

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

27/04/2022

Divergence and evolution of reproductive barriers among three allopatric populations of *Rhagoletis cingulata* across eastern North America and Mexico

Tadeo, E; Feder, JL; Egan, SP; Schuler, H; Aluja, M; Rull, J. 2015

Geography is often a key factor facilitating population divergence and speciation. In this regard, the geographic distributions of flies in the genus *Rhagoletis* (Diptera: Tephritidae) in temperate North America have been affected by cycles of Pleistocene glaciation and interglacial periods. Fluctuations in climatic conditions may have had their most dramatic effects on geographically isolating *Rhagoletis* flies in the central highland region of Mexico. During past periods of allopatry, a degree of post-zygotic reproductive isolation appears to have evolved between hawthorn-infesting populations of *Rhagoletis pomonella* (Walsh) in the central Eje Volcánico Trans Mexicano (EVTM) and those from the Sierra Madre Oriental Mountains (SMO) of Mexico, as well as hawthorn flies from the eastern USA. Here, we investigate the generality of this finding in the genus *Rhagoletis* by testing for reproductive isolation among populations of *Rhagoletis cingulata* (Loew) (Diptera: Tephritidae) collected from infested domesticated sweet cherry (*Prunus avium* L.) in the USA and black cherry [*Prunus serotina* Ehrh. (both Rosaceae)] from the SMO and EVTM. We report evidence for marked post-mating reproductive isolation among certain *R. cingulata* populations. The high levels of reproductive isolation were observed between *R. cingulata* flies from populations in the USA and SMO differed from the pattern seen for *R. pomonella*, primarily involving the EVTM. In addition, egg hatch was significantly reduced for crosses between SMO males and EVTM females, but not greatly in the opposite direction. We discuss potential causes for the different patterns of post-mating reproductive isolation among *Rhagoletis* flies.

LIFE-HISTORY PATTERNS OF NEARCTIC MOUNTAIN MIDGES (DIPTERA, DEUTEROPHLEBIIDAE)

COURTNEY, GW. 1991

Mountain midges (Diptera: Deuterophlebiidae) are widespread and seasonally abundant in streams of western North America. As part of a comprehensive systematic investigation of the Deuterophlebiidae, I examined the life histories of all Nearctic species, including aspects of habitat, reproductive biology, and phenology. Collection techniques were also evaluated. This paper is based on qualitative data obtained during a seven-year period (1981-1988) and from more than 500 collection sites, and on a quantitative phenological study in 1986 of 17 populations from the Pacific Northwest. Deuterophlebiids generally are considered stenobionts, requiring cold, mountain streams; however, many species inhabit a diversity of stream types, from small, high-gradient creeks to large, low-gradient rivers. The immature stages of all species are restricted to riffle habitats where current velocities typically exceed 1 m/s. Adult mountain midges exhibit marked diel synchrony, with emergence, flight, and mating during only the early morning. Flight behaviour of the short-lived adults is distinct and includes formation of male swarms. Field and laboratory observations show that females shed their wings and select oviposition sites underwater. Asynchronous life histories are typical of Nearctic species, with egg-hatching and adult emergence usually extending 2-3 mo. This strategy is adaptive for populations in the Pacific Northwest, where unpredictable spring spates may scour substrates and cause correspondingly high mortality of stream benthos. Univoltine life histories are typical of Nearctic mountain midges, though variation from this pattern was seen in three populations. Temporal, habitat and/or reproductive isolation of sympatric deuterophlebiids, blepharicerids, and other torrenticolous insects was evident in many streams.

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

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

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

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

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

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

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.

