this differentiation was stronger in the active than non-active compounds. This differentiation of the two species, especially in the emission of active compounds, appears to have evolved under selection for attraction of different suites of Lepidopteran pollinators.

Genomics of adaptation to host-plants in herbivorous insects

Simon, JC; d'Alençon, E; Guy, E; Jacquin-Joly, E; Jaquiere, J; Nouhaud, P; Peccoud, J; Sugio, A; Streiff, R. 2015

Herbivorous insects represent the most species-rich lineages of metazoans. The high rate of diversification in herbivorous insects is thought to result from their specialization to distinct host-plants, which creates conditions favorable for the build-up of reproductive isolation and speciation. These conditions rely on constraints against the optimal use of a wide range of plant species, as each must constitute a viable food resource, oviposition site and mating site for an insect. Utilization of plants involves many essential traits of herbivorous insects, as they locate and select their hosts, overcome their defenses and acquire nutrients while avoiding intoxication. Although advances in understanding insect-plant molecular interactions have been limited by the complexity of insect traits involved in host use and the lack of genomic resources and functional tools, recent studies at the molecular level, combined with large-scale genomics studies at population and species levels, are revealing the genetic underpinning of plant specialization and adaptive divergence in non-model insect herbivores. Here, we review the recent advances in the genomics of plant adaptation in hemipterans and lepidopterans, two major insect orders, each of which includes a large number of crop pests. We focus on how genomics and post-genomics have improved our understanding of the mechanisms involved in insect-plant interactions by reviewing recent molecular discoveries in sensing, feeding, digesting and detoxifying strategies. We also present the outcomes of large-scale genomics approaches aimed at identifying loci potentially involved in plant adaptation in these insects.

Wolbachia and reproductive conflict in *Exorista sorbillans*

Puttaraju, HP; Prakash, BM. 2005

Many arthropods harbour endosymbiotic bacteria of the genus *Wolbachia*. These endosymbionts are transmitted vertically from one generation to the next and are obligatory in several Dipterans that have been studied to date. These bacteria induce an array of reproductive isolation mechanisms that are implicated in pest management to evolutionary biology of respective hosts. The uzifly, *Exorista sorbillans*, a tachinid endoparasitoid of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), causes enormous losses to the silk industry, now it is known that it harbours *Wolbachia* endobacteria. The elimination of *Wolbachia* by antibiotics interrupts embryogenesis and causes various reproductive conflicts such as (1) a reduction of fecundity of uninfected female, (2) cytoplasmic incompatibility in the uninfected females crossed with infected males, (3) genomic incompatibility in crosses between males and females from uninfected population, and (4) sex-ratio distortion in uninfected females irrespective of the presence of *Wolbachia* in males. These results suggest that the relationship of *Wolbachia* with its uzifly host is one of mutual symbiosis as it controls the reproductive physiology of its host.

Homoploid hybrid speciation and genome evolution via chromosome sorting

Lukhtanov, VA; Shapoval, NA; Anokhin, BA; Saifitdinova, AF; Kuznetsova, VG. 2015

Genomes of numerous diploid plant and animal species possess traces of interspecific crosses, and many researches consider them as support for homoploid **hybrid** speciation (HHS), a process by which a new reproductively isolated species arises through **hybridization** and combination of parts of the parental genomes, but without an increase in ploidy. However, convincing evidence for a creative role of **hybridization** in the origin of reproductive isolation between **hybrid** and parental forms is extremely limited. Here, through studying *Agrodiaetus* butterflies, we provide proof of a previously unknown mode of HHS based on the formation of post-zygotic reproductive isolation via **hybridization** of chromosomally divergent parental species and subsequent fixation of a novel combination of chromosome fusions/fissions in **hybrid** descendants. We show that meiotic segregation, operating in the **hybrid** lineage, resulted in the formation of a new diploid genome, drastically rearranged in terms of chromosome number. We also demonstrate that during the heterozygous stage of the **hybrid** species formation, recombination was limited between rearranged chromosomes of different parental origin, representing evidence that the reproductive isolation was a direct consequence of **hybridization**.

Chemical Composition of Female Sexual Glands of *Spodoptera frugiperda* Corn and Rice Strains from Tolima, Colombia

Canas-Hoyos, N; Lobo-Echeverri, T; Saldamando-Benjumea, CI. 2017

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a Neotropical polyphagous moth that is an important pest of maize, *Zea mays* L., in the Western Hemisphere. In the Americas, including Colombia, the insect also is a pest of rice, *Oryza sativa* L.; sorghum, *Sorghum bicolor* (L.) Moench; cotton, *Gossypium hirsutum* L.; and sugarcane, *Saccharum officinarum* L. The species in Colombia has diverged into strains in maize and rice as their most frequent hosts. Studies of reproductive isolation in Colombia showed that females of the corn strain rarely mated with males of the rice strain while females of the rice strain mated with both strains. Because behavioral isolation can be influenced by chemical composition of female sexual glands, we examined the volatile compounds for both strains, considering the time of extraction and male stimulation in production of metabolites. Altogether, 27 compounds were detected by gas chromatography coupled to mass spectrometry in the female sexual glands of both strains under laboratory conditions. The most relevant were the pheromones (Z)-9-tetradecenyl acetate and (Z)-7-dodecenyl acetate, among other volatiles reported for Lepidoptera. While 11 constituents were exclusive for the rice strain and eight for the corn strain, nine were common to both strains. A detrended correspondence analysis associated a group of compounds with each strain, while no significant differences were found in the abundance of compounds in common (T-test/Mann Whitney). In addition, the production of compounds was optimal after 3 hours of the scotophase for the corn strain and after 6 hours for the rice strain, while the presence of males was a positive influence in the production of compounds for females of both strains.

Reproductive isolation and genetic variation between two "strains" of *Bracon hebetor* (Hymenoptera: Braconidae)

Heimpel, GE; Antolin, MF; Franqui, RA; Strand, MR. 1997

Bracon hebetor Say (Hymenoptera: Braconidae) is known primarily as a parasitoid of pyralid moth larvae infesting stored grain. In the 1970s, a parasitoid identified as *B. hebetor* was released for control of *Heliothis/Helicoverpa* spp. (Lepidoptera: Noctuidae) on the island of Barbados. Because life-history traits of this parasitoid differed from those reported for *B. hebetor* from the United States, we conducted a series of laboratory experiments to determine whether this parasitoid was (i) a population of *B. hebetor* that attacks noctuids in the held or (ii) a different species from *B. hebetor*. We confirmed that *Heliothis virescens* (F.) was a more suitable host for the Barbados strain than for *B. hebetor*. However, a stored-grain infesting pyralid, *Plodia interpunctella* (Hubner), was a more suitable host for the Barbados strain than was *H. virescens*. Reciprocal crosses between the Barbados strain and *B. hebetor* showed that the two populations were reproductively isolated. No mating was observed during a series of 30-min observations of reciprocal crosses, and the crosses produced only male offspring. Examination of each female's spermatheca confirmed that females were not fertilized. Sequence analysis of a 517-bp fragment of the mitochondrial 16S rRNA gene revealed that two populations of *B. hebetor* from our laboratory were identical but differed in sequence by 2% from the Barbados strain. Collectively, our results indicate that the Barbados strain is a distinct species from *B. hebetor*. (C) 1997 Academic Press.

Sex pheromone of Nuttall's sheep moth, *Hemileuca nuttalli*, from the eastern Sierra Nevada Mountains of California

McElfresh, JS; Millar, JG. 1999

The sex attractant pheromone blend of *Hemileuca nuttalli* (Lepidoptera: Saturniidae) from the eastern slope of the Sierra Nevada Mountains in California was determined to be a combination of E10,Z12-hexadeca-10, 12-dien-1-yl acetate (E10,Z12-16:Ac) and E10,E12-hexadeca-10, 12-dien-1-yl acetate (E10,E12-16:Ac). Ratios of the compounds in extracts of female pheromone glands varied around a mean of 100:48, although the ratio was not critical in field trials. Blends of synthetic E10,Z12-16:Ac and E10,E12-16:Ac in 100:50, 50: 100, and 100: 100 ratios attracted equal numbers of male moths. Field trials also indicated that E10,Z12-hexadeca-10,12-dien-1-ol (E10,Z12-16:OH) and E10,Z12-hexadeca-10,12-dienal (E10, Z12-16:Ald) were antagonistic at rates of 3.3% and 10%, respectively, of the E10,Z12-16:Ac, despite being found in female moth extracts. E10,E12-16:Ac, E10,Z12-16:OH, and E10,Z12-16:Ald all appear to have roles in maintenance of reproductive isolation between *H. nuttalli* and *H. eglanteria*.

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

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

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

Hybridization and postzygotic isolation patterns in pigeons and doves

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

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

The Male Sex Pheromone of the Butterfly *Bicyclus anynana*: Towards an Evolutionary Analysis

Nieberding, CM; de Vos, H; Schneider, MV; Lassance, JM; Estramil, N; Andersson, J; Bang, J; Hedenstrom, E; Lofstedt, C; Brakefield, PM. 2008

Background: Female sex pheromones attracting mating partners over long distances are a major determinant of reproductive isolation and speciation in Lepidoptera.

Males can also produce sex pheromones but their study, particularly in butterflies, has received little attention. A detailed comparison of sex pheromones in male butterflies with those of female moths would reveal patterns of conservation versus novelty in the associated behaviours, biosynthetic pathways, compounds, scent-releasing structures and receiving systems. Here we assess whether the African butterfly *Bicyclus anynana*, for which genetic, genomic, phylogenetic, ecological and ethological tools are available, represents a relevant model to contribute to such comparative studies. **Methodology/Principal Findings:** Using a multidisciplinary approach, we determined the chemical composition of the male sex pheromone (MSP) in the African butterfly *B. anynana*, and demonstrated its behavioural activity. First, we identified three compounds forming the presumptive MSP, namely (Z)-9-tetradecenol (Z9-14:OH), hexadecanal (16:Ald) and 6,10,14-trimethylpentadecan-2-ol (6,10,14-trime-15-2-ol), and produced by the male secondary sexual structures, the androconia. Second, we described the male courtship sequence and found that males with artificially reduced amounts of MSP have a reduced mating success in semi-field conditions. Finally, we could restore the mating success of these males by perfuming them with the synthetic MSP. **Conclusions/Significance:** This study provides one of the first integrative analyses of a MSP in butterflies. The toolkit it has developed will enable the investigation of the type of information about male quality that is conveyed by the MSP in intraspecific communication. Interestingly, the chemical structure of *B. anynana* MSP is similar to some sex pheromones of female moths making a direct comparison of pheromone biosynthesis between male butterflies and female moths relevant to future research. Such a comparison will in turn contribute to understanding the evolution of sex pheromone production and reception in butterflies.

'Becoming a species by becoming a pest' or how two maize pests of the genus *Ostrinia* possibly evolved through parallel ecological speciation events

Bourguet, D; Ponsard, S; Streiff, R; Meusnier, S; Audiot, P; Li, J; Wang, ZY. 2014

New agricultural pest species attacking introduced crops may evolve from pre-existing local herbivores by ecological speciation, thereby becoming a species by becoming a pest. We compare the evolutionary pathways by which two maize pests (the Asian and the European corn borers, ACB and ECB) in the genus *Ostrinia* (Lepidoptera, Crambidae) probably diverged from an ancestral species close to the current Adzuki bean borer (ABB). We typed larval *Ostrinia* populations collected on maize and dicotyledons across China and eastern Siberia, at microsatellite and mitochondrial loci. We found only two clusters: one on maize (as expected) and a single one on dicotyledons despite differences in male mid-tibia morphology, suggesting that all individuals from dicotyledons belonged to the ABB. We found evidence for migrants and **hybrids** on both host plant types. **Hybrids** suggest that field reproductive isolation is incomplete between ACB and ABB. Interestingly, a few individuals with an ABB-like microsatellite profile collected on dicotyledons had ACB mtDNA rather than ABB-like mtDNA, whereas the reverse was never found on maize. This suggests asymmetrical gene flow directed from the ACB towards the ABB. **Hybrids** and backcrosses in all directions were obtained in no-choice tests. In laboratory conditions, they survived as well as parental strain individuals. In Xinjiang, we found ACB and ECB in sympatry, but no **hybrids**. Altogether, our results suggest that reproductive isolation between ACB and ABB is incomplete and mostly prezygotic. This points to ecological speciation as a possible evolutionary scenario, as previously found for ECB and ABB in Europe.

Does divergent selection predict the evolution of mate preference and reproductive isolation in the tropical butterfly genus *Melinaea* (Nymphalidae: Ithomiini)?

McClure, M; Mahrouche, L; Houssin, C; Monllor, M; Le Poul, Y; Frerot, B; Furtos, A; Elias, M. 2019

Many studies have shown that speciation can be facilitated when a trait under divergent selection also causes assortative mating. In Mullerian mimetic butterflies, a change in wing colour pattern can cause reproductive isolation. However, colour pattern divergence does not always lead to reproductive isolation. Understanding how divergent selection affects speciation requires identifying the mechanisms that promote mate preference and/or choosiness. This study addresses whether shifts in wing colour pattern drives mate preference and reproductive isolation in the tropical butterfly genus *Melinaea* (Nymphalidae: Ithomiini), and focuses on five taxa that form a speciation continuum, from subspecies to fully recognized species. Using genetic markers, wing colour pattern quantification, male pheromone characterization and behavioural assays of mating preference, we characterize the extent of genetic and phenotypic differentiation between taxa and compare it to the level of reproductive isolation. We show strong premating isolation between the closely related species *M. satevis* and *M. marsaeus*, in addition to genetic and phenotypic (colour pattern and pheromones) differentiation. By contrast, *M. menophilus* and *M. marsaeus* consist of pairs of subspecies that differ for colour pattern but that cannot be differentiated genetically. Pheromonal differentiation of subspecies was significant only for *M. marsaeus*, although most individuals were indistinguishable. *Melinaea menophilus* and *M. marsaeus* also differ in the strength of assortative mating, suggesting that mate preference has evolved only in *M. marsaeus*, consistent with selection against maladaptive offspring, as subspecific **hybrids** of *M. marsaeus* have intermediate, non-mimetic colour patterns, unlike those of *M. menophilus* which display either parental phenotypes. We conclude that a shift in colour pattern per se is not sufficient for reproductive isolation, but rather, the evolution of assortative mating may be caused by selection against maladaptive intermediate phenotypes. This study suggests that mate preference and assortative mating evolve when adaptive, and that even in the early stages of divergence, reproductive isolation can be nearly complete due to mating preferences.

Radiation of pollination systems in the iridaceae of sub-Saharan Africa

Goldblatt, P; Manning, JC. 2006

center dot Background Seventeen distinct pollination systems are known for genera of sub-Saharan African Iridaceae and recurrent shifts in pollination system have evolved in those with ten or more species. Pollination by long-tongued anthophorine bees foraging for nectar and coincidentally acquiring pollen on some part of their bodies is the inferred ancestral pollination strategy for most genera of the large subfamilies Iridoideae and Crocoideae and may be ancestral for the latter. Derived strategies include pollination by long-proboscid flies, large butterflies, night-flying hovering and settling moths, hopliine beetles and sunbirds. Bee pollination is diverse, with active pollen collection by female bees occurring in several genera, vibratile systems in a few and non-volatile oil as a reward in one species. Long-proboscid fly pollination, which is apparently restricted to southern Africa, includes four separate syndromes using different sets of flies and plant species in different parts of the subcontinent. Small numbers of species use bibionid flies, short-proboscid flies or wasps for their pollination; only about 2 % of species use multiple pollinators and can be described as generalists. center dot Scope Using pollination observations for 375 species and based on repeated patterns of floral attractants and rewards, we infer pollination mechanisms for an additional 610 species. Matching pollination system to phylogeny or what is known about species relationships based on shared derived features, we infer repeated shifts in pollination system in some genera, as frequently as one shift for every five or six species of southern African *Babiana* or *Gladiolus*. Specialized systems using pollinators of one pollination group, or even a single pollinator species are the rule in the family. Shifts in pollination system are more frequent in genera of Crocoideae that have bilaterally symmetric flowers and a perianth tube, features that promote adaptive radiation by facilitating precise shifts in pollen placement, in conjunction with changes in flower colour, scent and tube length. center dot Conclusions Diversity of pollination systems explains in part the huge species diversity of Iridaceae in sub-Saharan Africa, and permits species packing locally. Pollination shifts are, however, seen as playing a secondary role in speciation by promoting reproductive isolation in peripheral, ecologically distinct populations in areas of diverse topography, climate and soils. Pollination of Iridaceae in Eurasia and the New World, where the family is also well represented, is poorly studied but appears less diverse, although pollination by both pollen- and oil-collecting bees is frequent and bird pollination rare.

Karyotypic diversity and speciation in *Agrodiaetus* butterflies

Kandul, NR; Lukhtanov, VA; Pierce, NE. 2007

That chromosomal rearrangements may play an important role in maintaining postzygotic isolation between well-established species is part of the standard theory of speciation. However, little evidence exists on the role of karyotypic change in speciation itself in the establishment of reproductive barriers between previously interbreeding populations. The large genus *Agrodiaetus* (Lepidoptera: Lycaenidae) provides a model system to study this question. *Agrodiaetus* butterflies exhibit unusual interspecific diversity in chromosome number, from $n = 10$ to $n = 134$; in contrast, the majority of lycaenid butterflies have $n = 23/24$. We analyzed the

evolution of karyotypic diversity by mapping chromosome numbers on a thoroughly sampled mitochondrial phylogeny of the genus. Karyotypic differences accumulate gradually between allopatric sister taxa, but more rapidly between sympatric sister taxa. Overall, sympatric sister taxa have a higher average karyotypic diversity than allopatric sister taxa. Differential fusion of diverged populations may account for this pattern because the degree of karyotypic difference acquired between allopatric populations may determine whether they will persist as nascent biological species in secondary sympatry. This study therefore finds evidence of a direct role for chromosomal rearrangements in the final stages of animal speciation. Rapid karyotypic diversification is likely to have contributed to the explosive speciation rate observed in *Agrodiaetus*, 1.6 species per million years.

Effects of different sex pheromone compositions and host plants on the mating behavior of two *Grapholita* species

Jung, CR; Jung, JK; Kim, Y. 2013

The two congener species *Grapholita molesta* and *Grapholita dimorpha* share two major sex pheromone components: cis-8-dodecenyl acetate (Z8-12Ac) and trans-8-dodecenyl acetate (E8-12Ac). In fact, commercial sex pheromone lures composed of only these two major components attract the males of both species. In this study, we aimed to determine the reproductive isolation components of these two species by analyzing the effects of the minor sex pheromone components and host plants. First, different ratios of the two major sex pheromone components were greatly favored by either male species. Sex pheromone gland extracts of *G. dimorpha* contained a lesser proportion of Z8-12Ac than that of *G. molesta*. In the three (apple, pear, and peach) orchards investigated in this study, a larger number of *G. molesta* males were attracted to the 95:5 pheromone mixture (Z8-12Ac and E8-12Ac, respectively), while a larger number of *G. dimorpha* males were attracted to the 85:15 mixture. Second, there was a significant variation in male attractions in different host plants. *G. molesta* males were more attracted to the sex pheromone lure in the apple orchards than that in the pear and peach orchards. In contrast, *G. dimorpha* males were more attracted to the lures in the pear and peach orchards than that in the apple orchard. Third, the minor sex pheromone components were important for reproductive isolation. Among the four minor components tested, addition of (Z)-8-dodecenol (Z8-12OH) to the major sex pheromone components significantly suppressed male attraction in *G. dimorpha* and slightly elevated male attraction in *G. molesta*. The discriminating effect of Z8-12OH was further validated using male electroantennogram analysis. These results suggest that reproductive isolation between two congeners can be achieved by variations in the minor sex pheromone components and in the host plants, as well as by changes in the ratio of the two major components. (C) 2013 Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society. Published by Elsevier B.V. All rights reserved.

MATE PREFERENCE ACROSS THE SPECIATION CONTINUUM IN A CLADE OF MIMETIC BUTTERFLIES

Merrill, RM; Gompert, Z; Dembeck, LM; Kronforst, MR; McMillan, WO; Jiggins, CD. 2011

Premating behavioral isolation is increasingly recognized as an important part of ecological speciation, where divergent natural selection causes the evolution of reproductive barriers. A number of studies have now demonstrated that traits under divergent natural selection also affect mate preferences. However, studies of single species pairs only capture a snapshot of the speciation process, making it difficult to assess the role of mate preferences throughout the entire process. *Heliconius* butterflies are well known for their brightly colored mimetic warning patterns, and previous studies have shown that these patterns are also used as mate recognition cues. Here, we present mate preference data for four pairs of sister taxa, representing different stages of divergence, which together allow us to compare diverging mate preferences across the continuum of *Heliconius* speciation. Using a novel Bayesian approach, our results support a model of ecological speciation in which strong premating isolation arises early, but continues to increase throughout the continuum from polymorphic populations through to "good," sympatric ecologically divergent species.