Hybridization in Phlebotominae (Diptera: Psychodidae): A mini-review

dos Reis, YV; Alevi, KCC. 2020

Taxonomy based only on morphology, although extremely important for the classification of sandflies, has been shown to be insufficient for the delimitation of some taxa. Thus, integrative taxonomy could play a fundamental role in clarifying these and other taxonomic issues, since data from different areas are used to aggregate greater reliability in species classification. Experimental crosses are important taxonomic tools, since the presence of reproductive barriers when associated with divergence between two evolutionary lineages, confirms the specific status of taxa based on the biological species concept. In the subfamily Phlebotominae, experimental crosses were mostly focused on the study of the *Lutzomyia longipalpis* complex, which helped to identify different evolutionary lineages for that group of vectors. Considering the difficulty of classifying some Phlebotominae species and the importance of **hybridization** studies for taxonomy, we grouped all the information associated with experimental crosses in sandflies in a mini-review. In view of the results grouped in this review, it is evident that i) experimental crossings are important tools to aggregate studies of integrative taxonomy in the Phlebotominae subfamily; ii) these analyses should be applied in the taxonomic studies of cryptic species; iii) *Lu. longipalpis* populations have pre and/or post-zygotic reproductive barriers; iv) *Lu. longipalpis* represents more than one species and efforts must be applied to differentiate the taxa of the *Lu. longipalpis* complex; v) *Phlebotomus* populations do not present intraspecific reproductive barriers; vi) the absence of reproductive barriers between *Ph. sergenti* from Israel and Turkey (representing populations of the same evolutionary lineage) does not rule out the possible existence of cryptic species, it being necessary to perform experimental crosses between the different strains indicated by the molecular markers; and finally, vii) different species of *Phlebotomus* have post-zygotic barriers, confirming the specific status of *Ph. duboscqi*, *Ph. papatasi*, and *Ph. bergeroti*.

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

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

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

Random Mating among *Anastrepha ludens* (Diptera: Tephritidae) Adults of Geographically Distant and Ecologically Distinct Populations in Mexico

Aluja, M; Rull, J; Perez-Staples, D; Diaz-Fleischer, F; Sivinski, J. 2009

The Mexican fruit fly *Anastrepha ludens* (Loew) (Diptera: Tephritidae) is a polyphagous pestiferous insect with a geographical range encompassing highly variable environmental conditions. Considering that cryptic species have been recently found among South American representatives of the same taxonomic group as *A. ludens*, we tested whether or not some populations of *A. ludens* have evolved assortative mating as an isolating mechanism that maintains intrapopulation genetic differences and behavioral adaptations to local conditions. Males and females stemming from widely separated locations with similar environmental conditions and males and females stemming from populations within individual-flight range, but collected in different hosts (a native and an exotic one), mated randomly amongst themselves when placed in a field cage. Despite the fact that sibling males and females from two distinct populations also mated randomly amongst themselves, siblings engaged in significantly longer copulations than non-siblings, indicating that perhaps adults discriminated mates with similar genetic compositions. Our results have important practical implications as *A. ludens* is the most devastating pest of citrus in Mexico and Central America, and large-scale releases of sterile flies are used to control it.

Variability and genetic structure of *Anastrepha ludens* Loew (Diptera: Tephritidae) populations from Mexico

Pecina-Quintero, V; Jimenez-Becerril, MF; Ruiz-Salazar, R; Nunez-Colin, CA; Loera-Gallardo, J; Hernandez-Delgado, S; Mayek-Perez, N. 2020

The Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), is the most devastating pest of citrus in Mexico. Knowledge of the genetic relationships and structure of Mexican *A. ludens* populations is limited. The aim of this research was to characterize the genetic variability and structure of the Mexican fruit fly by using AFLP markers (three EcoRI/MseI primer combinations). The specimens were collected from three hosts, namely, *Casimiroa edulis* La Llave & Lex [white sapote], *Casimiroa greggii* (S. Watson) F. Chiang [yellow chapote], and *Citrus Xparadisii* (Macfad.) [grapefruit], at four locations in the states of Veracruz (Teocelo and Jalapa), Nuevo Leon (El Jarro), and Tamaulipas (Troncones) in Mexico. A high polymorphism percentage (80.4%) allowed the differentiation of individuals from different *A. ludens* populations, and high intraspecific genetic variability (DI = 78.4%) was detected. The *A. ludens* populations clustered into two groups: group one included flies from Veracruz [grapefruit, Jalapa] and Nuevo Leon [yellow chapote, El Jarro], and group two included populations from Tamaulipas [yellow chapote, Troncones] and Veracruz [white sapote, Teocelo]. The populations from group one were from locations at lower altitudes (750 m above sea level), which had warmer (22 degrees C average temperature) and more humid (1470 mm of annual precipitation) climatic conditions than those of group two (1600 m, 18 degrees C and 965 mm). Both population structure and very high genetic differentiation (FST > 0.38) associated with geographical and/or host origin were found in *A. ludens* from Mexico. The genetic differentiation in Mexican *A. ludens* populations suggests reduced gene flow and reproductive isolation resulting either from habitat fragmentation and genetic drift or from natural selection for specific environmental (climate, geography, pest management strategies) or host (chemistry, phenology) condition. Genetic analysis could increase the success of *Anastrepha* control programs by improving knowledge of host-parasitoid population genetics, allowing matching of parasitoid species to their preferred pest populations.

Hidden in the urban parks of New York City: *Themira lohmanus*, a new species of Sepsidae described based on morphology, DNA sequences, mating behavior, and reproductive isolation (Sepsidae, Diptera)

Ang, YC; Rajaratnam, G; Su, KFY; Meier, R. 2017

New species from well-studied taxa such as Sepsidae (Diptera) are rarely described from localities that have been extensively explored and one may think that New York City belongs to this category. Yet, a new species of *Themira* (Diptera: Sepsidae) was recently discovered which is currently only known to reside in two of New York City's largest urban parks. Finding a new species of *Themira* in these parks was all the more surprising because the genus was revised in 1998 and is not particularly species-rich (13 species). Its status is confirmed as a new species based on morphology, DNA sequences, and reproductive isolation tests with a closely related species, and is described as *Themira lohmanus* Ang, sp. n. The species breeds on waterfowl dung and it is hypothesized that this makes the species rare in

natural environments. However, it thrives in urban parks where the public feeds ducks and geese. The mating behavior of *Themira lohmanus* was recorded and is similar to the behavior of its closest relative *T. biloba*.

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

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

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

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

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

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

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

PRICE, DK; BOAKE, CRB. 1995

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

Revision of Asian species of the *Drosophila melanica* species group (Diptera: Drosophilidae), with a description of a new species from Vietnam

Suwito, A; Toda, MJ; Takamori, H; Harada, K; Watabe, H. 2014

A new species of the *Drosophila* (*Drosophila*) *melanica* species group (Diptera: Drosophilidae), *Drosophila denruoi* Suwito & Watabe, sp. nov., is described from northern Vietnam. It is proved by crossing experiments that reproductive isolation is complete between this species and other morphologically similar species. Larval ganglion cells of *D. denruoi* possess $2n=12$ chromosomes, comprised of four pairs of acrocentric, one pair of metacentric and one pair of micro-chromosomes. This is the largest number of acrocentric chromosomes in the group and implies that the species has the most primitive chromosomal configuration in the *melanica* group. Based on the results of crossing experiments and close morphological examination, *Drosophila pengi* Okada & Kurokawa, 1957 from central Japan, once regarded as a synonym of *Drosophila tsigana* Burla & Gloor, 1952, is shown to be distinct from *D. tsigana*. *Drosophila pengi* is thus resurrected as a member of the *melanica* group. We synonymize *Drosophila bisetata* Toda, 1988, described from central Myanmar, with *D. pengi*. In addition, a population from Guizhou, southwestern China, once regarded as *D. tsigana*, is also identified as *D. pengi*. These populations from central Myanmar and southwestern China share the diagnostic characters of *D. pengi*. At the same time, it is reconfirmed that the Hokkaido population is conspecific with European *D. tsigana*. Furthermore, another species, *Drosophila longiserrata* Toda, 1988 described from central Myanmar, is synonymized with *Drosophila afer* Tan, Hsu & Sheng, 1949 from China, based on their having identical karyotypes and external morphology.