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

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

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

2-Methyl-(Z)-7-octadecene: Sex pheromone of allopatric *Lymantria lucescens* and *L. serva*

Gries, G; Schaefer, PW; Gries, R; Fan, YB; Higashiura, Y; Tanaka, B. 2002

Our objective was to identify the sex pheromone of *Lymantria lucescens* and *Lymantria serva* (Lepidoptera: Lymantriidae), whose larvae defoliate, respectively, *Quercus* spp. in temperate regions and *Ficus* spp. in the subtropics. Coupled gas chromatographic-electroantennographic (GC-EAD) detection analyses of pheromone gland extracts revealed one EAD active compound produced by female *L. lucescens* and by female *L. serva*. This was identified as 2-methyl-(Z)-7-octadecene (2me-Z7-18Hy) by retention index calculations on DB-5, DB-23, and DB-210 columns and by comparative GC-mass spectrometric (MS) and GC-EAD analyses of the insect-produced candidate pheromone and synthetic 2me-Z7-18Hy. In field experiments, traps baited with 2me-Z7-18Hy captured male *L. lucescens* near Toyota City, Japan, and male *L. serva* in Taipei, Taiwan. Allopatric distribution of *L. lucescens* and *L. serva* seems to allow both species to use the same sex pheromone without compromising its specificity.

Two Sympatric Spodoptera Species Could Mutually Recognize Sex Pheromone Components for Behavioral Isolation

Yan, Q; Liu, XL; Wang, YL; Tang, XQ; Shen, ZJ; Dong, SL; Den, JY. 2019

Spodoptera exigua and *S. litura* are two sympatric species in China and many other countries. Both moths employ a multiple component sex pheromone blend, including a common component Z9, E12-14:OAc, and two specific components Z9-14:OH and Z11-16:OAc for *S. exigua*, and one specific component Z9, E11-14:OAc for *S. litura*. For the two species, it has been well documented that males are able to recognize and behaviorally attracted by their species-specific sex pheromone, which functions as a means of reproductive isolation, but whether males could mutually recognize pheromone components of its sympatric species is unknown. In

the present study, the electroantennogram (EAG) and field evaluation were conducted to address this topic. The EAG recordings revealed that males of each species could significantly respond to specific components of its sympatric species, although the response values were lower than that to its own major component. In field tests, the specific components Z9-14:OH and Z11-16:OAc of *S. exigua* strongly inhibited the male catches of *S. litura* to its conspecific sex pheromone, while specific component Z9, E11-14:OAc of *S. litura* significantly reduced the male catches of *S. exigua* to its sex pheromone. Furthermore, the combined lure of the two species completely inhibited male catches of *S. litura*, and significantly decreased the male catches of *S. exigua*, compared to the species-specific lure alone. The results demonstrated that males of the two sibling species could perceive the specific components of its counterpart, suggesting that mutual recognition of pheromone components may function to strengthen the behavioral isolation between the two species. Our study has added new knowledge to the reproductive isolation via sex pheromone communication system in sympatric moth species, and provided a base for designing of mating disruption tactics targeting multispecies by using insect sex pheromones.

Host strain specific sex pheromone variation in *Spodoptera frugiperda*

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

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

Geographic contrasts between pre- and postzygotic barriers are consistent with reinforcement in *Heliconius* butterflies

Rosser, N; Queste, LM; Cama, B; Edelman, NB; Mann, F; Pezo, RM; Morris, J; Segami, C; Velado, P; Schulz, S; Mallet, JLB; Dasmahapatra, KK. 2019

Identifying the traits causing reproductive isolation and the order in which they evolve is fundamental to understanding speciation. Here, we quantify prezygotic and intrinsic postzygotic isolation among allopatric, parapatric, and sympatric populations of the butterflies *Heliconius elevatus* and *Heliconius pardalinus*. Sympatric populations from the Amazon (*H. elevatus* and *H. p. butleri*) exhibit strong prezygotic isolation and rarely mate in captivity; however, **hybrids** are fertile. Allopatric populations from the Amazon (*H. p. butleri*) and Andes (*H. p. sergestus*) mate freely when brought together in captivity, but the female F1 **hybrids** are sterile. Parapatric populations (*H. elevatus* and *H. p. sergestus*) exhibit both assortative mating and sterility of female F1s. Assortative mating in sympatric populations is consistent with reinforcement in the face of gene flow, where the driving force, selection against **hybrids**, is due to disruption of mimicry and other ecological traits rather than **hybrid** sterility. In contrast, the lack of assortative mating and **hybrid** sterility observed in allopatric populations suggests that geographic isolation enables the evolution of intrinsic postzygotic reproductive isolation. Our results show how the types of reproductive barriers that evolve between species may depend on geography.

Genetic mapping of two components of reproductive isolation between two sibling species of moths, *Ostrinia nubilalis* and *O. scapularis*

Streiff, R; Courtois, B; Meusnier, S; Bourguet, D. 2014

We report the quantitative trait loci (QTL) mapping of reproductive isolation traits between *Ostrinia nubilalis* (the European corn borer) and its sibling species *O. scapularis* (the Adzuki bean borer), focusing on two traits: mating isolation (mi) and pheromone production (Pher). Four genetic maps were generated from two backcross families, with two maps (one chromosomal map and one linkage map) per backcross. We located 165-323 AFLP markers on these four maps, resulting in the identification of 27-31 linkage groups, depending on the map considered. No-choice mating experiments with the offspring of each backcross led to the detection of at least two QTLs for mi in different linkage groups. QTLs underlying Pher were located in a third linkage group. The Z heterochromosome was identified by a specific marker (Tpi) and did not carry any of these QTLs. Finally, we considered the global divergence between the two sibling species, distortions of segregation throughout the genome, and the location and effect of mi and Pher QTLs in light of the known candidate genes for reproductive isolation within the genus *Ostrinia* and, more broadly, in phytophagous insects.

Plant odor and sex pheromone are integral elements of specific mate recognition in an insect herbivore

Borrero-Echeverry, F; Bengtsson, M; Nakamuta, K; Witzgall, P. 2018

Specific mate recognition relies on the chemical senses in most animals, and especially in nocturnal insects. Two signal types mediate premating olfactory communication in terrestrial habitats: sex pheromones, which blend into an atmosphere of plant odorants. We show that host plant volatiles affect the perception of sex pheromone in males of the African cotton leafworm *Spodoptera littoralis* and that pheromone and plant volatiles are not perceived as independent messages. In clean air, *S. littoralis* males are attracted to single synthetic pheromone components or even the pheromone of a sibling species, oriental cotton leafworm *S. litura*. Presence of host plant volatiles, however, reduces the male response to deficient or heterospecific pheromone signals. That plant cues enhance discrimination of sex pheromone quality confirms the idea that specific mate recognition in noctuid moths has evolved in concert with adaptation to host plants. Shifts in either female host preference or sex pheromone biosynthesis give rise to new communication channels that have the potential to initiate or contribute to reproductive isolation.

Genetic hitchhiking associated with life history divergence and colonization of North America in the European corn borer moth

Dopman, E. 2011

A primary goal for evolutionary biology is to reveal the genetic basis for adaptive evolution and reproductive isolation. Using Z and E pheromone strains the European corn borer (ECB) moth, I address this problem through multilocus analyses of DNA polymorphism. I find that the locus Triose phosphate isomerase (Tpi) is a statistically significant outlier in coalescent simulations of demographic histories of population divergence, including strict allopatric isolation, restricted migration,

secondary contact, and population growth or decline. This result corroborates a previous QTL study that identified the Tpi chromosomal region as a repository for gene(s) contributing to divergence in life history. Patterns of nucleotide polymorphism at Tpi suggest a recent selective sweep and genetic hitchhiking associated with colonization of North America from Europe similar to 200 generations ago. These results indicate that gene genealogies initially diverge during speciation because of selective sweeps, but differential introgression may play a role in the maintenance of differentiation for sympatric populations.

Reconsidering the taxonomy of several *Ostrinia* species in the light of reproductive isolation: a tale for Ernst Mayr

Frolov, AN; Bourguet, D; Ponsard, S. 2007

We reconsider the taxonomy of a group of closely related *Ostrinia* spp., illustrating how useful Mayr's biological species concept remains for studying speciation patterns and processes. We review and re-analyse recent data on *Ostrinia scapularis*, *Ostrinia nubilalis*, *Ostrinia narynensis* and *Ostrinia orientalis*, along with those obtained over > 45 years in the former Soviet Union. The ten species of the 'trilobed uncus' group in the *Ostrinia* genus are classified into subgroups according to male mid-tibia morphology. However, none of the characters that further discriminate between them (female sex pheromones, male genitalia and calling time) varies together with male mid-tibia morphology, and neither do molecular markers. Moreover, male mid-tibia morphology appears to depend on only two diallelic loci and seems to be unrelated to reproductive isolation between *Ostrinia* taxa. By contrast, reproductive isolation is strongly related to host-plant type. In accordance with Mayr's species concept, we thus propose a revision of the trilobed uncus *Ostrinia* spp. based primarily on host-plant type. We propose that *O. narynensis* Mutuura & Munroe, 1970 (syn. nov.) and *O. orientalis* Mutuura & Munroe, 1970 (syn. nov.) be synonymized with *O. scapularis* (Walker, 1859). We further demonstrate that *O. nubilalis* auctt. pro parte feeding on mugwort, hop, and several other dicotyledons (previously called the '*O. nubilalis* mugwort-race' in France) also belongs to *O. scapularis*. Consequently, we propose that only *O. nubilalis* specimens feeding on maize (the former French '*O. nubilalis* maize-race') belong to *O. nubilalis* (Hubner, 1796). The implications of this revision are discussed. (c) 2007 The Linnean Society of London.

Dobzhansky's rule and reinforcement of pre-zygotic reproductive isolation in zones of secondary contact

Lukhtanov, VA. 2010

It is well known that closely related sympatric species are usually more different in characters involved in species recognition (e.g. in visual and acoustic signals) than allopatric species of the same evolutionary age. In this article I call this phenomenon Dobzhansky's rule in accordance with the name of the scientist who first discovered it. There are two alternative explanations for this pattern. Under hypothesis of reinforcement by Dobzhansky, these species-specific differences evolve in situ, exactly in zone of overlap between two populations. Under hypothesis of differential fusion by Templeton, the differences originate in geographically separated regions, and only those populations that have evolved such differences can persist in secondary sympatry. These evolutionary scenarios are significantly different. The scenario by Dobzhansky is an essentially sympatric model, in which natural selection reinforces pre-zygotic isolation between divergent populations by selecting against unfit **hybrids**. The scenario by Templeton is based on classic allopatric speciation model that consider the formation of reproductive isolation to be a by-product of divergent evolution. In this work we show that the sympatric distribution of sister taxa of *Agrodiaetus* butterflies strongly correlates with differences in male wing colour. We also use a new quantitative phylogenetic test to distinguish between the models by Dobzhansky and by Templeton and to demonstrate that the pattern observed is, most likely, the result of reinforcement.

COMPONENTS OF REPRODUCTIVE ISOLATION BETWEEN NORTH AMERICAN PHEROMONE STRAINS OF THE EUROPEAN CORN BORER

Dopman, EB; Robbins, PS; Seaman, A. 2010

Of 12 potential reproductive isolating barriers between closely related Z- and E-pheromone strains of the European corn borer moth (*Ostrinia nubilalis*), seven significantly reduced gene flow but none were complete, suggesting that speciation in this lineage is a gradual process in which multiple barriers of intermediate strength accumulate. Estimation of the cumulative effect of all barriers resulted in nearly complete isolation (> 99%), but geographic variation in seasonal isolation allowed as much as similar to 10% gene flow. With the strongest barriers arising from mate-selection behavior or ecologically relevant traits, sexual and natural selection are the most likely evolutionary processes driving population divergence. A recent multilocus genealogical study corroborates the roles of selection and gene flow (Dopman et al. 2005), because introgression is supported at all loci besides Tpi, a sex-linked gene. Tpi reveals strains as exclusive groups, possesses signatures of selection, and is tightly linked to a QTL that contributes to seasonal isolation. With more than 98% of total cumulative isolation consisting of prezygotic barriers, Z and E strains of ECB join a growing list of taxa in which species boundaries are primarily maintained by the prevention of **hybridization**, possibly because premating barriers evolve during early stages of population divergence.

FORMATION OF REPRODUCTIVE ISOLATION BARRIERS IN THE EUROPEAN CORN-BORER, *OSTRINIA-NUBILALIS* - DIFFERENCES IN HOST-PLANT EXPLOITATION STRATEGIES

FROLOV, AN. 1994

Ethological reproductive isolation barriers between populations feeding on corns (*Ostrinia nubilalis*, *O. persica*) and on dicotyledonous host plants (*O. nubilalis*, *O. narynensis*, *O. nubilalis* x *narynensis*, *O. scapularis*) were tested in lab using pair mating analysis. The corn dwelling populations of *O. nubilalis* were shown to form two groups according to their ability to breed with dicotyledon dwellers. In one group, populations which crossbred freely were clustered; another group contained populations with lower fertility rate. Formation of the reproductive barrier was correlated with caterpillars adaptations to feed upon corn leaves at whorl stage, which is peculiar to northern populations. Contrary to this, in the south where caterpillars used to feed upon leaves after their tasseling, there was no sign of barriers. It is supposed that differentiation of the *O. nubilalis* populations is caused by their selecting one of two possible strategies of the fodder plants. One of these is species-generalized and ontogenetic stage-specialized strategy implying feeding on a wide score of plant species but at flowering stage only. Another is species-specialized and ontogenetic stage-generalized strategy, feeding occurring on few plant species but at different stages, including those when antibiotic mechanisms are in effect.

Sex pheromone components of nettle caterpillar, *Setora nitens*

Sasaerila, Y; Gries, R; Gries, G; Khaskin, G; Hardi. 2000

Gas chromatographic-electroantennographic detection (GC-EAD) analyses of pheromone gland extracts of Female nettle caterpillars, *Setora nitens*, revealed four compounds that consistently elicited responses from male moth antennae. Retention indices on three fused silica columns (DB-5, DB-23, and DB-210) of two EAD-active compounds were almost identical to those of (E)-9-dodecenal (E9-12: Ald) and (E)-9,11-dodecadienal (E9,11-12: Ald), two pheromone components previously identified in congeneric *Setothosea* species. However, comparative GC, CC-EAD, and GC-mass spectrometry of extracted *S. nitens* compounds and authentic standards revealed that the candidate pheromone components were (Z)-9-dodecenal (Z9-12:Ald) and (Z)-9,11-dodecadienal (Z9,11-12:Ald). The two other EAD-active compounds in pheromone gland extracts proved to be the corresponding alcohols to these aldehydes. In field-trapping experiments in Tawau, Malaysia, synthetic Z9-12:Ald and Z9,11-12:Ald at a 1:1 ratio, but not singly, attracted male *S. nitens*. Attractiveness of these two aldehydes could not be enhanced through the addition of their corresponding alcohols. Whether these differences in pheromone biology and chemistry between *S. nitens* and *S. asina* are sufficient to prevent cross-attraction of heterospecific males or whether nonpheromonal mechanisms are required to maintain reproductive isolation is currently being studied.

When the Rule Becomes the Exception. No Evidence of Gene Flow between Two *Zerynthia* Cryptic Butterflies Suggests the Emergence of a New Model Group

Zinetti, F; Dapporto, L; Vovlas, A; Chelazzi, G; Bonelli, S; Balletto, E; Ciofi, C. 2013

There is increasing evidence that most parapatric cryptic/sister taxa are reproductively compatible across their areas of contact. Consequently, the biological species concept, which assumes absence of interbreeding, is becoming a not so effective criterion in evolutionary ecology. Nevertheless, the few parapatric sister taxa showing complete reproductive barriers represent interesting models to study speciation processes and the evolution of reproductive isolation. In this study, we examined contact populations in northwestern Italy of two butterfly species, *Zerynthia polyxena* and *Z. cassandra*, characterized by different genitalic morphotypes. We studied levels of divergence among 21 populations distributed from Sicily to France using three genetic markers (the mitochondrial COI and ND1 genes and the nuclear wingless gene) and genitalic geometric morphometrics. Moreover, we performed species distribution modelling to estimate different climatic requirements of *Z. polyxena* and *Z. cassandra*. We projected climatic data into glacial maximum scenarios in order to verify if and to which extent glacial cycles could have contributed to speciation processes. Genetic and morphometric analyses identified two main groups. All specimens showed a concordant pattern of diversification, including those individuals sampled in the contact area. Haplotype distribution and climatic models showed that during glacial maxima both species experienced a strong range contraction and presumably remained separated into different microrefugia in southern France, in the Italian Peninsula and on the islands of Elba and Sicily. Long term separation was probably favoured by reduced dispersal ability and high phylopatry, while genitalic diversification probably favoured interbreeding avoidance. Conversely, the aposematic wing pattern remained almost identical. We compared our results with those obtained in other species and concluded that *Z. polyxena* and *Z. cassandra* represent a valuable model in the study of speciation.

Geographic variation in male courtship acoustics and genetic divergence of populations of the *Cotesia flavipes* species complex

Joyce, AL; Bernal, JS; Vinson, SB; Hunt, RE; Schulthess, F; Medina, RF. 2010

Courtship behaviors of insect populations can vary across the range of a species. Populations exhibiting divergent courtship behavior may indicate genetic divergence or cryptic species. Courtship acoustic signals produced by male wing fanning and genetic structure (using amplified fragment length polymorphisms) were examined for seven allopatric populations of the *Cotesia flavipes* (Hymenoptera: Braconidae) species complex, using four *C. sesamiae* (Cameron) and three *C. flavipes* Cameron populations. Members of this species complex parasitize lepidopteran pests in gramineous crops including sugarcane, maize, and rice. Significant variation was detected in courtship acoustic signals and genetic structure among populations of both species. For *C. sesamiae*, courtship acoustic signals varied more between populations of two biotypes that were collected near an area of sympatry. The two biotypes of *C. sesamiae* were also genetically divergent. For *C. flavipes*, significant differences in acoustic signals and genetic structure occurred among allopatric populations; these differences support the recent designation of one population as a new species. Courtship acoustics play a role in reproductive isolation in this species complex, and are likely used in conjunction with chemical signals. Ecological factors such as host range and host plant use may also influence the divergence of both courtship acoustic signals and genetic structure among populations in the *C. flavipes* complex.

Characterization of sympatric *Platanthera bifolia* and *Platanthera chlorantha* (Orchidaceae) populations with intermediate plants

Esposito, F; Vereecken, NJ; Gammella, M; Rinaldi, R; Laurent, P; Tyteca, D. 2018

Platanthera bifolia and *P. chlorantha* are terrestrial and rewarding orchids with a wide Eurasian distribution. Although genetically closely related, they exhibit significant morphological, phenological and ecological differences that maintain reproductive isolation between the species. However, where both species co-occur, individuals with intermediate phenotypic traits, often considered as **hybrids**, are frequently observed. Here, we combined neutral genetic markers (AFLPs), morphometrics and floral scent analysis (GC-MS) to investigate two mixed *Platanthera* populations where morphologically intermediate plants were found. Self-pollination experiments revealed a low level of autogamy and artificial crossings combined with assessments of fruit set and seed viability, showed compatibility between the two species. The results of the genetic analyses showed that morphologically intermediate plants had similar genetic patterns as the *P. bifolia* group. These results are corroborated also by floral scent analyses, which confirmed a strong similarity in floral scent composition between intermediate morphotypes and *P. bifolia*. Therefore, this study provided a much more detailed picture of the genetic structure of a sympatric zone between two closely allied species and supports the hypothesis that intermediate morphotypes in sympatry could reflect an adaptive evolution in response to local pollinator-mediated selection.

Herbivorous insects: model systems for the comparative study of speciation ecology

Funk, DJ; Filchak, KE; Feder, JL. 2002

Does ecological divergence drive species-level evolutionary diversification? How so and to what degree? These questions were central to the thinking of the evolutionary synthesis. Only recently, however, has the ecology of speciation become an important focus of empirical study. Here, we argue that ecologically specialized, phylogenetically diverse, and experimentally tractable herbivorous insect taxa offer great opportunities to study the myriad mechanisms by which ecology may cause reproductive isolation and promote speciation. We call for the development and integrated experimental study of a taxonomic diversity of herbivore model systems and discuss the availability and recent evaluation of suitable taxa. Most importantly, we describe a general comparative framework that can be used to rigorously test a variety of hypotheses about the relative contributions and the macroevolutionary generality of particular mechanisms. Finally, we illustrate important issues for the experimental analysis of speciation ecology by demonstrating the consequences of specialized host associations for ecological divergence and premating isolation in *Neochlamisus bebbianae* leaf beetles.

Does hunger lead to hybridization in a genus of sexually cannibalistic insects (Orthoptera: Prophalangopsidae)?

Dupuis, JR; Judge, KA; Brunet, BMT; Chan, SO; Sperling, FAH. 2020

Allochronic isolation can be a strong mechanism for reproductive isolation and speciation. However, imperfect allochrony and the expression of phenological plasticity can erode temporal barriers to gene flow and result in **hybridization** between divergent lineages. Here, we combine behavioural ecology and genomics to investigate this scenario in two closely related species of grigs in the genus *Cyphoderris*. These species exhibit a unique mating system whereby females feed on the fleshy hind wings of the male during copulation, and copulation with conspecific males is more likely in food-restricted females than in well-fed females. In western Canada, *Cyphoderris buckelli* and *Cyphoderris monstrosa* are sympatric but largely allochronically separated, with *C. buckelli* breeding earlier. However, their breeding seasons can overlap, leading to potential for older *C. buckelli* females to mate with young *C. monstrosa* males to obtain resources via sexual cannibalism. We used behavioural assays to test whether female feeding status affects the propensity for interspecific mating between *C. buckelli* females and *C. monstrosa* males. We then tested for **hybridization** and gene exchange in wild populations of both species, using morphology, mitochondrial DNA and genome-wide nuclear markers. We found that interspecific courtship and mating can occur, but the relationship between food restriction and increased propensity for **hybridization** was not significant. Although we observed intraspecific population genetic structure in both species, we found no signatures of **hybridization** in the morphological or genetic datasets, which suggests that postmating reproductive barriers might be preventing successful **hybridization** in the wild.

A genetic switch for male UV iridescence in an incipient species pair of sulphur butterflies

Ficarrotta, V; Hanly, JJ; Loh, LS; Francescutti, CM; Ren, AN; Tunstrom, K; Wheat, CW; Porter, AH; Counterman, BA; Martin, A. 2022

Mating cues evolve rapidly and can contribute to species formation and maintenance. However, little is known about how sexual signals diverge and how this variation integrates with other barrier loci to shape the genomic landscape of reproductive isolation. Here, we elucidate the genetic basis of ultraviolet (UV) iridescence, a courtship signal that differentiates the males of *Colias eurytheme* butterflies from a sister species, allowing females to avoid costly heterospecific matings. Anthropogenic range expansion of the two incipient species established a large zone of secondary contact across the eastern United States with strong signatures of genomic admixtures spanning all autosomes. In contrast, Z chromosomes are highly differentiated between the two species, supporting a disproportionate role of sex chromosomes in speciation known as the large-X (or large-Z) effect. Within this chromosomewide reproductive barrier, linkage mapping indicates that cis-regulatory variation of *bric a brac* (*bab*) underlies the male UV-iridescence polymorphism between the two species. *Bab* is expressed in all non-UV scales, and butterflies of either species or sex acquire widespread ectopic iridescence following its CRISPR knockout, demonstrating that *Bab* functions as a suppressor of UV-scale differentiation that potentiates mating cue divergence. These results highlight how a genetic switch can regulate a premating signal and integrate with other reproductive barriers during intermediate phases of speciation.

Minor Components Play an Important Role in Interspecific Recognition of Insects: A Basis to Pheromone Based Electronic Monitoring Tools for Rice Pests

Chen, QH; Zhu, F; Tian, ZH; Zhang, WM; Guo, R; Liu, WC; Pan, LM; Du, YJ. 2018

Several lepidopteran species share the same pheromone blend consisting of (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-hexadecenal (Z9-16:Ald) at different ratios and active doses. In rice pest *Chilo suppressalis*, (Z)-11-hexadecenal, (Z11-16:OH) and octadecenal (18:Ald) were identified as minor components in the pheromone gland of female moths, and these components were previously not considered as part of the sex pheromone of *C. suppressalis*. Z11-16:Ald, Z9-16:Ald and (Z)-13-octadecenal (Z13-18:Ald) frequently trapped other lepidopteran species, such as rice pests *Scirpophaga incertulas* and *Mythimna separata*, corn and vegetable pests *Helicoverpa armigera* in the field, suggesting a lack of specificity in the pheromone blend. Our data showed that the minor component Z11-16:OH did not have a synergistic effect on the attractiveness of the blend to *C. suppressalis*; however, pheromone mixtures containing Z11-16:OH failed in trapping male *H. armigera* moths. We confirmed the identity and specificity of the *C. suppressalis* sex pheromone and demonstrated that Z11-16:OH plays a key role in the reproductive isolation of *C. suppressalis*, *M. separata*, and *H. armigera* moths, and a similar role of Z9-18:Ald in that of *S. incertulas* and *C. suppressalis*. This phenomenon could be more widely applicable to interspecific interactions in the pheromone communication between insects, which is crucial to developing the electronic automatic counting device for automatically monitoring the pest population by pheromone trapping based on its species specificity.

Influence of host plant, geography and pheromone strain on genomic differentiation in sympatric populations of *Ostrinia nubilalis*

Coates, BS; Kozak, GM; Kim, KS; Sun, J; Wang, YZ; Fleischer, SJ; Dopman, EB; Sappington, TW. 2019

Patterns of mating for the European corn borer (*Ostrinia nubilalis*) moth depend in part on variation in sex-pheromone blend. The ratio of (E)-11- and (Z)-11-tetradecenyl acetate (E11- and Z11-14:OAc) in the pheromone blend that females produce and males respond to differs between strains of *O. nubilalis*. Populations also vary in female oviposition preference for and larval performance on maize (C4) and nonmaize (C3) host plants. The relative contributions of sexual and ecological trait variation to the genetic structure of *O. nubilalis* remains unknown. Host-plant use (C-13/C-14 ratios) and genetic differentiation were estimated among sympatric E and Z pheromone strain *O. nubilalis* males collected in sex-pheromone baited traps at 12 locations in Pennsylvania and New York between 2007 and 2010. Among genotypes at 65 single nucleotide polymorphism marker loci, variance at a position in the pheromone gland fatty acyl-reductase (*pgfar*) gene at the locus responsible for determining female pheromone ratio (Pher) explained 64% of the total genetic differentiation between males attracted to different pheromones (male response, Resp), providing evidence of sexual inter-selection at these unlinked loci. Principal coordinate, Bayesian clustering, and distance-based redundancy analysis (dbRDA) demonstrate that host plant history or geography does not significantly contribute to population variation or differentiation among males. In contrast, these analyses indicate that pheromone response and *pgfar*-defined strain contribute significantly to population genetic differentiation. This study suggests that behavioural divergence probably plays a larger role in driving genetic variation compared to host plant-defined ecological adaptation.

Frequency of hybridization between *Ostrinia nubilalis* E- and Z-pheromone races in regions of sympatry within the United States

Coates, BS; Johnson, H; Kim, KS; Hellmich, RL; Abel, CA; Mason, C; Sappington, TW. 2013

Female European corn borer, *Ostrinia nubilalis*, produce and males respond to sex pheromone blends with either E- or Z-Delta 11-tetradecenyl acetate as the major component. E- and Z-race populations are sympatric in the Eastern United States, Southeastern Canada, and the Mediterranean region of Europe. The E- and Z-pheromone races of *O. nubilalis* are models for incipient species formation, but **hybridization** frequencies within natural populations remain obscure due to lack of a high-throughput phenotyping method. Lassance et al. previously identified a pheromone gland-expressed fatty-acyl reductase gene (*pgfar*) that controls the ratio of Delta 11-tetradecenyl acetate stereoisomers. We identified three single nucleotide polymorphism (SNP) markers within *pgfar* that are differentially fixed between E- and Z-race females, and that are >= 98.2% correlated with female pheromone ratios measured by gas chromatography. Genotypic data from locations in the United States demonstrated that *pgfar*-z alleles were fixed within historically allopatric Z-pheromone race populations in the Midwest, and that **hybrid** frequency ranged from 0.00 to 0.42 within 11 sympatric sites where the two races co-occur in the Eastern United States (mean **hybridization** frequency or heterozygosity (H-O) = 0.226 +/- 0.279). Estimates of **hybridization** between the E- and Z-races are important for understanding the dynamics involved in maintaining race integrity, and are consistent with previous estimates of low levels of genetic divergence between E- and Z-races and the presence of weak prezygotic mating barriers.

Impacts of climate warming on hybrid zone movement: Geographically diffuse and biologically porous "species borders"

Scriber, JM. NA

The ecology and evolutionary biology of insect-plant associations has realized extensive attention, especially during the past 60 years. The classifications (categorical designations) of continuous variation in biodiversity, ranging from global patterns (e.g., latitudinal gradients in species richness/diversity and degree of herbivore feeding specialization) to localized insect-plant associations that span the biospectrum from polyphenisms, polymorphisms, biotypes, demes, host races, to cryptic species, remain academically contentious. Semantic and biosystematic (taxonomical) disagreements sometimes detract from more important ecological and evolutionary processes that drive diversification, the dynamics of gene flow and local extinctions. This review addresses several aspects of insect specialization, host-associated divergence and ecological (including "**hybrid**") speciation, with special reference to the climate warming impacts on species borders of **hybridizing** swallowtail butterflies (Papilionidae). Interspecific **hybrid** introgression may result in collapse of multi-species communities or increase species numbers via homoploid **hybrid** speciation. We may see diverging, merging, or emerging genotypes across **hybrid** zones, all part of the ongoing processes of evolution. Molecular analyses of genetic mosaics and genomic dynamics with "divergence hitchhiking", combined with ecological, ethological and physiological studies of "species porosity", have already begun to unveil some answers for some important ecological/evolutionary questions. (i) How rapidly can host-associated divergence lead to new species (and why doesn't it always do so, e.g., resulting in "incomplete" speciation)? (ii) How might "speciation genes" function, and how/where would we find them? (iii) Can oscillations from specialists to generalists and back to specialists help explain global diversity in herbivorous insects? (iv) How could recombinant interspecific **hybridization** lead to divergence and speciation? From ancient phytochemically defined angiosperm affiliations to recent and very local geographical mosaics, the Papilionidae (swallowtail butterflies) have provided a model for enhanced understanding of ecological patterns and evolutionary processes, including

host-associated genetic divergence, genomic mosaics, genetic hitchhiking and sex-linked speciation genes. Apparent homoploid **hybrid** speciation in *Papilio* appears to have been catalyzed by climate warming-induced interspecific introgression of some, but not all, species diagnostic traits, reflecting strong divergent selection (discordant), especially on the Z (= X) chromosome. Reproductive isolation of these novel recombinant **hybrid** genotypes appears to be accomplished via a delayed post-diapause emergence or temporal isolation, and is perhaps aided by the thermal landscape. Changing thermal landscapes appear to have created (and may destroy) novel recombinant **hybrid** genotypes and **hybrid** species.

Assortative mating in sympatric host races of the European corn borer

Malausa, T; Bethenod, MT; Bontemps, A; Bourguet, D; Cornuet, JM; Ponsard, S. 2005

Although a growing body of work supports the plausibility of sympatric speciation in animals, the practical difficulties of directly quantifying reproductive isolation between diverging taxa remain an obstacle to analyzing this process. We used a combination of genetic and biogeochemical markers to produce a direct field estimate of assortative mating in phytophagous insect populations. We show that individuals of the same insect species, the European corn borer *Ostrinia nubilalis*, that develop on different host plants can display almost absolute reproductive isolation-the proportion of assortative mating was >95%-even in the absence of temporal or spatial isolation.

Comparative transcriptomics of the pheromone glands provides new insights into the differentiation of sex pheromone between two host populations of *Chilo suppressalis*

Guo, S; Tian, Z; Quan, WL; Sun, D; Liu, W; Wang, XP. 2020

Reproductive isolation between different host populations is often based on intraspecific sex pheromone differences. The mechanisms underlying these differences have not been thoroughly elucidated to date. Previous studies suggested that *Chilo suppressalis* has differentiated into rice and water-oat host populations, and these two populations manifest clear differences in sex pheromone titer and mating rhythm. Hence, this moth is an ideal model to investigate the endogenous mechanisms of intraspecific reproductive isolation. Here, we identified a series of putative genes associated with sex pheromone biosynthesis based on the *C. suppressalis* pheromone gland transcriptome data. Transcripts of most genes were at higher level in the rice population. Then we obtained 11 pivotal differentially expressed genes (DEGs). The expression levels of these DEGs exhibited a distinct increase in the rice population. Moreover, we also observed the expression rhythm of these DEGs is discrepant between two host populations. Our study offers a new understanding to elucidate the mechanisms of intraspecific reproductive isolation.

DIFFERENCES IN ULTRAVIOLET WING PATTERNS IN THE NEW-ZEALAND LYCAENID BUTTERFLIES *LYCAENA-SALUSTIUS*, *L-RAUPARAHA*, AND *L-FEREDAYI* AS A LIKELY ISOLATING MECHANISM

MEYERROCHOW, VB. 1991

The dorsal and ventral surfaces of fore- and hind wings in *Lycaena salustius*, *L. rauparaha*, and *L. feredayi* were examined under visible and ultraviolet (UV) light. Differences between the males and females of all three species, invisible to the human eye, appear under UV-radiation. Males of *L. salustius* exhibit extensive UV-reflecting regions on the dorsal sides of both wing pairs. On female *L. salustius*, these UV-reflecting areas are reduced to tiny submarginal spots. Males and females of both *L. rauparaha* and *L. feredayi* are UV-absorbing. Scales examined under the scanning electron microscope confirmed the correlation between scale-ultrastructure and UV-reflectance or absorbance known from other butterflies. The geographical distribution of the sexually UV-dichromatic *L. salustius* and either the UV-absorbing *L. rauparaha* or *L. feredayi* (rarely both) overlap, and the latter two are not found together on their own. Therefore I suggest that the UV-pattern is an isolating mechanism, similar to the one found in the well-studied American sympatric UV-reflecting pierid *Colias eurytheme* and the UV-absorbing *C. philodice*. Intermittant 'flickers' of UV-patterns, as generated by beating wings, possibly combined with a certain degree of polarization of the reflected light, provide an insect eye with a powerful stimulus useful in mating and territorial ritual displays.

Patterns of divergence across the geographic and genomic landscape of a butterfly hybrid zone associated with a climatic gradient

Ryan, SF; Fontaine, MC; Scriber, JM; Pfrender, ME; O'Neil, ST; Hellmann, JJ. 2017

Hybrid zones are a valuable tool for studying the process of speciation and for identifying the genomic regions undergoing divergence and the ecological (extrinsic) and nonecological (intrinsic) factors involved. Here, we explored the genomic and geographic landscape of divergence in a **hybrid** zone between *Papilio glaucus* and *Papilio canadensis*. Using a genome scan of 28,417 ddRAD SNPs, we identified genomic regions under possible selection and examined their distribution in the context of previously identified candidate genes for ecological adaptations. We showed that differentiation was genomewide, including multiple candidate genes for ecological adaptations, particularly those involved in seasonal adaptation and host plant detoxification. The Z chromosome and four autosomes showed a disproportionate amount of differentiation, suggesting genes on these chromosomes play a potential role in reproductive isolation. Cline analyses of significantly differentiated genomic SNPs, and of species-diagnostic genetic markers, showed a high degree of geographic coincidence (81%) and concordance (80%) and were associated with the geographic distribution of a climate-mediated developmental threshold (length of the growing season). A relatively large proportion (1.3%) of the outliers for divergent selection were not associated with candidate genes for ecological adaptations and may reflect the presence of previously unrecognized intrinsic barriers between these species. These results suggest that exogenous (climate-mediated) and endogenous (unknown) clines may have become coupled and act together to reinforce reproductive isolation. This approach of assessing divergence across both the genomic and geographic landscape can provide insight about the interplay between the genetic architecture of reproductive isolation and endogenous and exogenous selection.

Does pheromone biology of *Lambdina athasaria* and *L-pellucidaria* contribute to their reproductive isolation?

Duff, CM; Gries, G; Mori, K; Shirai, Y; Seki, M; Takikawa, H; Sheng, T; Slessor, KN; Gries, R; Maier, CT; Ferguson, DC. 2001

Recently, 7-methylheptadecane and 7,11-dimethylheptadecane have been reported as sex pheromone components of both spring hemlock looper (SHL), *Lambdina athasaria*, and pitch pine looper (PPL), *Lambdina pellucidaria*. Our objective was to test the hypothesis that SHL and PPL are reproductively isolated, in part, through species specificity in: (1) absolute configuration of pheromone components, (2) diel periodicity of pheromonal communication, and/or (3) seasonal flight period. In coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses of stereoselectively synthesized (7S)- and (7R)-7-methylheptadecane [7S; 7R] as well as (7S,11S)-, (7R,11R)-, and (meso-7,11)-7,11-dimethylheptadecane [7S,11S; 7R,11R, meso-7,11], only 7S and meso-7,11 elicited responses by male SHL and PPL antennae. In field experiments, male SHL and PPL were attracted only to lures containing 7S plus meso-7,11. In hourly recordings of trap-captured males, SHL and PPL in their respective habitats were trapped between 24:00 and 03:00 hr. Capture of both SHL and PPL in pheromone-baited traps throughout June indicated overlapping seasonal flight periods. These findings of identical absolute configuration of pheromonal components, diel periodicity of pheromonal communication, and overlap of seasonal flight periods support synonymy of SHL and PPL. Finite taxonomic classification of PPL and SHL must await careful assessment of further criteria. such as morphometrics, molecular comparisons and ecological analyses.

Cryptic speciation associated with geographic and ecological divergence in two Amazonian *Heliconius* butterflies

Rosser, N; Freitas, AVL; Huertas, B; Joron, M; Lamas, G; Merot, C; Simpson, F; Willmott, KR; Mallet, J; Dasmahapatra, KK. 2019

The evolution of reproductive isolation via a switch in mimetic wing coloration has become the paradigm for speciation in aposematic *Heliconius* butterflies. Here, we provide a counterexample to this, by documenting two cryptic species within the taxon formerly considered *Heliconius demeter* Staudinger, 1897. Amplified fragment length polymorphisms identify two sympatric genotypic clusters in northern Peru, corresponding to subspecies *Heliconius demeter ucayalensis* H. Holzinger & R. Holzinger, 1975 and *Heliconius demeterjoroni* ssp. nov. These subspecies are reciprocally monophyletic for the mitochondrial genes COI and COII and the nuclear gene *Ef1 alpha*, and exhibit marked differences in larval morphology and host plant use. COI sequences from 13 of the 15 currently recognized subspecies show that mtDNA differences are reflected across the range of *H. demeter*, with a deep phylogenetic split between the southern and northern Amazonian races. As such, our data suggest vicariant speciation driven by disruptive selection for larval performance on different host plants. We raise *Heliconius demeter eratosignis* (Joicey & Talbot, 1925) to *Heliconius eratosignis* based on nomenclatural priority, a species also comprising *H. eratosignis ucayalensis* comb. nov. and three other southern Amazonian races. *Heliconius demeterjoroni* spp. nov. remains within *H. demeter* s.s., along with northern Amazonian and Guianan subspecies.

TEMPORAL SEPARATION OF FLIGHT TIME OF 2 SYMPATRIC MALACOSOMA SPECIES

SAMPLE, BE. 1992

Malacosoma americanum (F.) and *M. disstria* Hubner were collected by black-light traps in eastern West Virginia in 1988-1990. Ten locations were sampled in 1988 and 1989 and 27 locations in 1990. Traps were operated at each location for 0.5-h intervals centered at 2100, 2300, 0100, 0300, and 0500 hours. Factorial analyses of variance were performed on the quantity of each species collected in each time interval. Peak flight time for *M. americanum* occurred at 2300 and 0100 hours whereas most *M. disstria* flew during 0300 and 0500 hours. Flight activity of *M. americanum* was unrelated to the presence of *M. disstria*. However, *M. disstria* flew significantly later at night, and was less abundant when *M. americanum* was present. Other research suggests that species within *Malacosoma* use similar sex pheromones. Separate flight activities of these species may help reduce interspecific pheromone attraction and aid the reproductive isolation of these species.

Difference in diel mating time contributes to assortative mating between host plant-associated populations of *Chilo suppressalis*

Quan, WL; Liu, W; Zhou, RQ; Chen, R; Ma, WH; Lei, CL; Wang, XP. 2017

Behavioral isolation in animals can be mediated by inherent mating preferences and assortative traits, such as divergence in the diel timing of mating activity. Although divergence in the diel mating time could, in principle, promote the reproductive isolation of sympatric, conspecific populations, there is currently no unequivocal evidence of this. We conducted different mate-choice experiments to investigate the contribution of differences in diel mating activity to the reproductive isolation of the rice and water-oat populations of *Chilo suppressalis*. The results show that inter-population difference in diel mating activity contributes to assortative mating in these populations. In the rice population, most mating activity occurred during the first half of the scotophase, whereas in the water-oat population virtually all mating activity was confined to the second half of the scotophase. However, when the photoperiod of individuals from the water-oat population was altered to more closely align their mating activity with that of the rice population, mate choice was random. We conclude that inter-population differences in diel mating time contribute to assortative mating, and thereby the partial reproductive isolation, of these host-associated populations of *C. suppressalis*.

Differentiation in life cycle of sympatric populations of two forms of *Hyphantria* moth in central Missouri

Takeda, M. 2005

Wing patterns of *Hyphantria* adult male moths collected in central Missouri were examined throughout the breeding season. Three major peaks of adult flight were observed: the first peak consisted mainly of adults with spotted wings, while the second and third peaks consisted of immaculate adults. Black-headed larvae appeared in the field following the first major peak of moth flight, and red-headed larvae appeared in the field following the second peak. Sympatric red-headed and black-headed forms were collected in the field and subsequently reared on an artificial diet under conditions of 16 h light : 8 h dark (LD 16:8) at 25 degrees C. The larval period of the black-headed form was shorter than the red-headed, whereas the pupal period of the black-headed form was longer than the red-headed. Pupal development is retarded in some individuals at high temperatures in the black-headed form. Photoperiodic response curves for pupal diapause were different between the two forms. The critical photoperiod for pupal diapause was 15 h 10 min in the red-headed form, which was longer than that for the black-headed form (14 h 40 min). The two forms responded to shifts in photoperiod differently. These developmental responses temporally separate the two forms in the field; the red-headed and black-headed forms represent a set of adaptations favoring univoltinism and bivoltinism, respectively. Red-headed larvae fed mainly at night, while the black-headed larvae fed without a clear day-night rhythm. Nocturnal feeding in the red-headed form is adaptive to protection against predation, but fails to fully utilize heat units and thus to produce a second generation.

Host-plant specialization in pheromone strains of the European corn borer *Ostrinia nubilalis* in France

Pelozuelo, L; Malosse, C; Genestier, G; Guenego, H; Frerot, B. 2004

European corn borer (ECB) feeding on maize (*Zea mays*); mugwort (*Artemisia vulgaris*), and hop (*Humulus lupulus*) are genetically different in France and referred to as host-plant races. Here, we investigated sex pheromone composition as a possible trait linked to the host plant. ECB host races were sampled from 13 different sites in France. GC-MS analysis of female pheromone showed that 175 out of 176 maize females belonged to the Z type with one **hybrid**. In contrast, mugwort and hop females belonged almost exclusively to the E type. No Z females were found on these plants and only 2 females out of 169 were **hybrids**. In the three sites of sympatry, the **hybrid** proportion was far from Hardy-Weinberg expectations. Wind tunnel experiments showed that 76-79% of maize males from three populations were attracted by Z females, whereas neither mugwort nor hop males were. Mugwort males from Toussus-le-Noble were attracted by E females originating from an American maize strain. These data showed that maize, mugwort, and hop host races of *O. nubilalis* differ not only in their host plant but also in the sex pheromone they use. Because mugwort and hop are putative ancestral host plants, these results are discussed from the point of view of evolutionary scenarios for the emergence of Z and E strains.

r/K-like trade-off and voltinism discreteness: The implication to allochronic speciation in the fall webworm, *Hyphantria cunea* complex (Arctiidae)

Yang, F; Kawabata, E; Tufail, M; Brown, JJ; Takeda, M. 2017

North America has distinct types of *Hyphantria* moths (Arctiidae) characterized by red (RD)- and black (BL)-headed larvae, of which the taxonomic status is unresolved. Genetic divergence of 26 populations, based on 710bp of the mtCOI sequence, showed two phylogenetic lineages, which could not be connected in the haplotype network with 95% confidence. The two lineages are separated by 3.1% sequence divergence and should be considered for full species status. The estimated split occurred 1.2-1.6million years ago. The range of the RD type covered most of the continent, whereas that of the BL type was limited to eastern deciduous forests. Several biological characteristics were differentiated in the zone of cohabitation where BL had more annual generations than RD. Spring

emergence of BL precedes that of RD in the field by at least 1 month, because the diapause in BL was shallow, whereas it was deep in RD. Voltinism requires discreteness of numbers, which functions as a sink of **hybrids** between the two parental lines that have distinct but equally adaptive reproductive strategies; BL may be more r-strategist-like and RD more K-strategist-like, because fast-developing BL has multivoltine life cycle, investing less silk proteins as the round-the-clock feeder, and slow-developing RD univoltine one investing more silk as the nocturnal feeder. Also, intensity of diapause, deep in RD and weak in BL, was grossly different, which may enforce segregation of spring adults. Allochronic speciation avoiding coincidental occurrence of adult stages is therefore the most likely scenario. Because the adults never meet in nature, large morphological differentiation is not required.

Moths passing in the night: Phenological and genomic divergences within a forest pest complex

Nelson, TD; MacDonald, ZG; Sperling, FAH. 2022

Temporal separation of reproductive timing can contribute to species diversification both through allochronic speciation and later reinforcement of species boundaries. Such phenological differences are an enigmatic component of evolutionary divergence between two major forest defoliator species of the spruce budworm complex: *Choristoneura fumiferana* and *C. occidentalis*. While these species interbreed freely in laboratory settings, natural **hybridization** rates have not been reliably quantified due to their indistinguishable morphology. To assess whether temporal isolation is contributing to reproductive isolation, we collected adult individuals throughout their expected zone of sympatry in western Canada at 10-day intervals over two successive years, assigning taxonomic identities using thousands of single nucleotide polymorphisms. We found unexpectedly broad sympatry between *C. fumiferana* and *C. occidentalis* biennis and substantial overlap of regional flight periods. However, flight period divergence was much more apparent on a location-by-location basis, highlighting the importance of considering spatial scale in these analyses. Phenological comparisons were further complicated by the biennial life cycle of *C. o. biennis*, the main subspecies of *C. occidentalis* in the region, and the occasional occurrence of the annually breeding subspecies *C. o. occidentalis*. Nonetheless, we demonstrate that biennialism is not a likely contributor to reproductive isolation within the species complex. Overall, interspecific F-1 **hybrids** comprised 2.9% of sequenced individuals, confirming the genomic distinctiveness of *C. fumiferana* and *C. occidentalis*, while also showing incomplete reproductive isolation of lineages. Finally, we used F-ST-based outlier and genotype-environment association analyses to identify several genomic regions under putative divergent selection. These regions were disproportionately located on the Z linkage region of *C. fumiferana*, and contained genes, particularly antifreeze proteins, that are likely to be associated with overwintering success and diapause. In addition to temporal isolation, we conclude that other mechanisms, including ecologically mediated selection, are contributing to evolutionary divergence within the spruce budworm species complex.

Temperature coupling as an emergent property: Parallel thermal effects on male song and female response do not contribute to species recognition in an acoustic moth

Greenfield, MD; Medlock, C. 2007

Temperature coupling exists when changes in male signal production with temperature are paralleled by changes in female response. Such thermal effects have been observed in various ectothermic animals producing acoustic, visual, and electric signals in which the signal rate may be subject to stabilizing selection imposed by female preference. Often, coupling was considered as an adaptive function wherein male and female thermal effects coevolved under selection pressure favoring species recognition, although this assumption has not been tested definitively. We investigated thermal effects on pulse-pair rate in male song and female acceptance threshold for male song rate in an acoustic moth, *Achroia grisella*, in which male song rate is subject to directional selection. Male song rate and female acceptance threshold do exhibit parallel increases as temperature rises from 18 degrees C to 30 degrees C, but female thresholds are much lower than male song rates and the thermal effect on female response cannot augment species recognition. In further investigations using inbred lines of *A. grisella* we found that the male and female thermal effects are genetically correlated, and we discuss the likely sources of this covariance. We consider several explanations for the occurrence of temperature coupling in this species and suggest that it represents an emergent property arising from the neuromuscular responses to temperature that are common to several physiological systems.

RATIOS OF GEOMETRICAL AND OPTICAL ISOMERS OF PHEROMONES - IRRELEVANT OR IMPORTANT IN SCOLYTIDS

GRIES, G. 1992

A specific ratio of exo- to endo-brevicomin has been found in airborne volatiles of male *Dendroaonus ponderosae* and *Dryocoetes confusus*. Moreover, a specific ratio of enantiomers of seudenol and 1-methyl-cyclohex-2-en-1-ol had been found in airborne volatiles of female *D. rufipennis*. It is hypothesized that scolytid beetles may utilize specific ratios of both geometrical and optical isomers of pheromones for optimal attraction of conspecifics and means of reproductive isolation in the use of pheromone channels between species.

Transcriptome comparison of the sex pheromone glands from two sibling Helicoverpa species with opposite sex pheromone components

Li, ZQ; Zhang, S; Luo, JY; Wang, CY; Lv, LM; Dong, SL; Cui, JJ. 2015

Differences in sex pheromone component can lead to reproductive isolation. The sibling noctuid species, *Helicoverpa armigera* and *Helicoverpa assulta*, share the same two sex pheromone components, Z9-16:Ald and Z11-16:Ald, but in opposite ratios, providing an typical example of such reproductive isolation. To investigate how the ratios of the pheromone components are differently regulated in the two species, we sequenced cDNA libraries from the pheromone glands of *H. armigera* and *H. assulta*. After assembly and annotation, we identified 108 and 93 transcripts putatively involved in pheromone biosynthesis, transport, and degradation in *H. armigera* and *H. assulta*, respectively. Semi-quantitative RT-PCR, qRT-PCR, phylogenetic, and mRNA abundance analyses suggested that some of these transcripts involved in the sex pheromone biosynthesis pathways perform. Based on these results, we postulate that the regulation of desaturases, KPSE and LPAQ, might be key factor regulating the opposite component ratios in the two sibling moths. In addition, our study has yielded large-scale sequence information for further studies and can be used to identify potential targets for the bio-control of these species by disrupting their sexual communication.

Trends in the changes of the postgonites in the frit fly genus Meromyza (Diptera: Chloropidae)

Yatsuk, AA; Safonkin, AF. 2018

The common patterns and trends of microevolutionary transformations in the shape of the front appendix of postgonites in 27 species of *Meromyza* frit flies are revealed using morphometric analysis. Geometric morphometry was performed using program packages TPS and MorphoJ. To digitize shapes, there were placed 24 landmarks, reflecting contours of the front appendix of postgonites, allowing to detect any change in the shape that affects the reproductive isolation of species. The intercluster variability is characterized by three main components, attributable to: I (47.75%) changes in the shape of the lower edge, II (16.64%) elongation of the protruding part, III (14.83%) expansion of the line of the postgonites affixing. Within each cluster, the pattern of species separation through the changes in the shape of postgonites front appendix is investigated. In clusters plurisetia, nigriseta and, especially, atletica and zachvatkini, the variability of the upper edge of postgonites plays the most important role. In cluster groups meigeni and inornata variability was noticed along the whole contour of postgonites, while in clusters saltatrix and pratorum along the bottom edge and at the end of the protruding part. Mahalanobis distance calculation showed that in the studied contours of postgonites front

appendix there are no matching clusters, and in each of them there are no fully overlapping contours. The morphometric analysis confirms species grouping, previously suggested by the results of the mtDNA locus CO1 studies, into 8 clusters.

Molecular differentiation at nuclear loci in French host races of the European corn borer (*Ostrinia nubilalis*)

Malausa, T; Leniaud, L; Martin, JF; Audiot, P; Bourguet, D; Ponsard, S; Lee, SF; Harrison, RG; Dopman, E. 2007

French populations of the European corn borer consist of two sympatric and genetically differentiated host races. As such, they are well suited to study processes that could be involved in sympatric speciation, but the initial conditions of host-race divergence need to be elucidated. Gene genealogies can provide insight into the processes involved in speciation. We used DNA sequences of four nuclear genes to (1) document the genetic structure of the two French host races previously delineated with allozyme markers, (2) find genes directly or indirectly involved in reproductive isolation between host races, and (3) estimate the time since divergence of the two taxa and see whether this estimate is compatible with this divergence being the result of a host shift onto maize after its introduction into Europe similar to 500 years ago. Gene genealogies revealed extensive shared polymorphism, but confirmed the previously observed genetic differentiation between the two host races. Significant departures from the predictions of neutral molecular evolution models were detected at three loci but were apparently unrelated to reproductive isolation between host races. Estimates of time since divergence between French host races varied from similar to 75,000 to similar to 150,000 years, suggesting that the two taxa diverged recently but probably long before the introduction of maize into Europe.

AN INTERPRETATION OF THE IMPACT OF THE WISCONSIN GLACIATION ON THE HEMILEUCA MAIA/NEVADENSIS COMPLEX (SATURNIIDAE) OF THE GREAT LAKES REGION

Tuttle, JP; Carr, TW; Collins, MM. 2020

The biology of the *Hemileuca maia* group of "buck moths" in the Great Lakes region is summarized, especially with respect to the northern "maia complex" where adult phenotype is not well-correlated with either pheromone response or perceived host plant associations. Published field trials of pheromone response are updated with respect to host plant associations. We found a reciprocal lack of response to pheromone between willow-feeding "complex" moths in lowland, glaciated habitats to the north and oak-feeding populations in non-glaciated habitats to the south. We discuss how post-glacial host plant distributions - expansion from previous refugia and forestation of the Great Lakes terrain - may have played a key role in structuring current Great Lakes *Hemileuca* populations. In addition, the subsequent rapid and extreme drying during the mid-Holocene (8,000-3,200 years BP) led to the expansive tall grass prairie, producing a finger-like eastern extension known as the Prairie Peninsula, which probably provided an additional ecological barrier separating these oak and willow-feeding populations. Rapid expansion of willow-feeding populations from Pleistocene refugia after 18,000 years BP offers a more likely explanation, compared to gene flow, for the general lack of genetic divergence previously found in the buck moths of the Great Lakes region.

Chirally Dimorphic Male Genitalia in Praying Mantids (Ciulfina: Liturgusidae)

Holwell, GI; Herberstein, ME. 2010

Although male polymorphisms occur widely in nature and have received considerable recent attention from studies of alternative mating strategies, male genital polymorphisms are less well known. Here, we describe a dimorphism in the orientation of the male genitalic complex of the praying mantid genus *Ciulfina*. Populations of *Ciulfina* species vary in the proportion of males with dextral (right-oriented) and sinistral (left-oriented) genitalia, ranging from directional asymmetry (single orientation only) to apparent antisymmetry (equal proportions of both orientations). The proportion of dextral males varied between species (*C. baldersoni*: 46%; *C. rentzi*: 24%; *C. klassi*: 100%; *C. biseriata*: 83%) and between populations. We used elliptic Fourier analysis to quantify shape and size variation between the genitalia of dextral and sinistral males and determined that the two forms were mirror images of one another in two species. We found that the level of mechanical reproductive isolation between heterospecific populations of opposite genital orientation was no greater than that between heterospecific populations with the same orientation or of mixed orientation. Genital orientation therefore did not influence premating isolation between these species, despite complete postmating isolation. The geographic proximity of populations to heterospecifics also showed no particular pattern with respect to genital orientation. These results suggest that reversible trait asymmetry in *Ciulfina* is not driven by reproductive isolation, and add to the growing evidence against the species isolation hypothesis for rapid genital evolution. *J. Morphol.* 271:1176-1184, 2010. (C) 2010 Wiley-Liss, Inc.

Breeding barriers at a diploid-hexaploid contact zone in *Aster amellus*

Castro, S; Munzbergova, Z; Raabova, J; Loureiro, J. 2011

Polyploidization is an important mechanism of sympatric speciation, but few studies have addressed breeding barriers between polyploids and their diploid progenitors in the field, and the available data have been mainly obtained from diploid-tetraploid contact zones. In contrast to diploid-tetraploid complexes, **hybridization** between diploid and hexaploid individuals may lead to viable fertile tetraploid offspring, and thus the interactions between these ploidy levels can be more complex. We investigated the breeding barriers operating between diploid and hexaploid individuals of *Aster amellus* at a contact zone in Central Europe to understand the absence of **hybrids** (i.e., tetraploids) and mixed populations. Phenological segregation, assortative mating mediated by pollinators and crossing ability were assessed under natural and controlled conditions in diploid and hexaploid populations growing in close proximity. The results revealed low levels of reproductive isolation (RI) due to flowering phenology (RI = 11-45%) and pollinator behavior (RI = 17%), so that pollen transfer between diploids and hexaploids is possible. In contrast, almost complete reproductive isolation was observed due to a series of post-pollination barriers that significantly reduced the production of offspring from inter-cytotype crosses (RI = 99.9%), even though some tetraploids were detected in seeds and seedlings. We conclude that the absence of tetraploids at the contact zone is probably due to a combination of several factors, including spatial segregation, strong post-pollination barriers (such as gametic isolation, low viability of tetraploid seeds and/or inability of tetraploid plants to reach the flowering stage), and to a lesser extent, temporal and behavioral segregation. Future studies should explore the fitness of tetraploids and the effect of different traits on the reproductive success and fitness of each cytotype. This will enable a fuller understanding of the dynamics and mechanisms acting in contact zones.

Biological Flora of the British Isles: *Gymnadenia conopsea* s.l.

Meekers, T; Hutchings, MJ; Honnay, O; Jacquemyn, H. 2012

This account presents information on all aspects of the biology of *Gymnadenia conopsea* (L.) R. Brown s.l. (Fragrant Orchid) that are relevant to understanding its ecological characteristics and behaviour. The main topics are presented within the framework of the Biological Flora of the British Isles: distribution, habitat, communities, responses to biotic factors, responses to environment, structure and physiology, phenology, floral and seed characteristics, herbivores and disease, history and conservation. Most available information was published before recent revisions in taxonomy split what had been known as *Gymnadenia conopsea* into three separate species, now designated *G. borealis* (Druce) R.M. Bateman, Pridgeon & M.W. Chase, *G. conopsea* (L.) R. Brown and *G. densiflora* (Wahlenb.) Dietrich. Although the three newly accepted species have at least partial reproductive isolation, they have numerous morphological and ecological similarities. Some recent work still refers to *G. conopsea* s.l. because of difficulties in distinguishing between the species, and inertia in adoption of the new taxonomy. Wherever possible,

differences between *G. borealis*, *G. conopsea* s.s. and *G. densiflora* are highlighted. *Gymnadenia conopsea* s.l. is a perennial, terrestrial orchid that is widely distributed across Eurasia. The three segregate species are all considered native. *G. conopsea* s.s. (Chalk Fragrant Orchid) occurs mainly in the southern half of Britain, where it is most commonly observed in dry calcareous grasslands and pastures. *G. densiflora* (Marsh Fragrant Orchid) occurs in damp calcareous marshes, fens and dune slacks in England and Wales; it is rare in Scotland but frequent in Ireland. *G. borealis* (Heath Fragrant Orchid) is mainly found on hill pastures in Wales, northern England and Scotland. The three species all avoid shade, being most abundant in open habitats. Although the three segregate species cannot always be reliably distinguished on the basis of morphological characteristics, diagnostic features include plant height, leaf width and disposition, density of flowers in the inflorescence, shape of the labellum, spur width, and odour and colour of the flowers. Additionally, several cytotypes within *G. conopsea* s.s. have been observed in Central Europe. These often grow in mixed-ploidy populations; in contrast, the species appears to be predominantly diploid in Britain. *Gymnadenia conopsea* s.l. is a non-bulbous geophyte. Leaves emerge in spring, and flowering takes place between the end of June and the beginning of August. Vegetative adult dormancy the failure of above-ground parts to appear in a growing season, followed by reappearance of full-sized photosynthetic plants in subsequent years has been observed. Such dormancy rarely lasts longer than 1 year. The flowers of *G. conopsea* s.l. are arranged in a spike. The species is self-compatible but dependent on pollinators for successful pollination and fruit set. The most frequent pollinators are diurnal and nocturnal Lepidoptera. Pollinators are rewarded with abundant nectar produced within the long floral spur. In many populations, fruit set is > 90%. Although *G. conopsea* s.l. is widely distributed and can be locally abundant, the number of populations and size of populations in Britain have declined markedly during recent decades, mostly due to habitat destruction, ploughing of old fields and drainage. Because the distribution of the segregate species has been poorly documented, their conservation status is not well known. Conservation of remaining populations and restoration of degraded populations require reinstatement of appropriate management regimes (late summer/autumn mowing or grazing), combined with more detailed inventories of the three species.

Experimental evidence for heritable reproductive time in 2 allochronic populations of pine processionary moth

Branco, M; Paiva, MR; Santos, HM; Burban, C; Kerdelhue, C. 2017

Phenology allows organisms to overcome seasonally variable conditions through life-cycle adjustment. Changes in phenology can drastically modify the evolutionary trajectory of a population, while a shift in the reproductive time may cause allochronic differentiation. The hypothesis of heritable reproductive time was experimentally tested, by studying a unique population of the pine processionary moth *Thaumetopoea pityocampa* (Den. & Schiff.) which has a shifted phenology, and however co-occurs with the typical population following the classical life cycle. When populations of both types were reared under controlled conditions, the reproductive time was maintained asynchronous, as observed in the field. The shifted population was manipulated in the laboratory to reproduce later than usual, yet the offspring emerged in the next year at the expected dates thus coming back to the usual cycle. **Hybrids** from crosses performed between the 2 populations showed an intermediate phenology. From the emergence times of parents and offspring, a high heritability of the reproductive time ($h = 0.76$) was observed. The offspring obtained from each type of cross was genetically characterized using microsatellite markers. Bayesian clustering analysis confirmed that **hybrids** can be successfully identified and separated from the parental genetic classes by genotyping. Findings support the hypothesis that, for this particular population, incipient allochronic speciation is due to a heritable shift in the reproductive time that further causes assortative mating and might eventually cause ecological adaptation/maladaptation in response to environmental changes.

Study on host plants for reproduction of *Chilo suppressalis*

Jiang, WH; Li, HD; Cheng, XF; Ye, JR; Feng, YB; Han, ZJ. 2015

The rice stem borer, *Chilo suppressalis*, is generally considered to be a polyphagous pest. The current study challenges this view by investigating its oviposition preference, larval survival and development on different host plants under both laboratory and field conditions. Rice and water-oat populations of the borer inhabiting on corresponding plants respectively have partial reproductive isolation based on previous studies. In a laboratory multiple-choice test, *C. suppressalis* adults from rice population laid most of eggs on water-oat (46.5% of total eggs) and rice (43.8%), with very few laid on wheat (3.6%), sugarcane (4.0%) and maize (2.0%). Field surveys supported the laboratory study and found no egg on the plants other than rice and water-oat. Neonate inoculation experiments performed in field and laboratory showed that larval survival rate was much higher on rice (49.1%-51.2%) and wheat (36.5%-44.1%) than that on water-oat (10.7%-10.8%), maize (1.2%-7.2%), sugarcane (0-1.5%) and weeds (2.4%). These results were discussed with the data reported from water-oat population and it was concluded that *C. suppressalis* is not a typical polyphagous pest. Rice population mainly reproduces on rice and use water-oat only as minor host, and water-oat population breeds better on water-oat than on rice as reported. Neither population could thrive on the other recorded host plants, which are used for the supplementary nutrition sources of larvae. These findings provide useful information for the development of control strategies to prevent *C. suppressalis* laying eggs on rice seedlings in early spring, hence effectively reducing population density of this pest in rice fields. (C) 2015 Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society. Published by Elsevier B.V. All rights reserved.

Concordance of the spectral properties of dorsal wing scales with the phylogeographic structure of European male *Polyommatus icarus* butterflies

Piszer, G; Kertesz, K; Sramko, G; Krizsik, V; Balint, Z; Biro, LP. 2021

The males of more than 80% of the *Lycaenidae* species belonging to the tribe *Polyommata* exhibit structural coloration on their dorsal wing surfaces. These colors have a role in reinforcement in prezygotic reproductive isolation. The species-specific colors are produced by the cellular self-assembly of chitin/air nanocomposites. The spectral position of the reflectance maximum of such photonic nanoarchitectures depends on the nanoscale geometric dimensions of the elements building up the nanostructure. Previous work showed that the coloration of male *Polyommatus icarus* butterflies in the Western and Eastern Palearctic exhibits a characteristic spectral difference (20 nm). We investigated the coloration and the de novo developed DNA microsatellites of 80 *P. icarus* specimens from Europe from four sampling locations, spanning a distance of 1621 km. Remarkably good concordance was found between the spectral properties of the blue sexual signaling color (coincident within 5 nm) and the population genetic structure as revealed by 10 microsatellites for the *P. icarus* species.

Separation possibilities and genital measurement variations in two cryptic species of European pierid butterflies, *Leptidea juvernica* Williams, 1946 and *L. sinapis* (Linnaeus, 1758)

Sachanowicz, K. 2013

Of the three recently separated cryptic butterfly species of the *Leptidea sinapis* complex, the two species *L. sinapis* and *L. juvernica* occur sympatrically and syntopically in central Europe. As the separation of these species requires genital or genetic characters, their correct identification is a fundamental problem to be solved prior to any biological and distributional studies. In the present study a morphometric approach was applied to test for separation possibilities and to examine genital measurement variations based on large population samples of the two species (347 females and 636 males). Butterflies were collected at 456 localities distributed across Poland. Specimens of both sexes could be separated using either the shape or the length of the antrum bursae (females), phallus and saccus (males). Intraspecific seasonal differences in these measurements may affect the discrimination of males only. Genital characters were significantly larger in butterflies of the spring brood than in those of the second brood. In females, antrum bursae length ranges were separated by an interval, allowing for the exact identification of each specimen. In males, no such intervals were found between phallus and saccus length ranges, which slightly overlapped. Discriminant analysis resulted in 100% of males correctly classified. The present study confirmed the validity of interspecific differences in the shape of phallus and saccus for species identification without measurements. The length of these two characters discriminates the two species when separation is limited to specimens from the same generations. As an alternative for discriminant analysis, either phallus length and vinculum width or phallus length and the ratio of the ventral edge of genital capsule

length and saccus length (VEL/SL ratio) is recommended for the practical separation of the two species. The differences in the shape and size of the copulatory organs of the two species seem to indicate reproductive isolation due to mechanical incompatibility of their genitals. (c) 2013 Elsevier GmbH. All rights reserved.

Rational Design of a Novel Hawkmoth Pollinator Interaction in *Mimulus* Section *Erythranthe*

Byers, KJRP; Bradshaw, HD. 2021

Diversification of the ca. 275,000 extant flowering plant species has been driven in large part by coevolution with animal pollinators. A recurring pattern of pollinator shifts from hummingbird to hawkmoth pollination has characterized plant speciation in many western North American plant taxa, but in the genus *Mimulus* (monkeyflowers) section *Erythranthe* the evolution of hawkmoth pollination from hummingbird-pollinated ancestors has not occurred. We manipulated two flower color loci and tested the attractiveness of the resulting four color phenotypes (red, yellow, pink, and white) to naive hawkmoths (*Manduca sexta*). Hawkmoths strongly prefer derived colors (yellow, pink, white) over the ancestral red when choosing an initial flower to visit, and generally preferred derived colors when total visits and total visit time were considered, with no hawkmoth preferring ancestral red over derived colors. The simple flower color genetics underlying this innate pollinator preference suggests a potential path for speciation into an unfilled hawkmoth-pollinated niche in *Mimulus* section *Erythranthe*, and the deliberate design of a hawkmoth-pollinated flower demonstrates a new, predictive method for studying pollination syndrome evolution.

8-Methyldecan-2-yl acetate inhibits response to the pheromone in the western corn rootworm *Diabrotica v. virgifera*

Toth, M; Ujvary, I; Imrei, Z. 2010

Compounds that are structurally closely related to the western corn rootworm (WCR) (*Diabrotica v. virgifera*, Coleoptera: Chrysomelidae) pheromone were prepared and screened for biological activity in the field, presented alone or in combination with the pheromone 8-methyldecan-2-yl propanoate. None of the synthetic compounds showed attraction when presented alone. However, when presented in combination with the pheromone, catches in traps containing 8-methyldecan-2-yl acetate as a second component were dramatically reduced, suggesting strong inhibitory activity for this compound. The addition of the inhibitory acetate to the known floral WCR lure (4-methoxycinnamaldehyde plus indole) did not influence male (or female) catches suggesting that the inhibitor interferes in the perception process of the pheromone and not by exerting repellency per se. To our knowledge, this is the first report on an inhibitor of response to pheromone in WCR. 8-Methyldecan-2-yl acetate has previously been described as a sex attractant of *Diabrotica cristata*, so its inhibitory activity towards males of WCR may reflect a role in maintaining reproductive isolation between the two taxa.

Assortative Mating between European Corn Borer Pheromone Races: Beyond Assortative Meeting

Pelozuelo, L; Meusnier, S; Audiot, P; Bourguet, D; Ponsard, S. 2007

Background. Sex pheromone communication systems may be a major force driving moth speciation by causing behavioral reproductive isolation via assortative meeting of conspecific individuals. The 'E' and 'Z' pheromone races of the European corn borer (ECB) are a textbook example in this respect. 'Z' females produce and 'Z' males preferentially respond to a 'Z' pheromone blend, while the 'E' race communicates via an 'E' blend. Both races do not freely **hybridize** in nature and their populations are genetically differentiated. A straightforward explanation would be that their reproductive isolation is a mere consequence of "assortative meeting" resulting from their different pheromones specifically attracting males towards same-race females at long range. However, previous laboratory experiments and those performed here show that even when moths are paired in a small box - i.e., when the meeting between sexual partners is forced - inter-race couples still have a lower mating success than intra-race ones. Hence, either the difference in attractivity of E vs. Z pheromones for males of either race still holds at short distance or the reproductive isolation between E and Z moths may not only be favoured by assortative meeting, but must also result from an additional mechanism ensuring significant assortative mating at close range. Here, we test whether this close-range mechanism is linked to the E/Z female sex pheromone communication system. Methodology/Principal Findings. Using crosses and backcrosses of E and Z strains, we found no difference in mating success between full-sisters emitting different sex pheromones. Conversely, the mating success of females with identical pheromone types but different coefficients of relatedness to the two parental strains was significantly different, and was higher when their genetic background was closer to that of their male partner's pheromone race. Conclusions/Significance. We conclude that the close-range mechanism ensuring assortative mating between the E and Z ECB pheromone races is unrelated to the difference in female sex pheromone. Although the nature of this mechanism remains elusive, our results show that it is expressed in females, acts at close range, segregates independently of the autosome carrying Pher and of both sex chromosomes, and is widely distributed since it occurs both in France and in the USA.

Patterns of genomic and allochronic strain divergence in the fall armyworm, *Spodoptera frugiperda* (JE Smith)

Tessnow, AE; Raszick, TJ; Porter, P; Sword, GA. 2022

Speciation is the process through which reproductive isolation develops between distinct populations. Because this process takes time, speciation studies often necessarily examine populations within a species that are at various stages of divergence. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is comprised of two strains (R = Rice & C = Corn) that serve as a novel system to explore population divergence in sympatry. Here, we use ddRADSeq data to show that fall armyworm strains in the field are largely genetically distinct, but some interstrain **hybridization** occurs. Although we detected F1 **hybrids** of both R- and C-strain maternal origin, only **hybrids** with R-strain mtDNA were found to contribute to subsequent generations, possibly indicating a unidirectional barrier to gene flow. Although these strains have been previously defined as "host plant-associated," we recovered an equal proportion of R- and C-strain moths in fields dominated by C-strain host plants. As an alternative to host-associated divergence, we tested the hypothesis that differences in nightly activity patterns could account for reproductive isolation by genotyping temporally collected moths. Our data indicates that strains exhibit a significant shift in the timing of their nightly activities in the field. This divergence in phenology creates a prezygotic reproductive barrier that likely maintains the genetic isolation between strains. Thus, we conclude that it may be ecologically inaccurate to refer to the C- and R- strain as "host-associated" and they should more appropriately be considered "allochronic strains."

Mixed life cycles in the transitional zone between voltinisms in the fall webworm, *Hyphantria cunea*

Gomi, T. 1996

The fall webworm, *Hyphantria cunea* Drury, was introduced from North America into Japan at half a century ago. At present, the populations north of 36 degrees N are bivoltine and those south of 36 degrees N are trivoltine. In the present study, the life cycle in the transitional zone between voltinisms was clarified in Tsukuba (36.1 degrees N). When fourth instar larvae of the second generation were collected in the field and maintained in the laboratory, the incidence of pupal diapause increased when the sampling date was delayed from 16 August to 5 September. The fact that some developed without diapause indicates the occurrence of a trivoltine life cycle in this locality, where a bivoltine life cycle is predominant. The critical photoperiod for diapause induction at 20 and 25 degrees C was 14 h 13 min and 14 h 10 min, respectively. The photoperiodic response may explain the rapid increase in diapause incidence in late summer. In laboratory-reared diapause pupae, the time and period of chilling (5 degrees C) greatly influenced the time required for adult emergence at the final incubation temperature of 25 degrees C. However, diapause pupae chilled for a sufficiently long period developed to adulthood rapidly, irrespective of the conditions before chilling. Therefore, the difference in timing of entering diapause between pupae of the second and third generations would not result in their temporal reproductive isolation in the following spring. It is

concluded that the mixed voltinism in Tsukuba is not a result of a mixture of genetically distinct bivoltine and trivoltine populations, but a result of the phenotypic plasticity in a genetically rather homogeneous population.

Evolution of multiple sex-chromosomes associated with dynamic genome reshuffling in *Leptidea wood-white* butterflies 10.1038/s41437-020-0325-9

Yoshido, A; Sichova, J; Pospisilov, K; Nguyen, P; Volenikov, A; Safar, J; Provaznik, J; Vila, R; Marec, F. 2020

Sex-chromosome systems tend to be highly conserved and knowledge about their evolution typically comes from macroevolutionary inference. Rapidly evolving complex sex-chromosome systems represent a rare opportunity to study the mechanisms of sex-chromosome evolution at unprecedented resolution. Three cryptic species of wood-white butterflies-*Leptidea juvernica*, *L. sinapis* and *L. reali*-have each a unique set of multiple sex-chromosomes with 3-4 W and 3-4 Z chromosomes. Using a transcriptome-based microarray for comparative genomic **hybridisation** (CGH) and a library of bacterial artificial chromosome (BAC) clones, both developed in *L. juvernica*, we identified Z-linked *Leptidea* orthologs of *Bombyx mori* genes and mapped them by fluorescence in situ **hybridisation** (FISH) with BAC probes on multiple Z chromosomes. In all three species, we determined synteny blocks of autosomal origin and reconstructed the evolution of multiple sex-chromosomes. In addition, we identified W homologues of Z-linked orthologs and characterised their molecular differentiation. Our results suggest that the multiple sex-chromosome system evolved in a common ancestor as a result of dynamic genome reshuffling through repeated rearrangements between the sex chromosomes and autosomes, including translocations, fusions and fissions. Thus, the initial formation of neo-sex chromosomes could not have played a role in reproductive isolation between these *Leptidea* species. However, the subsequent species-specific fissions of several neo-sex chromosomes could have contributed to their reproductive isolation. Then, significantly increased numbers of Z-linked genes and independent neo-W chromosome degeneration could accelerate the accumulation of genetic incompatibilities between populations and promote their divergence resulting in speciation.

A combination of sexual and ecological divergence contributes to rearrangement spread during initial stages of speciation

Kozak, GM; Wadsworth, CB; Kahne, SC; Bogdanowicz, SM; Harrison, RG; Coates, BS; Dopman, EB. 2017

Chromosomal rearrangements between sympatric species often contain multiple loci contributing to assortative mating, local adaptation and **hybrid** sterility. When and how these associations arise during the process of speciation remains a subject of debate. Here, we address the relative roles of local adaptation and assortative mating on the dynamics of rearrangement evolution by studying how a rearrangement co-varies with sexual and ecological trait divergence within a species. Previously, a chromosomal rearrangement that suppresses recombination on the Z (sex) chromosome was identified in European corn borer moths (*Ostrinia nubilalis*). We further characterize this recombination suppressor and explore its association with variation in sex pheromone communication and seasonal ecological adaptation in pairs of populations that are divergent in one or both of these characteristics. Direct estimates of recombination suppression in pedigree mapping families indicated that more than 39% of the Z chromosome (encompassing up to similar to 10 megabases and similar to 300 genes) resides within a non-recombining unit, including pheromone olfactory receptor genes and a major quantitative trait locus that contributes to ecotype differences (Pdd). Combining direct and indirect estimates of recombination suppression, we found that the rearrangement was occasionally present between sexually isolated strains (E vs. Z) and between divergent ecotypes (univoltine vs. bivoltine). However, it was only consistently present when populations differed in both sexual and ecological traits. Our results suggest that independent of the forces that drove the initial establishment of the rearrangement, a combination of sexual and ecological divergence is required for rearrangement spread during speciation.

The Genetic Structure of Asian Corn Borer, *Ostrinia furnacalis*, Populations in China: Haplotype Variance in Northern Populations and Potential Impact on Management of Resistance to Transgenic Maize

Li, J; Coates, BS; Kim, KS; Bourguet, D; Ponsard, S; He, KL; Wang, ZY. 2014

Asian corn borer, *Ostrinia furnacalis* (Guenee), is a severe pest that infests cultivated maize in the major production regions of China. Populations show genotype-by-environment variation in voltinism, such that populations with a single generation (univoltine) are fixed in Northern China where growing seasons are short. Low genetic differentiation was found among samples from 33 collection sites across China and one site from North Korea ($n = 1673$) using variation at 6 nuclear microsatellite loci (ENA corrected global $F_{ST} = 0.020$; P value < 0.05). Analysis of molecular variance indicated that geographic region, number of generations or voltinism accounted for $< 0.38\%$ of the total genetic variation at nuclear loci and was corroborated by clustering of co-ancestries among genotypes using the program STRUCTURE. In contrast, a mitochondrial haplotype network identified 4 distinct clusters, where 70.5% of samples from univoltine populations were within a single group. Univoltine populations were also placed into a unique cluster using Population Graph and Principal component analyses, which showed significant differentiation with multivoltine populations ($\phi(ST) = 0.400$; P value < 0.01). This study suggests that gene flow among *O. furnacalis* in China may be high among regions, with the exception of northeastern localities. Haplotype variation may be due to random genetic drift resulting from partial reproductive isolation between univoltine and multivoltine *O. furnacalis* populations. Such reproductive isolation might impact the potential spread of alleles that confer resistance to transgenic maize in China.

Genetic basis to divergence of sex pheromones in two closely related moths, *Ostrinia scapularis* and *O. zealis*

Tabata, F; Ishikawa, Y. 2005

Crossing experiments between two closely related moths, *Ostrinia scapularis* and *O. zealis*, were conducted to gain insight into the genetic basis of the divergence of female sex pheromones. The sex pheromone of *O. scapularis* comprises (E)-11- and (Z)-11-tetradecenyl acetates (E11 and Z11), and distinct genetic variation is found in the blend of components. This variation is largely controlled by a single autosomal locus with two alleles, A(E(sca)) and A(Z(sca)). E-type (A(E(sca))A(E(sca))) females produce a pheromone with a mean E11:Z11 ratio of 99:1, whereas Z-type (A(Z(sca))A(Z(sca))) and I-type (A(E(sca))A(Z(sca))) females produce a pheromone with a mean of 3:97 and 64:36, respectively. *O. zealis* is distinctive in that it has a third pheromone component, (Z)-9-tetradecenyl acetate (Z9), in addition to E11 and Z11, and the typical blend ratio is 60:35:5 (Z9:E11:Z11). Our study revealed that Z9 production in *O. zealis* is mainly regulated by an autosomal recessive gene *phr((zea))*, which is suggested to be involved in the chain-shortening of a pheromone precursor fatty acid, and linked to A(E(zca)), a gene corresponding to A(E(sca)) in *O. scapularis*. A few mutations in a gene involved in pheromone production could explain the dramatic shift between a two-component pheromone communication system in *O. scapularis* and a three-component system in *O. zealis*.

Facultative pupal mating in *Heliconius erato*: Implications for mate choice, female preference, and speciation

Thurman, TJ; Brodie, E; Evans, E; McMillan, WO. 2018

Mating systems have broad impacts on how sexual selection and mate choice operate within a species, but studies of mating behavior in the laboratory may not reflect how these processes occur in the wild. Here, we examined the mating behavior of the neotropical butterfly *Heliconius erato* in the field by releasing larvae and virgin females and observing how they mated. *H. erato* is considered a pupal-mating species (i.e., males mate with females as they emerge from the pupal case). However, we observed only two teneral mating events, and experimentally released virgins were almost all mated upon recapture. Our study confirms the presence of some pupal-mating behavior in *H. erato*, but suggests that adult mating is likely the prevalent mating strategy in this species. These findings have important

implications for the role of color pattern and female mate choice in the generation of reproductive isolation in this diverse genus.

Genetics of female mate discrimination of heterospecific males in *Nasonia* (Hymenoptera, Pteromalidae)

Velthuis, B.J.; Yang, W.C.; van Opijnen, T.; Werren, J.H. 2005

Despite its importance to mating system evolution, sexual selection and reproductive isolation, little is known about the genetic basis of female mate choice. We studied the behavioural and genetic basis of interspecific mate discrimination in *Nasonia* wasps, using two strains of *N. longicornis* (LCa and LId) that differ in female acceptance of males from the sibling species *N. vitripennis*. Both strains showed low acceptance ($\leq 2\%$) of *N. vitripennis* males in the first courtship, but acceptance by LId females subsequently increased (11% by the second and 15% by the third courtship), whereas acceptance by LCa females remained low (! 2%). After 2 h of exposure to a *N. vitripennis* male, 59% of LId females mated compared with only 8% of LCa females. Interstrain cross F1 females showed significantly higher acceptance of *N. vitripennis* males than either parental strain in the first courtship (15-29%) and after 2 h (79-82%). A genetic analysis of acceptance at 2 h revealed three major quantitative trait loci (QTL). For each major QTL, the allele that increased female mate acceptance was dominant. The QTL with the largest effect accounted for half the strain difference, and was confirmed by additional crosses. This QTL was involved in the females' continued reluctance to mate with *N. vitripennis* males during multiple courtships. Results are discussed in terms of the behavioural and genetic architecture of female mate discrimination and possible implications to the evolution of courtship, mating systems and reproductive isolation. (c) 2005 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Female mate choice is a reproductive isolating barrier in *Heliconius* butterflies

Southcott, L.; Kronforst, M.R. 2018

In sexually reproducing organisms, speciation involves the evolution of reproductive isolating mechanisms that decrease gene flow. Premating reproductive isolation, often the result of mate choice, is a major obstacle to gene flow between species because it acts earlier in the life cycle than other isolating barriers. While female choice is often considered the default mode in animal species, research in the butterfly genus *Heliconius*, a frequent subject of speciation studies, has focused on male mate choice. We studied mate choice by *Heliconius cydno* females by pairing them with either conspecific males or males of the closely related species *Heliconius pacheus*. Significantly more intraspecific trials than interspecific trials resulted in mating. Because male courtship rates did not differ between the species when we excluded males that never courted, we attribute this difference to female choice. Females also performed more acceptance behaviours towards conspecific males. Premating isolation between these two species thus entails both male and female mate choice, and female choice may be an important factor in the origin of *Heliconius* species.

Phenotypic divergence in reproductive traits of a moth population experiencing a phenological shift

Santos, H.M.; Paiva, M.R.; Rocha, S.; Kerdelhue, C.; Branco, M. 2013

Allochrony that is reproductive isolation by time may further lead to divergence of reproductive adaptive traits in response to different environmental pressures over time. A unique summer population of the pine processionary moth *Thaumetopoea pityocampa*, reproductively isolated from the typical winter populations by allochronic differentiation, is here analyzed. This allochronically shifted population reproduces in the spring and develops in the summer, whereas winter populations reproduce in the late summer and have winter larval development. Both summer and winter populations coexist in the same pine stands, yet they face different climatic pressures as their active stages are present in different seasons. The occurrence of significant differences between the reproductive traits of the summer population and the typical winter populations (either sympatric or allopatric) is thus hypothesized. Female fecundity, egg size, egg covering, and egg parasitism were analyzed showing that the egg load was lower and that egg size was higher in the summer population than in all the studied winter populations. The scales that cover the egg batches of *T. pityocampa* differed significantly between populations in shape and color, resulting in a looser and darker covering in the summer population. The single specialist egg parasitoid species of this moth was almost missing in the summer population, and the overall parasitism rates were lower than in the winter population. Results suggest the occurrence of phenotypic differentiation between the summer population and the typical *T. pityocampa* winter populations for the life-history traits studied. This work provides an insight into how ecological divergence may follow the process of allochronic reproductive isolation.

Subspecific differentiation in male reproductive traits and virgin queen preferences, in *Bombus terrestris*

Lecocq, T.; Coppee, A.; Mathy, T.; Lhomme, P.; Cammaerts-Tricot, M.C.; Urbanova, K.; Valterova, I.; Rasmont, P. 2015

Many species display local variations in pre-mating signals and in mating preferences. This may lead to discrimination against potential foreign mates that may ultimately lead to reproductive isolation. However, the extent to which population differentiation in mating cues affects the species recognition has received little empirical support. Here, we investigate the consequence of geographic differentiation in male reproductive traits on female preferences to these traits in *Bombus terrestris*. We characterise (1) the geographic differentiation in male cephalic labial gland secretions (CLGS), a key trait for mate attraction, and (2) the preference of virgin females to the CLGS of different subspecies. Our results show geographic CLGS differences parallel with divergences in female preferences for these secretions. This geographic CLGS differentiation in males, along with female preference for sympatric males, could lead to or reflect a pre-mating isolation among subspecies.

Phylogenomic Analyses Clarify True Species within the Butterfly Genus *Speyeria* despite Evidence of a Recent Adaptive Radiation

Thompson, E.; Baumsteiger, J.; Hill, R.L. 2019

When confronted with an adaptive radiation, considerable evidence is needed to resolve the evolutionary relationships of these closely related lineages. The North American genus *Speyeria* is one especially challenging radiation of butterflies due to potential signs of incomplete lineage sorting, ongoing **hybridization**, and similar morphological characters between species. Previous studies have found species to be paraphyletic and have been unable to disentangle taxa, often due to a lack of data and/or incomplete sampling. As a result, *Speyeria* remains unresolved. To achieve phylogenetic resolution of the genus, we conducted phylogenomic and population genomic analyses of all currently recognized North American *Speyeria* species, as well as several subspecies, using restriction-site-associated DNA sequencing (RADseq). Together, these analyses confirm the 16 canonical species, and clarify many internal relationships. However, a few relationships within *Speyeria* were poorly supported depending on the evolutionary model applied. This lack of resolution among certain taxa corroborates *Speyeria* is experiencing an ongoing adaptive radiation, with incomplete lineage sorting and lack of postzygotic reproductive barriers contributing to **hybridization** and further ambiguity. Given that many *Speyeria* taxa are under duress from anthropogenic factors, their legal protection must be viewed cautiously and on a case by case basis in order to properly conserve the diversity being generated.

Sympatric, temporally isolated populations of the pine white butterfly *Neophasia menapia*, are morphologically and genetically differentiated

Bell, KL; Hamm, CA; Shapiro, AM; Nice, CC. 2017

Temporal isolation remains an understudied, and potentially under-appreciated, mechanism of reproductive isolation. Phenological differences have been discovered in populations of the pine white butterfly (*Neophasia menapia*), a typically univoltine species found throughout western North America. At two locations in the Coast Range of California there are two periods of adult emergence per year, one in early summer (July) and one in late summer/ autumn (September/October). Differences in flight time are accompanied by differences in wing shape and pigmentation. Here we use a combination of population genomics and morphological analyses to assess the extent to which temporal isolation is able to limit gene flow between sympatric early and late flights. Not only did we detect both genetic and morphological differences between early and late flights at the two sites, we also found that the patterns of differentiation between the two flights were different at each location, suggesting an independent origin for the two sympatric flights. Additionally, we found no evidence that these sympatric flights originated via colonization from any of the other sampled localities. We discuss several potential hypotheses about the origin of these temporally isolated sympatric flights.

Host-plant dependent wing phenotypic variation in the neotropical butterfly *Heliconius erato*

Jorge, LR; Cordeiro-Estrela, P; Klaczko, LB; Moreira, GRP; Freitas, AVL. NA

Most phytophagous insects feed on a single plant during development, and this may influence not only performance-linked traits, but also more subtle morphological differences. Insect-plant interactions are thus valuable for studying environmental influences on phenotypes. By using geometric morphometrics, we investigated the variation in forewing size and shape in the butterfly *Heliconius erato* phyllis reared on six species of passion vines (*Passiflora* spp.). We detected wing shape sexual dimorphism, for which the adaptive significance deserves further investigation. There was size as well as wing shape variation among individuals fed on different hosts. These subtle differences in shape were interpreted as environmental effects on development, which should be under weak natural selection for these traits, and therefore not strongly canalized. This result reinforces the role of plasticity on host-plant use, as well as the corresponding consequences on developmental variability among phytophagous insects. We propose that this variation can be an important factor in resource specialization and partner recognition, possibly triggering reproductive isolation and sympatric speciation in phytophagous insects. This interaction also shows itself as a good model for studying the role of environmental and interaction diversity in evolution. (c) 2011 The Linnean Society of London, Biological Journal of the Linnean Society, 2011, 102, 765-774.

A single point mutation causes one-way alteration of pheromone receptor function in two *Heliothis* species

Cao, S; Liu, Y; Wang, B; Wang, GR. 2021

The sex pheromone processing system of moths has been a major focus of research on olfaction and speciation, as it is highly specific and closely related to reproductive isolation. The two noctuid moths *Heliothis virescens* and *Heliothis subflexa* have been used as a model for deciphering the mechanisms underlying differentiation in pheromone communication, but no information exist regarding the functions of the pheromone receptors (PRs) of *H. subflexa*. Here, we functionally characterized all candidate PRs of *H. subflexa*, and found that only the response profile of OR6 differed between the two species. Through domain swapping and site-directed mutation followed by functional characterization, we identified a critical amino acid in OR6 caused a one-way alteration in specificity. This result suggests HsubOR6 evolved from an ancestral OR6 gene with a HvirOR6-like function and implies that the evolutionary direction of the receptor specificity was from the *H. virescens*-like pattern to *H. subflexa*-like pattern.

Response of *Mythimna unipuncta* Males to Components of the *Sesamia nonagrioides* Pheromone

Eizaguirre, M; Lopez, C; Sans, A; Bosch, D; Albajes, R. 2009

Several sympatric lepidopteran species feed on maize plants, and the different components of their species-specific female sex pheromones may play a role in attracting conspecifics and/or deter heterospecific males. In this study, we analyzed the content of *Mythimna unipuncta* pheromone glands and tested the response of males to components of their own pheromone blend and that of *Sesamia nonagrioides* in the wind tunnel. Whole pheromone glands, and lures where (Z)-9-hexadecenyl acetate, (Z)-11-hexadecen-1-ol or (Z)-9-hexadecenyl acetate + (Z)-11-hexadecen-1-ol were added to the major component, (Z)-11-hexadecenyl acetate, elicited significantly higher responses by *M. unipuncta* males than lures with main component alone, although the levels varied with concentration. In the field a rather different outcome was observed, as the addition of other compounds found in the female pheromone gland did not improve trap catch over lures with only (Z)-11-hexadecenyl acetate. The addition of (Z)-11-hexadecenal, a compound of the *S. nonagrioides* pheromone, to (Z)-11-hexadecenyl acetate significantly reduced attraction of *M. unipuncta* males both in the wind tunnel and in the field, as well as the number of sympatric clover cutworm, *Discestra trifolii*, under field conditions. The addition of (Z)-9-hexadecenyl acetate, a minor component of the *M. unipuncta* pheromone blend, reduced the number of *S. nonagrioides* captured in field traps that were baited with the *S. nonagrioides* lure. The significance of such inhibition in the reproductive isolation of sympatric species that attack maize is discussed.

A Delta 11 desaturase gene genealogy reveals two divergent allelic classes within the European corn borer (*Ostrinia nubilalis*)

Geiler, KA; Harrison, RG. 2010

Background: Moth pheromone mating systems have been characterized at the molecular level, allowing evolutionary biologists to study how changes in protein sequence or gene expression affect pheromone phenotype, patterns of mating, and ultimately, the formation of barriers to gene exchange. Recent studies of *Ostrinia* pheromones have focused on the diversity of sex pheromone desaturases and their role in the specificity of pheromone production. Here we produce a Delta 11 desaturase genealogy within *Ostrinia nubilalis*. We ask what has been the history of this gene, and whether this history suggests that changes in Delta 11 desaturase have been involved in the divergence of the E and Z *O. nubilalis* pheromone strains. Results: The Delta 11 desaturase gene genealogy does not differentiate *O. nubilalis* pheromone strains. However, we find two distinct clades, separated by 2.9% sequence divergence, that do not sort with pheromone strain, geographic origin, or emergence time. We demonstrate that these clades do not represent gene duplicates, but rather allelic variation at a single gene locus. Conclusions: Analyses of patterns of variation at the Delta 11 desaturase gene in ECB suggest that this enzyme does not contribute to reproductive isolation between pheromone strains (E and Z). However, our genealogy reveals two deeply divergent allelic classes. Standing variation at loci that contribute to mate choice phenotypes may permit novel pheromone mating systems to arise in the presence of strong stabilizing selection.

POPULATION-STRUCTURE AND DETERMINATIONS OF EVOLUTION WITHIN THE GENUS *OSTRINIA*

FROLOV, AN. 1994

The analysis of evolution modes in the genus *Ostrinia* was carried out basing on comparison of literature data on biology of *Ostrinia* spp. and pheromone races in *O. nubilalis* with obtained by author experimental data on the population structure and factors determining it. The conclusion is drawn that evolution in the genus *Ostrinia* had gone in the direction of adaptation to less humid areas, and within, the group of species with trilobed uncus at least two evolutionary trends can be marked out. The evidence produced by us show that origin of different pheromone strains in European corn borer was caused by adaptations to different levels of

humidity; the hydrophilous E-race is found in humid regions of the Alps, the Carpathians, and the Caucasus. Relatively xerophilous Z-race - in less humid areas with the sea climate. The variable climate of the continent stimulate population polymorphism within the species. Capable to intergradation *O. nubilalis*, *O. narynensis*, *O. scapularis* and *O. persica* initially developing on the same range of dicotyledonous host plants, seem to be the semispecies adapted to different non-overlapping diapasons of humidity which ensure effective utilization of heterogenous living space by the system (*O. nubilalis* s. 1.); **hybrid** zones cannot be considered as the tension ones, but they are integral parts of the system. The adaptation of some European corn borer populations to feeding on cereals resulted in formation of reproductive isolation and promoted the borer's expansion into less humid regions.

Ehrlich and Raven Revisited: Mechanisms Underlying Codiversification of Plants and Enemies

Janz, N. 2011

After almost 50 years of scrutiny, the ideas that Ehrlich and Raven presented in their classical paper on the coevolution between butterflies and plants are still very much alive. Much of this interest has involved the potential for codiversification, both in how the interaction itself diversifies and how the interaction affects modes and rates of speciation. Despite high levels of conservatism and specialization, diversification of the interaction appears to be mainly a consequence of host shifts, but this somewhat paradoxical conclusion can be understood by an appreciation of the ecological as well as genetic mechanisms behind host shifts. There are several ways that the interaction can influence speciation, with or without host-plant-based divergent selection on reproductive barriers. One current debate is over the relative importance of radiations following shifts to new adaptive zones and elevated rates of speciation in groups with plastic and diverse host use.

A role for both ecology and geography as mechanisms of genetic differentiation in specialized butterflies

Downey, MH; Nice, CC. 2013

An important mechanism of speciation for specialized phytophagous insects is host-associated differentiation, in which natural selection drives the evolution of reproductive isolation. Here we use molecular population genetics data to test the hypothesis that regional and local specialization on three alternate hosts restricts gene flow in the *Mitoura gryneus* species complex of butterflies. Over half of the variation in mitochondrial DNA sequences is explained by host plant association (I center dot(CT) = 0.57, P = 0.002) consistent with the hypothesis of host-associated divergence on the three hosts. AFLP analyses revealed the number of clusters of individuals was $K = 2$, with all individuals associated with one host grouping separately from all other host-associated individuals. Combined with previous experimental results, these findings present evidence of varying levels of differentiation among host associations and identify a role for both isolation in allopatry as well as ecological factors in limiting gene exchange. The *Mitoura* species complex includes multiple, differentiated lineages at varying stages of divergence, providing an opportunity to examine the multifarious mechanisms that generate biodiversity in phytophagous insects.

Molecular population genetics of a host-associated sibling species complex of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachninae)

Kobayashi, N; Kumagai, M; Minegishi, D; Tamura, K; Aotsuka, T; Katakura, H. 2011

To clarify evolutionary relationships and assess the intensity and persistence of reproductive isolation because of host fidelity in three closely related host-associated phytophagous ladybird beetles (*Henosepilachna pustulosa*, *Henosepilachna niponica* and *Henosepilachna yasutomii*), we determined sequences of part of the nuclear DNA internal transcribed spacer-II (ITS-2) region (388 bp long) for 70 individuals from seven populations, and part of the mitochondrial cytochrome c oxidase subunit I (COI) gene (985 bp long) for 280 individuals from 28 populations across the three species. We failed to detect any polymorphic sites in the ITS-2 region. We detected 59 COI haplotypes, among which two were shared by different species. While a haplotype network and a phylogenetic gene tree of the COI haplotypes did not support monophyly for any of the three species, the results of AMOVA did not invalidate the classification of these beetles into three species. The isolation-with-migration analytic (IMa) test suggested that gene flow between allopatric species (i.e., *H. pustulosa* versus *H. niponica*; *H. pustulosa* versus *H. yasutomii*) was well prevented, probably because of their limited dispersal power. On the other hand, the IMa test detected a low level of unidirectional gene flow between the sympatric species *H. niponica* and *H. yasutomii*, from the former to the latter. This result was consistent with a survival rate of F1 **hybrids** between the two species that is higher on the host plant of *H. yasutomii* than on the host plant of *H. niponica*. High F_{ST} values among two sympatric and three nearly sympatric population pairs of *H. niponica* and *H. yasutomii*, however, indicated that gene flow between the two species has been quite restricted even if it has occurred. Because no reproductive barriers other than the difference in host plants is known for sympatric *H. niponica* and *H. yasutomii*, our results suggest that host differentiation and associated ecological divergence function as an effective reproductive barrier between the two species. Resume Genetique moleculaire des populations d'un complexe d'especes jumelles de coccinelles phytophages avec specificite d'hote (Coleoptera: Coccinellidae: Epilachninae) En vue d'eclaircir les relations phylogenetiques et de mesurer l'intensite et la persistance de l'isolement reproductif du a la specificite d'hote chez trois coccinelles phytophages (*Henosepilachna pustulosa*, *H. niponica* et *H. yasutomii*), nous avons determine d'une part, les sequences de la region ITS-2 (388 pb) de l'ADN nucleaire chez 70 individus dans 7 populations et d'autre part, d'une partie du gene mitochondrial cytochrome c oxydase sous unite I (COI; 985 pb) chez 280 individus provenant de 28 populations. Aucun site polymorphique n'a ete detecte dans la region ITS-2. Nous avons detecte 59 haplotypes COI dont deux etaient communs a des especes differentes. Un reseau d'haplotypes pour les trois especes n'a pas montre leur monophylie mais une AMOVA n'a pas refute l'hypothese de l'existence de trois especes. Le test analytique de migration-avec-isolement (IMa) a suggere que l'echange genique entre des especes allopatriques (*H. pustulosa* versus *H. niponica*; *H. pustulosa* versus *H. yasutomii*) est bien empeche, sans doute a cause de leur pouvoir de dispersion limite. Par ailleurs, le test IMa a detecte un flux genique unidirectionnel faible entre les especes sympatriques *H. niponica* et *H. yasutomii*, du premier au second. Ce resultat est en accord avec le fait que le taux de survie des **hybrides** F1 entre ces deux especes est superieur sur la plante hote de *H. yasutomii* que sur celle de *H. niponica*. Cependant, des valeurs elevees de F_{ST} pour deux paires de populations sympatriques et trois paires presque sympatriques de *H. niponica* et *H. yasutomii* ont montre des echanges de genes nuls ou tres restreints entre ces deux especes. Malgre l'absence de barriere reproductrice autre que la difference de plantes hotes entre les populations sympatriques de *H. niponica* et *H. yasutomii*, nos resultats suggerent que la specificite d'hote et la divergence ecologique qui en resulte constituent une barriere reproductrice efficace entre les deux especes.

Exposure to Conspecific and Heterospecific Sex-Pheromones Modulates Gustatory Habituation in the Moth *Agrotis ipsilon*

Hostachy, C; Couzi, P; Portemer, G; Hanafi-Portier, M; Murmu, M; Deisig, N; Dacher, M. 2019

In several insects, sex-pheromones are essential for reproduction and reproductive isolation. Pheromones generally elicit stereotyped behaviors. In moths, these are attraction to conspecific sex-pheromone sources and deterrence for heterospecific sex-pheromone. Contrasting with these innate behaviors, some results in social insects point toward effects of non-sex-pheromones on perception and learning. We report the effects of sex-pheromone pre-exposure on gustatory perception and habituation (a non-associative learning) in male *Agrotis ipsilon* moths, a non-social insect. We also studied the effect of Z5-decenyl acetate (Z5), a compound of the sex-pheromone of the related species *Agrotis segetum*. We hypothesized that conspecific sex-pheromone and Z5 would have opposite effects. Pre-exposure to either the conspecific sex-pheromone or Z5 lasted 15 min and was done either immediately or 24 h before the experiments, using their solvent alone (hexane) as control. In a sucrose responsiveness assay, pre-exposure to the conspecific sex-pheromone had no effect on the dose-response curve at either delays. By contrast, Z5 slightly improved sucrose responsiveness 15 min but not 24 h after pre-exposure. Interestingly, the conspecific sex-pheromone and Z5 had time-dependent effects on gustatory habituation: pre-exposing the moths with Z5 hindered learning after immediate but not 24-h pre-exposure, whereas pre-exposure to the conspecific sex-pheromone hindered learning at 24-h but not immediate pre-exposure. They did not have opposite effects. This is the first time a sex-pheromone is reported to affect learning in a non-social insect. The difference in modulation between conspecific sex-pheromone and Z5 suggests that con- and hetero-specific sex-pheromones act on plasticity through different cerebral pathways.

Population genetic structure and assessment of allochronic divergence in the Macoun's Arctic (*Oeneis macounii*) butterfly

Gradish, AE; Keyghobadi, N; Sperling, FAH; Otis, GW. 2019

Patterns in the genetic variation of species can be used to infer their specific demographic and evolutionary history and provide insight into the general mechanisms underlying population divergence and speciation. The Macoun's Arctic (*Oeneis macounii* (W.H. Edwards, 1885); MA) butterfly occurs across Canada and parts of the northern United States in association with jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*Pinus contorta* Douglas ex Loudon). MA's current distribution is highly fragmented, and the extent of reproductive isolation among allopatric populations is unknown. Furthermore, although MA is biennial, adults emerge every year in some populations. These populations presumably consist of two alternate-year cohorts, providing the opportunity for sympatric divergence via allochronic isolation. Using mitochondrial DNA (mtDNA) and amplified fragment length polymorphism (AFLP) markers, we analyzed MA's genetic structure to determine the current and historical role of allopatric and allochronic isolation in MA population divergence. Both markers revealed high diversity and a low, but significant, degree of spatial structure and pattern of isolation by distance. Phylogeographic structure was generally absent, with low divergence among mtDNA haplotypes. MA likely exhibits low dispersal and gene flow among most allopatric populations; however, there was no evidence of differentiation resulting from allochronic isolation for sympatric cohorts.

Genetic mechanisms preventing the fusion of ecotypes even in the face of gene flow

Ohshima, I. 2012

Understanding the genetics behind adaptation and reproductive isolation contributes to our knowledge about how biodiversity is created and maintained. Host races of phytophagous insects are host-associated ecotypes and have been considered as candidates for ecological speciation, but very little is known about the genetic backgrounds of host adaptations. A leaf-mining moth, *Acrocercops transecta*, consists of *Juglans*- and *Lyonia*-associated host races. This study assesses the genetic bases of oviposition preference and larval performance using F-1, F-2 and backcross **hybrids** between the two host races. Segregation patterns in the **hybrid** generations revealed that larval performance on *Juglans* is dominant, but oviposition preference for *Lyonia* is dominant. This result indicates that genetic components introgressed from the *Lyonia* race are removed from the *Juglans* race even though **hybrid** larvae are viable on *Juglans*. Thus, simple genetic controls with contrasting dominance directions in host-adaptation traits function as barriers to prevent a fusion of host races.

Changes of Sex Pheromone Communication Systems Associated with Tebufenozide and Abamectin Resistance in Diamondback Moth, *Plutella xylostella* (L.)

Xu, Z; Cao, GC; Dong, SL. 2010

Many insect pests have evolved resistance to insecticides. Along with this evolution, the sex pheromone communication system of insects also may change, and subsequently reproductive isolation may occur between resistant and susceptible populations. In this study of the diamondback moth, we found that resistant females (especially Abamectin resistant females) produced less sex pheromone and displayed a lower level of calling behavior. Resistant males showed higher EAG responsiveness to the sex pheromone mixture of females, and responded to a broader range of ratios between the two major components compared to the responses of susceptible moths. In addition, wind tunnel experiments indicated that changes associated with insecticide resistance in the Abamectin resistant strain (Aba-R) significantly reduced female attractiveness to susceptible males. Furthermore, mating choice experiments confirmed that non-random mating occurred between the two different strains. Aba-R females with an abnormal pheromone production and blend ratio exhibited significantly lower mating percentages with males from either their own strain or other strains, which corroborates the results obtained by the wind tunnel experiments. The implications of this non-random mating for insect speciation and insecticide resistance management are discussed.

Integration of molecular, ecological, morphological and endosymbiont data for species delimitation within the *Pnigalio soemius* complex (Hymenoptera: Eulophidae)

Gebiola, M; Gomez-Zurita, J; Monti, MM; Navone, P; Bernardo, U. 2012

Integrative taxonomy is a recently developed approach that uses multiple lines of evidence such as molecular, morphological, ecological and geographical data to test species limits, and it stands as one of the most promising approaches to species delimitation in taxonomically difficult groups. The *Pnigalio soemius* complex (Hymenoptera: Eulophidae) represents an interesting taxonomical and ecological study case, as it is characterized by a lack of informative morphological characters, deep mitochondrial divergence, and is susceptible to infection by parthenogenesis-inducing *Rickettsia*. We tested the effectiveness of an integrative taxonomy approach in delimiting species within the *P. soemius* complex. We analysed two molecular markers (COI and ITS2) using different methods, performed multivariate analysis on morphometric data and exploited ecological data such as hostplant system associations, geographical separation, and the prevalence, type and effects of endosymbiont infection. The challenge of resolving different levels of resolution in the data was met by setting up a formal procedure of data integration within and between conflicting independent lines of evidence. An iterative corroboration process of multiple sources of data eventually indicated the existence of several cryptic species that can be treated as stable taxonomic hypotheses. Furthermore, the integrative approach confirmed a trend towards host specificity within the presumed polyphagous *P. soemius* and suggested that *Rickettsia* could have played a major role in the reproductive isolation and genetic diversification of at least two species.

Size and shape differences in genitalia of males from sympatric and reproductively isolated populations of *Anthocoris antevolens* White (Heteroptera : Anthocoridae) in the Yakima Valley, Washington

Horton, DR; Lewis, TM. 2005

Anthocoris antevolens White (Heteroptera: Anthocoridae) is a widespread predatory bug in North America commonly associated with deciduous trees and shrubs. Unpublished observations showed that there is considerable geographic variation in male genitalia in this species and that the variation may lead to reproductive isolation among geographically separated populations. We show that male bugs from two sympatric populations in the Yakima Valley, Washington, one occurring on oak (*Quercus garryana* Douglas) and the other collected from willow (*Salix* sp.), differed in size and shape of the phallus and clasper. Mating trials showed that males from the oak source successfully inseminated females from the oak source in 75% of pairings; insemination success for males from the willow source paired with females from the willow source was somewhat lower at 62%. In nonlike crosses (oak X willow, willow X oak), males failed to inseminate the female in 100% of pairings, despite vigorous mating attempts by the males. Copulation duration was independent of population source. However, males from the willow source initiated copulation attempts significantly sooner in the assay than males from the oak source, irrespective of female source. We interrupted copulating pairs by freezing them with liquid nitrogen and showed that males in nonlike crosses generally had failed to fully inflate the phallus in the female. Results support statements made elsewhere that *A. antevolens* is actually composed of an unknown number of reproductively isolated cryptic species.

Assortative mating in two pheromone strains of the cabbage looper moth, *Trichoplusia ni*

Zhu, JW; Chastain, BB; Spohn, BG; Haynes, KF. 1997

The evolution of animal communication systems is an integral part of speciation. In moths, species specificity of the communication channel is largely a result of unique sex pheromone blends produced by females and corresponding specificity of male behavioral response. Insights into the process of speciation may result from studies of pheromone strains within a species in which reproductive isolation is not complete. Toward this end we investigated assortative mating based on female pheromone phenotypes and male response specificity between mutant and normal colonies of the cabbage looper moth, *Trichoplusia ni*. There was no evidence of assortative mating in small cages in which the density of moths was high. In larger cages with lower densities of moths, assortative mating was evident. In these larger cages, matings between normal males and normal females and mutant males and mutant females were more frequent than interstrain matings. Wind tunnel tests indicated that normal males responded preferentially to pheromone released by normal females, whereas, mutant males did not discriminate between normal and mutant pheromone blends. In large field cages, pheromone traps baited with normal females caught equal numbers of mutant and normal males, while pheromone traps baited with mutant females caught primarily mutant males. The overall pattern of assortative mating could be explained primarily based on the normal males' preference for the pheromone blend released by normal females.

Ostrinia revisited: Evidence for sex linkage in European Corn Borer *Ostrinia nubilalis* (Hubner) pheromone reception

Olsson, SB; Kesevan, S; Groot, AT; Dekker, T; Heckel, DG; Hansson, BS. 2010

Background: The European Corn Borer, *Ostrinia nubilalis* (Hubner), is a keystone model for studies on the evolution of sex pheromone diversity and its role in establishing reproductive isolation. This species consists of two sympatric races, each utilizing opposite isomers of the same compound as their major pheromone component. Female production and male response are congruent in each race, and males from each strain exhibit phenotypic differences in peripheral physiology. Both strains possess co-localized pheromone-sensitive olfactory sensory neurons characterized by a larger amplitude action potential (spike) responding to the major pheromone component, and a smaller spike amplitude cell responding to the minor component, i.e. the opposite isomer. These differences in amplitude correspond to differences in dendritic diameter between the two neurons. Previous studies showed that behavioral response to the pheromone blend was sex-linked, but spike amplitude response to pheromone components matched autosomal, not sex-linked inheritance. Results: As part of a larger study to finely map the loci responsible for pheromone communication in this species, we have reanalyzed peripheral physiology among parental, and first and second generation **hybrids** between the two pheromone strains using tungsten electrode electrophysiology. Our results reveal that differences in spike amplitude ratio between male pheromone-sensitive sensory neurons in *O. nubilalis* races are controlled, at least partially, by sex-linked genes that exhibit E-strain dominance. Conclusions: We propose that peripheral olfactory response in *O. nubilalis* may be affected both by autosomal and sex-linked genes exhibiting a cross-locus dominance effect, and suggest that the genetic basis for pheromone reception and response in the species is more closely linked than previously thought.

Consequences of coupled barriers to gene flow for the build-up of genomic differentiation

Kunrath, HD; Bogdanowicz, SM; Searle, JB; Harrison, RG; Coates, BS; Kozak, GM; Dopman, EB. NA

Theory predicts that when different barriers to gene flow become coincident, their joint effects enhance reproductive isolation and genomic divergence beyond their individual effects, but empirical tests of this "coupling" hypothesis are rare. Here, we analyze patterns of gene exchange among populations of European corn borer moths that vary in the number of acting barriers, allowing for comparisons of genomic variation when barrier traits or loci are in coincident or independent states. We find that divergence is mainly restricted to barrier loci when populations differ by a single barrier, whereas the coincidence of temporal and behavioral barriers is associated with divergence of two chromosomes harboring barrier loci. Furthermore, differentiation at temporal barrier loci increases in the presence of behavioral divergence and differentiation at behavioral barrier loci increases in the presence of temporal divergence. Our results demonstrate how the joint action of coincident barrier effects leads to levels of genomic differentiation that far exceed those of single barriers acting alone, consistent with theory arguing that coupling allows indirect selection to combine with direct selection and thereby lead to a stronger overall barrier to gene flow. Thus, the state of barriers-independent or coupled-strongly influences the accumulation of genomic differentiation.

Specific olfactory neurons and glomeruli are associated to differences in behavioral responses to pheromone components between two *Helicoverpa* species

Wu, H; Xu, M; Hou, C; Huang, LQ; Dong, JF; Wang, CZ. 2015

Sex pheromone communication of moths helps to understand the mechanisms underlying reproductive isolation and speciation. *Helicoverpa armigera* and *Helicoverpa assulta* use (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-hexadecenal (Z9-16:Ald) as pheromone components in reversed ratios, 97:3 and 5:95, respectively. *H. armigera* also produces trace amount of (Z)-9-tetradecenal (Z9-14:Ald) in the sex pheromone gland, but *H. assulta* does not. Wind tunnel studies revealed that the addition of small amounts (0.3%) of Z9-14:Ald to the main pheromone blend of *H. armigera* increased the males' attraction, but at higher doses (1%, 10%) the same compound acted as an inhibitor. In *H. assulta*, Z9-14:Ald reduced male attraction when presented as 1% to the pheromone blend, but was ineffective at lower concentrations (0.3%). Three types (A-C) of sensilla trichodea in antennae were identified by single sensillum recording, responding to Z11-16:Ald, Z9-14:Ald, and both Z9-16:Ald and Z9-14:Ald, respectively. Calcium imaging in the antennal lobes (ALs) revealed that the input information of the three chemicals was transmitted to three units of the macroglomerular complex (MGC) in ALs in both species: a large glomerulus for the major pheromone components, a small one for the minor pheromone components, and a third one for the behavioral antagonists. The type A and C neurons tuned to Z11-16:Ald and Z9-16:Ald had a reversed target in the MGC between the two species. In *H. armigera*, low doses (1, 10 μ g) of Z9-14:Ald dominantly activated the glomerulus which processes the minor pheromone component, while a higher dose (100 μ g) also evoked an equal activity in the antagonistic glomerulus. In *H. assulta*, instead, Z9-14:Ald always strongly activated the antagonistic glomerulus. These results suggest that Z9-14:Ald plays different roles in the sexual communication of two *Helicoverpa* species through activation of functionally different olfactory pathways.

High background noise shapes selective auditory filters in a tropical cricket

Schmidt, AKD; Riede, K; Romer, H. 2011

Because of call frequency overlap and masking interference, the airborne sound channel represents a limited resource for communication in a species-rich cricket community like the tropical rainforest. Here we studied the frequency tuning of an auditory neuron mediating phonotaxis in the rainforest cricket *Paroecanthus podagrosus*, suffering from strong competition, in comparison with the same homologous neuron in two species of European field crickets, where such competition does not exist. As predicted, the rainforest species exhibited a more selective tuning compared with the European counterparts. The filter reduced background nocturnal noise levels by 26. dB, compared with only 16 and 10. dB in the two European species. We also quantified the performance of the sensory filter under the different filter regimes by examining the representation of the species-specific amplitude modulation of the male calling song, when embedded in background noise. Again, the filter of the rainforest cricket performed significantly better in terms of representing this important signal parameter. The neuronal representation of the calling song pattern within receivers was maintained for a wide range of signal-to-noise ratios because of the more sharply tuned sensory system and selective attention mechanisms. Finally, the rainforest cricket also showed an almost perfect match between the filter for sensitivity and the peripheral filter for directional hearing, in contrast to its European counterparts. We discuss the consequences of these adaptations for intraspecific acoustic communication and reproductive isolation between species.

Trade-offs between melanisation and life-history traits in *Helicoverpa armigera*

Ma, WH; Chen, LZ; Wang, M; Li, XC. 2008

1. Previously we established a homozygous melanic strain (JBM) with 16 black pupae spontaneously occurring within a laboratory population (JBW) of *Helicoverpa armigera* and demonstrated that the melanisation is controlled by a single recessive autosomal gene. 2. Data obtained indicate that the melanisation is globally expressed in the pupal and adult stages (except for the body hairs of adults) but not in the egg and larval stages. No differences in body colour can be found between the melanic JBM and the wild-type JBW strains before the metamorphic pupation moult. After pupation, the JBM pupae gradually blacken, whereas the wild-type JBW pupae gradually turn brown, indicating that the biosynthetic steps leading to brown pigments are shut off in the JBM strain. In the adult stage, wings are darker and hairs on the abdomen and tergum are lighter in the melanic moths than in the wild-type individuals. 3. Life-table experiments reveal that the melanism is associated with slower development in all life stages, smaller body weight, lower mating rate and fecundity, less mating time, and accordingly, lower net reproduction rate and population trend index. 4. Single pair inbreeding and reciprocal crosses show that the mating rate is much lower in the inter-strain crosses than in the intra-strain crosses, indicating the presence of mating preference for its own colour morph and the presence to some degree of reproductive isolation between the two colour morphs.

Biosynthetic pathways of the pheromone of the Egyptian armyworm *Spodoptera littoralis*

Munoz, L; Rosell, G; Quero, C; Guerrero, A. 2008

Most insect pheromones comprise multicomponent blends of geometric or optical isomers, and one major question is how insects produce species-specific ratios of components for successful reproductive isolation. Key enzymes suggested to be involved in pheromone biosynthesis are acetyl-coenzyme A carboxylase and fatty acyl synthetase, chain-shortening enzymes, desaturases, elongases, reductases, oxidases, and alcohol acetyl transferases. The female pheromone composition of the Egyptian armyworm *Spodoptera littoralis* (Boisd.) is highly dependent on the origin of the strain. In this review, we present a summary of the different reported pheromone compositions of the moth, including from our recent studies on this subject, as well as the biosynthetic routes to the different components and the molecular approaches involved. In addition, the key role played in the proposed biosynthetic pathways by a number of important biosynthetic enzymes, such as chain shortening enzymes, desaturases and alcohol acetyl transferases, is outlined, as well as the latest developments on the inhibition of these enzymes.

Non-Pleiotropic Coupling of Daily and Seasonal Temporal Isolation in the European Corn Borer

Levy, RC; Kozak, GM; Dopman, EB. 2018

Speciation often involves the coupling of multiple isolating barriers to produce reproductive isolation, but how coupling is generated among different premating barriers is unknown. We measure the degree of coupling between the daily mating time and seasonal mating time between strains of European corn borer (*Ostrinia nubilalis*) and evaluate the hypothesis that the coupling of different forms of allochrony is due to a shared genetic architecture, involving genes with pleiotropic effects on both timing phenotypes. We measure differences in gene expression at peak mating times and compare these genes to previously identified candidates that are associated with changes in seasonal mating time between the corn borer strains. We find that the E strain, which mates earlier in the season, also mates 2.7 h earlier in the night than the Z strain. Earlier daily mating is correlated with the differences in expression of the circadian clock genes *cycle*, *slimb*, and *vrille*. However, different circadian clock genes associate with daily and seasonal timing, suggesting that the coupling of timing traits is maintained by natural selection rather than pleiotropy. Juvenile hormone gene expression was associated with both types of timing, suggesting that circadian genes activate common downstream modules that may impose constraint on future evolution of these traits.

Quantitative genetics of signal evolution: A comparison of the pheromonal signal in two populations of the cabbage looper, *Trichoplusia ni*

Gemeno, C; Moore, AJ; Preziosi, RF; Haynes, KF. 2001

Pheromones are important in reproductive isolation among populations of moths, but the genetics associated with diversification of pheromonal signals is poorly understood. To gain insight into processes that may lead to diversification we examined the genetic architecture underlying the production of the sex pheromone of the cabbage looper moth, *Trichoplusia ni*. We compared genetic parameters of two populations; one with a wild-type pheromone phenotype (N) and one where a single-gene mutation affecting the pheromone blend produced by females had been established (M). Using a half-sib breeding design we estimated heritabilities, coefficients of additive genetic variation, and phenotypic, genetic, and environmental correlations of the pheromone components. In both populations, narrow sense heritabilities were generally moderate and genetic correlations were mostly positive. Comparisons between the two populations showed that, while the pattern of phenotypic correlations showed significant agreement between populations, the patterns of genetic (co)variation (i.e. the shapes of the within population matrix) were dissimilar between the two populations. The presence of additive genetic variation in both populations indicates that there is the potential for further evolution of individual pheromone components. However, because of the differences between the populations in the pattern of genetic variation and covariation, the populations will evolve along different evolutionary trajectories even under identical selection pressures. These results suggest that single gene mutations, once established, can be associated with further alterations in the genetic architecture and this has implications for the evolution of pheromone communication.

Inter-geographic hybridization in the corn borer *Ostrinia furnacalis* reduced the fitness of hybrids

He, HM; Tang, JJ; Huang, LL; Gao, YL; Xue, FS. 2019

It is commonly observed that if the genetic divergences between parental lineages are too marked, the intermixed offspring might not be successful or might present reduced fitness, i.e., **hybrid** inferiority. In the present study, we performed an intraspecific **hybridization** using two populations of the Asian corn borer, *Ostrinia furnacalis*: a southern population (S) from Huizhou city (23.09 degrees N, 114.4 degrees E) and a northern population (N) from Harbin city (44.9 degrees N, 127.2 degrees E). The key life-history traits were compared between the two populations with geographical reproductive isolation and their two **hybrid** populations (SfxNo and NfxSo populations) at 20, 24, 28 and 32 degrees C. Our results showed that there were significant differences in life-history traits in the two parental populations, with the S population performing better. However, the two **hybrid** populations exhibited delayed larval development time, relatively reduced body weight, lower growth rate and sex ratio distortion compared with their parents. Our results provided further evidence that intraspecific **hybridization** between the two geographical populations reduced the fitness of the **hybrids**. The sexual size dimorphism was also different between the pure populations and the **hybrid** populations. We also found that the sex ratios in the two **hybrid** populations were mainly influenced by the genotype of the mothers. The negative effects of intraspecific **hybridization** on the key life-history traits may provide a genetic means of pest management to suppress pest populations.

Ongoing ecological speciation in *Cotesia sesamiae*, a biological control agent of cereal stem borers

Kaiser, L; Le Ru, BP; Kaoula, F; Paillusson, C; Capdevielle-Dulac, C; Obonyo, JO; Herniou, EA; Jancek, S; Branca, A; Calatayud, PA; Silvain, JF; Dupas, S. 2015

To develop efficient and safe biological control, we need to reliably identify natural enemy species, determine their host range, and understand the mechanisms that

drive host range evolution. We investigated these points in *Cotesia sesamiae*, an African parasitic wasp of cereal stem borers. Phylogenetic analyses of 74 individual wasps, based on six mitochondrial and nuclear genes, revealed three lineages. We then investigated the ecological status (host plant and host insect ranges in the field, and host insect suitability tests) and the biological status (cross-mating tests) of the three lineages. We found that one highly supported lineage showed all the hallmarks of a cryptic species. It is associated with one host insect, *Sesamia nonagrioides*, and is reproductively isolated from the other two lineages by pre- and postmating barriers. The other two lineages had a more variable phylogenetic support, depending on the set of genes; they exhibited an overlapping and diversified range of host species and are not reproductively isolated from one another. We discuss the ecological conditions and mechanisms that likely generated this ongoing speciation and the relevance of this new specialist taxon in the genus *Cotesia* for biological control.

Historical and current patterns of gene flow in the butterfly *Pararge aegeria*

Livraghi, L; Voda, R; Evans, LC; Gibbs, M; Dinca, V; Holland, PWH; Shreeve, TG; Vila, R; Dapporto, L; Breuker, CJ. 2018

Aim We have investigated the phylogeography and genetic structure of the Speckled Wood butterfly (*Pararge aegeria*) across its entire distribution range and studied its dispersal both on mainland and across sea straits. The apparent lack of gene flow between Sardinia and Corsica was further investigated by means of mating experiments. Location Europe and North Africa. **Methods** We sampled 345 individuals and sequenced one mitochondrial gene (Cytochrome c Oxidase subunit I, COI) for all samples and two nuclear genes (wingless and zerknullt) for a subset of the specimens. A total of 22 females from Corsica and Sardinia were used to establish a series of crosses to investigate reproductive compatibility and were screened for the presence of *Wolbachia*. Bayesian inference (BI) and haplotype networks were employed to infer phylogenetic relationships and a Principal Coordinate Analysis (PCoA) was used to represent geographical patterns of genetic diversity. Mating and courtship data were analysed using linear mixed effect models. **Results** We detected two main COI lineages separated by the Mediterranean Sea and maintained over relatively short sea straits. While nuclear gene variation was generally in agreement with that of COI, this was not the case in all areas (e.g. Iberian Peninsula and Corsica/Sardinia). Mating experiments revealed no evidence of reproductive isolation between the lineages, nor clear relation to *Wolbachia* infection status. **Main conclusions** We propose that following the post-glacial recolonization of Europe, the ancestral COI lineage of *P. aegeria* was maintained in North Africa and Mediterranean islands, while a new lineage colonized from Eastern Europe, replacing and apparently out-competing the ancestral variant. Several hypotheses are discussed that may explain the local discordance between the nuclear genes and COI, including sex-specific dispersal, selection and differential rates of gene evolution.

A major locus controls a biologically active pheromone component in *Heliconius melpomene*

Byers, KJRP; Darragh, K; Musgrove, J; Almeida, DA; Garza, SF; Warren, IA; Rastas, PM; Kucka, M; Chan, YF; Merrill, RM; Schulz, S; McMillan, WO; Jiggins, CD. 2020

Understanding the production, response, and genetics of signals used in mate choice can inform our understanding of the evolution of both intraspecific mate choice and reproductive isolation. Sex pheromones are important for courtship and mate choice in many insects, but we know relatively little of their role in butterflies. The butterfly *Heliconius melpomene* uses a complex blend of wing androconial compounds during courtship. Electroantennography in *H. melpomene* and its close relative *Heliconius cydno* showed that responses to androconial extracts were not species specific. Females of both species responded equally strongly to extracts of both species, suggesting conservation of peripheral nervous system elements across the two species. Individual blend components provoked little to no response, with the exception of octadecanal, a major component of the *H. melpomene* blend. Supplementing octadecanal on the wings of octadecanal-rich *H. melpomene* males led to an increase in the time until mating, demonstrating the bioactivity of octadecanal in *Heliconius*. Using quantitative trait locus (QTL) mapping, we identified a single locus on chromosome 20 responsible for 41% of the parental species' difference in octadecanal production. This QTL does not overlap with any of the major wing color or mate choice loci, nor does it overlap with known regions of elevated or reduced F-ST. A set of 16 candidate fatty acid biosynthesis genes lies underneath the QTL. Pheromones in *Heliconius* carry information relevant for mate choice and are under simple genetic control, suggesting they could be important during speciation.

Concerted evolution of male and female display traits in the European corn borer, *Ostrinia nubilalis*

Lassance, JM; Lofstedt, C. 2009

Background: Sexual reproduction entails the encounter of the sexes and the multiplicity of rituals is parallel to the diversity of mating systems. Evolutionary mechanisms such as sexual selection and sexual conflict have led to the elaboration of traits to gain attention and favours from potential partners. A paradox exists about how coordinated systems can evolve and diverge when there would seem to be a stabilising selection acting. Moth display traits - pheromones - constitute an advantageous model with which to address questions about the evolution of mating systems in animals. Both males and females can possess pheromones that are involved either in close- or long-range communication. Female and male pheromones appear to have different origins and to be under different evolutionary constraints, thus they might be envisioned as independently evolving traits. We conducted laboratory experiments to explore the role of scents released during courtship by males of the European corn borer, *Ostrinia nubilalis*. **Results:** Information provided by the male pheromone appears critical for female acceptance. The composition of this male pheromone varies in an age-dependent manner and females show mating preference towards older males in choice experiments. Furthermore, male signals may allow species discrimination and reinforce reproductive isolation. Finally, we found evidence for a genetic correlation between male and female signals, the evolution of which is best explained by the constraints and opportunities resulting from the sharing of gene products. **Conclusion:** In this study we used an integrative approach to characterise the male sex pheromone in a moth. Interestingly, the male chemical signal is analogous to the female signal in that structurally similar compounds are being used by both sexes. Hence, in systems where both sexes possess display traits, the pleiotropy of genes generating the traits could influence the evolutionary trajectories of sexual signals and lead to their divergence, with speciation being the ultimate result.

Assessing Genomic Admixture between Cryptic *Plutella* Moth Species following Secondary Contact

Ward, CM; Baxter, SW. 2018

Cryptic species are genetically distinct taxa without obvious variation in morphology and are occasionally discovered using molecular or sequence data sets of populations previously thought to be a single species. The world-wide Brassica pest, *Plutella xylostella* (diamondback moth), has been a problematic insect in Australia since 1882, yet a morphologically cryptic species with apparent endemism (*P. australiana*) was only recognized in 2013. *Plutella xylostella* and *P. australiana* are able to **hybridize** under laboratory conditions, and it was unknown whether introgression of adaptive traits could occur in the field to improve fitness and potentially increase pressure on agriculture. Phylogenetic reconstruction of 29 nuclear genomes confirmed *P. xylostella* and *P. australiana* are divergent, and molecular dating with 13 mitochondrial genes estimated a common *Plutella* ancestor 1.96 +/- 60.175 Ma. Sympatric Australian populations and allopatric Hawaiian *P. xylostella* populations were used to test whether neutral or adaptive introgression had occurred between the two Australian species. We used three approaches to test for genomic admixture in empirical and simulated data sets including 1) the *f*₃ statistic at the level of the population, 2) pairwise comparisons of Nei's absolute genetic divergence (*d*(XY)) between populations, and 3) changes in phylogenetic branch lengths between individuals across 50-kb genomic windows. These complementary approaches all supported reproductive isolation of the *Plutella* species in Australia, despite their ability to **hybridize**. Finally, we highlight the most divergent genomic regions between the two cryptic *Plutella* species and find they contain genes involved with processes including digestion, detoxification, and DNA binding.

Variation in Courtship Ultrasounds of Three *Ostrinia* Moths with Different Sex Pheromones

Takanashi, T; Nakano, R; Surlykke, A; Tatsuta, H; Tabata, J; Ishikawa, Y; Skals, N. 2010

Moths use ultrasounds as well as pheromones for sexual communication. In closely related moth species, variations in ultrasounds and pheromones are likely to profoundly affect mate recognition, reproductive isolation, and speciation. The European corn borer, *Ostrinia nubilalis*, and its Asian congeners, *Ostrinia furnacalis* and *Ostrinia scapularis*, exhibit within-species and between-species variation in their pheromone communication. Recently, we reported ultrasound communication in *O. furnacalis*; however, variations in ultrasounds in the three congeners have not been addressed to date. Here we investigated features of ultrasound production and hearing in *O. nubilalis* and *O. scapularis*, and compared them with those of *O. furnacalis*. As in *O. furnacalis*, males of *O. nubilalis* and *O. scapularis* produced ultrasounds during courtship by rubbing specialized scales on the wings against scales on the thorax. The covering of these scales with nail polish muffled the sounds and significantly reduced mating success in *O. nubilalis*, showing the importance of ultrasound signaling in mating. The ultrasounds produced by *O. nubilalis* and *O. scapularis* were similar, consisting of long trains of pairs of pulses with a main energy at 40 kHz, but distinctly different from the ultrasound produced by *O. furnacalis*, consisting of groups of pulses peaking at 50 kHz and with substantially more energy up to 80 kHz. Despite overall similarities, temporal features and patterns of amplitude modulation differed significantly among the geographic populations of *O. nubilalis* and *O. scapularis*, which differed in pheromone type. In contrast, no significant difference in hearing was found among the three species with regard to the most sensitive frequencies and hearing threshold levels. The patterns of variations in the songs and pheromones well reflected those of the phylogenetic relationships, implying that ultrasound and pheromone communications have diverged concordantly. Our results suggest that concordant evolution in sexual signals such as courtship ultrasounds and sex pheromones occurs in moths.

Evolution of oviposition strategies and speciation in the globeflower flies *Chiastocheta* spp. (Anthomyiidae)

Despres, L; Jaeger, N. 1999

Trollius europaeus (Ranunculaceae) is involved in an intimate interaction with several species of *Chiastocheta* flies (Anthomyiidae) that are both seed predators and pollinators. In this paper, we analyse the oviposition strategy of the six *Chiastocheta* species found to coexist on *T. europaeus* in 19 populations from the French Alps. We show that the species are not equivalent in their oviposition behaviour.; *C. rotundiventris* usually deposits no more than one egg per flower in first-day flowers whereas *C. dentifera* aggregates its eggs on fruits and thus does not contribute to pollination at all; the four remaining species deposit eggs sequentially during the flowering period from the 2nd to the 7th day. Hence, the outcomes of the interaction in terms of net seed production for the plant greatly depend on the *Chiastocheta* species visiting it, ranging from a mutualistic to a purely-parasitic interaction. We assessed mitochondrial divergence between *Chiastocheta* spp. by sequencing a 1320-bp mitochondrial DNA fragment. The low divergence observed between species (0-4-15%) suggests that genus diversification took place recently. Unlike in other plant-insect systems where diversification is usually thought to be driven by cospeciation or host shifts we propose that *Chiastocheta* speciation took place within the host plant. Basal separation of a particularly mutualistic species provided favourable conditions for plant specialization on this seed-parasite as: a pollinator early in the evolution of the association. The parasitic species ovipositing on fruits derived from a species ovipositing on flowers. Diversification of the intermediate strategies probably occurred in relation with the Pleistocene climatic events, reproductive isolation between species being reinforced by niche partitioning for oviposition and/or sexual selection.

Positive selection alone is sufficient for whole genome differentiation at the early stage of speciation process in the fall armyworm

Nam, K; Nhim, S; Robin, S; Bretaudeau, A; Negre, N; D'Alençon, E. 2020

Background The process of speciation involves differentiation of whole genome sequences between a pair of diverging taxa. In the absence of a geographic barrier and in the presence of gene flow, genomic differentiation may occur when the homogenizing effect of recombination is overcome across the whole genome. The fall armyworm is observed as two sympatric strains with different host-plant preferences across the entire habitat. These two strains exhibit a very low level of genetic differentiation across the whole genome, suggesting that genomic differentiation occurred at an early stage of speciation. In this study, we aim at identifying critical evolutionary forces responsible for genomic differentiation in the fall armyworm. **Results** These two strains exhibit a low level of genomic differentiation ($F_{ST} = 0.0174$), while 99.2% of 200 kb windows have genetically differentiated sequences ($F_{ST} > 0$). We found that the combined effect of mild positive selection and genetic linkage to selectively targeted loci are responsible for the genomic differentiation. However, a single event of very strong positive selection appears not to be responsible for genomic differentiation. The contribution of chromosomal inversions or tight genetic linkage among positively selected loci causing reproductive barriers is not supported by our data. Phylogenetic analysis shows that the genomic differentiation occurred by sub-setting of genetic variants in one strain from the other. **Conclusions** From these results, we concluded that genomic differentiation may occur at the early stage of a speciation process in the fall armyworm and that mild positive selection targeting many loci alone is sufficient evolutionary force for generating the pattern of genomic differentiation. This genomic differentiation may provide a condition for accelerated genomic differentiation by synergistic effects among linkage disequilibrium generated by following events of positive selection. Our study highlights genomic differentiation as a key evolutionary factor connecting positive selection to divergent selection.

From sympatry to parapatry: a rapid change in the spatial context of incipient allochronic speciation

Burban, C; Rocha, S; Leblois, R; Rossi, JP; Saune, L; Branco, M; Kerdelhue, C. 2020

Speciation is nowadays recognized as a dynamic process in which the respective roles of forces driving ecological differentiation and reproductive isolation can change through time and space. Incipient speciation events are particularly useful to follow such processes that are not tractable when considering well-differentiated taxa. A promising case study was discovered in the pine processionary moth, *Thaumetopoea pityocampa*, a Mediterranean defoliator of *Pinus* species, for which allochrony acting as an automatic magic trait was recognized as the major driver of an incipient speciation process. In Portugal, a unique population with a shifted phenology, known as the summer population (SP), co-occurs with a population following the typical life cycle, known as the winter population (WP). We monitored male activity of both populations in the Leiria region, i.e. over the whole SP distribution range using a systematic sampling along two transects, and studied Portuguese WPs at a larger geographical scale to explore their genetic diversity and spatial pattern of differentiation. Results showed that the WPs were genetically more diverse than the SP, with a strong pattern of isolation by distance both at large and small spatial scales, while the SP was very homogeneous over its whole range, without signature of its recent spatial expansion. Contrary to our expectations, no F1 hybrids were identified, even though we found an extended flight period of the SP, overlapping with the beginning of the WP reproductive period. Interestingly, the SP was found to be mostly limited to the sea shore where the WP is now scarce or absent, which could suggest competitive exclusion. Once clearly occurring in a sympatric context, the allochronic differentiation tends to develop nowadays in parapatry.

Sex pheromone components of *Casuarina* moth, *Lymantria xyliana*

Gries, G; Schaefer, PW; Khaskin, G; Hahn, R; Gries, R; Chao, JT. 1999

cis-7,8-Epoxy-2-methyleicosane is a sex pheromone component of the *Casuarina* moth, *Lymantria xyliana* Swinhoe. The compound was extracted from pheromone glands of female moths and was identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometry. In field experiments in Taiwan, traps baited with either or both of (7R,8S)-cis-7,8-epoxy-2-methyleicosane (>99% eel [termed here (+)-xylinalure] and (7S,8R)-cis-7,8-epoxy-2-methyleicosane (>99% eel [termed here (-)-xylinalure] captured male *L. xyliana*. Addition of further candidate pheromone components to xylinalure did not enhance its attractiveness. Demonstration of whether or not female *L. xyliana* produce both optical isomers of xylinalure, and determination of the ratio, will require pheromone

extract analyses on a chiral, enantiomer-separating column (as yet unavailable) or derivatization of epoxides in accumulated gland extracts. Attraction of male *L. xyli* to either enantiomer of xylinalure contrasts with enantiospecific production of, and/or response to, epoxy pheromones in congeners. With no other nocturnal lymantriid moth known in Taiwan to utilize xylinalure for pheromonal communication, enantiospecific "fine tuning" of xylinalure, or evolution of a more complex pheromone blend, may not have been necessary for *L. xyli* to maintain specificity of sexual communication. Racemic xylinalure will be appropriate for pheromone-based detection surveys of *L. xyli* in North America.

Hologenomic speciation: synergy between a male-killing bacterium and sex-linkage creates a 'magic trait' in a butterfly hybrid zone

Gordon, IJ; Ileri, P; Smith, DAS. 2014

Danaus chrysippus (L.) in Africa comprises four substantially isolated semispecies that are migratory and **hybridize** on a seasonal basis throughout the eastern and central part of the continent. In the **hybrid** zone (but not elsewhere), the butterfly is commonly host to a male killing endosymbiotic bacterium, *Spiroplasma* sp., which principally infects one semispecies, *Danaus chrysippus chrysippus* in Kenya. A W-autosome mutation, inherited strictly matrilineally, links B and C colour gene loci, which have thus gained sex-linkage in *chrysippus*. We have monitored variation in sex ratio and genotype at the A and C colour gene loci for two extended periods of 18 months (2004-5) and 12 months (2009-10) in adults reared from wild eggs laid on trap plants in Kasarani, near Nairobi, Kenya. Additionally, in 2009-10, all surviving adult butterflies were screened for *Spiroplasma* infection. The **hybridizing** Kasarani population is highly atypical in three respects, and has apparently been so for some 30 years: first, the sex ratio is permanently female-biased (as expected), although subject to seasonal fluctuation, being lowest (male/female) when *D.c. chrysippus* (cc) peaks and highest when *Danaus chrysippus dorippus* (CC) predominates; second, the population is invariably dominated by Cc heterozygotes of both sexes but especially females; and third, cc males are always scarce because they are systematically eliminated by male killing, whereas the CC genotype is male-biased. It is this imbalance of sex versus genotype that determines the massive departure from Hardy-Weinberg equilibrium in the population, in part because cc females have little choice but to pair with C- males. We suggest that: first, Cc **hybrids** of both sexes fail to disperse in the company of either parental semispecies; second, *Spiroplasma* positive females carrying the W-autosome mutation have a selective advantage over females that lack the translocation; third, the endoparasite and the translocation create a 'magic trait' linkage group that underlies hologenomic reproductive isolation between two emerging species, *D.c. chrysippus* and *D.c. dorippus*; and, fourth, that the predominance of males in *dorippus* suggests that individuals must be protected by a male-killing suppressor gene. By contrast to the C locus, Aa heterozygotes are in substantial and permanent deficit, suggesting either assortative mating between AA (*chrysippus* and *dorippus*) and aa (*Danaus chrysippus alcippus*), or heterozygote unfitness, or both. (c) 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, 111, 92-109.

Inheritance of central neuroanatomy and physiology related to pheromone preference in the male European corn borer

Karpati, Z; Olsson, S; Hansson, BS; Dekker, T. 2010

Background: The European corn borer (ECB), *Ostrinia nubilalis*, is a textbook example of pheromone polymorphism. Males of the two strains (Z and E) prefer opposite ratios of the two pheromone components, Z11- and E11-tetradecenyl acetate, with a sex-linked factor underlying this difference in preference. The male antennal lobes of the two strains contain a pheromone sensitive macroglomerular complex (MGC) that is identical in morphology, but reversed in functional topology. However, **hybrids** prefer intermediate ratios. How a topological arrangement of two glomeruli can accommodate for an intermediate preference was unclear. Therefore we studied the neurophysiology of **hybrids** and paternal backcrosses to see which factors correlated with male behavior. Results: Projection neuron (PN) recordings and stainings in **hybrids** and backcrosses show a dominance of the E-type MGC topology, notwithstanding their intermediate preference. Apparently, the topological arrangement of glomeruli does not directly dictate preference. However, two other factors did correlated very well with preference. First, volumetric measurements of MGC glomeruli demonstrate that, whereas in the parental strains the medial MGC glomerulus is more than 2 times larger than the lateral, in **hybrids** they are intermediate between the parents, i.e. equally sized. Paternal backcrosses showed that the volume ratio is sex-linked and co-dominant. Second, we measured the summed potential difference of the antennae in response to pheromone stimulation using electroantennogram recordings (EAG). Z-strain antennae responded 2.5 times stronger to Z11 than to E11-14: OAc, whereas in E-strain antennae the ratio was approximately equal. **Hybrid** responses were intermediate to the parents, and also here the antennal response of the paternal backcrosses followed a pattern similar to the behavioral phenotype. We found no differences in frequency and types of projection and local interneurons encountered between the two strains and their **hybrids**. Conclusions: Male pheromone preference in the ECB strains serves as a strong prezygotic reproductive isolation mechanism, and has contributed to population divergence in the field. Our results demonstrate that male pheromone preference is not directly affected by the topological arrangement of olfactory glomeruli itself, but that male preference may instead be mediated by an antennal factor, which causes the MGC glomeruli to be differentially sized. We postulate that this factor affects readout of blend information from the MGC. The results are an illustration of how pheromone preference may be 'spelled out' in the ALs, and how evolution may modulate this.