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

Hagele, K. 1999

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

Reproductive isolation between sympatric and allopatric Brazilian populations of *Lutzomyia longipalpis* s.l. (Diptera : Psychodidae)

Souza, NA; Andrade-Coelho, CA; Vigoder, FM; Ward, RD; Peixoto, AA. 2008

Lutzomyia longipalpis s.l., the main vector of *Leishmania chagasi* in Latin America, is a species complex although the exact number of siblings is yet unknown. In Brazil, the siblings differ in male copulatory courtship songs and pheromones that most certainly act as pre-zygotic reproductive barriers. Here we analysed the reproductive isolation between three allopatric and two sympatric populations of *Lu. longipalpis* s.l. from Brazil. The results indicate a strong copulatory and pre-mating isolation between the three allopatric populations. In addition, the results also indicate a stronger pre-mating isolation between the two sympatric siblings than between the three allopatric ones, suggesting a role for reinforcement in the speciation of the *Lu. longipalpis* s.l. complex.

Cryptic diversity and gene flow among three African agricultural pests: *Ceratitis rosa*, *Ceratitis fasciventris* and *Ceratitis ananæ* (Diptera, Tephritidae)

Virgilio, M; Delatte, H; Quilici, S; Backeljau, T; De Meyer, M. 2013

The *Ceratitis* FAR complex' is a species complex of African fruit flies (Diptera, Tephritidae) including the major agricultural pest *Ceratitis rosa* and the morphologically similar *Ceratitis fasciventris* and *Ceratitis ananæ*. To resolve their intra- and interspecific genetic relationships and to estimate gene flow within this complex, we surveyed allelic variation at 16 microsatellite loci in 27 African populations of the three morphospecies. Interpopulation genetic distances and individual Bayesian assignments distinguished five genotypic clusters: two involving *C.rosa* (R1, R2; that may occur in sympatry), two involving *C.fasciventris* (F1, F2; with parapatric distributions) and one involving *C.ananæ* (A). Intra- and interspecific patterns of genetic differentiation were not hierarchically structured and genetic differentiation between conspecific clusters (F1F2 and R1R2) was higher or comparable with differentiation between heterospecific clusters (e.g. F1-A or R2-A). In some cases, gene flow estimates among morphospecies or among heterospecific genotypic clusters were significantly different from zero, showing the lack of reproductive isolation. Genetic differentiation between genotypic clusters was partly supported by morphological differences observed a posteriori in male secondary sexual characters. These results suggest important revisions to current models of ecological niche requirements and invasion risk of the major agricultural pest *C.rosa* and provide a basis for a taxonomic re-interpretation of the FAR complex.

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

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

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

Period Gene of *Bactrocera cucurbitae* (Diptera: Tephritidae) Among Strains with Different Mating Times and Sterile Insect Technique

Matsumoto, A; Ohta, Y; Itoh, TQ; Sanada-Morimura, S; Matsuyama, T; Fuchikawa, T; Tanimura, T; Miyatake, T. 2008

The efficacy of sterile insect technique (SIT) depends on successful mating of released males with wild females. If the time of mating in a day of mass-reared and released males differs from those of wild females, the efficiency of SIT decreases. Therefore, understanding the molecular mechanisms controlling mating time of the target pests is particularly important for SIT. The period (*per*) gene, which has been considered as a key clock gene controlling the mating time of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), was cloned from two strains having different times of mating during the day. DNA sites varied in the 5' and 3' untranslated regions and at synonymous sites, although protein sequences were identical. We also provide phylogenetic relationships among *PER* protein sequences of dipteran species including several tephritid pest species. The functional domains of *PER* in the melon fly are very similar to those in other tephritid species. A luciferase reporter assay showed that the melon fly *PER* can functionally complement that of *Drosophila melanogaster* (Meigen). The results implicate that the major genetic cause of the difference in circadian periods, and thus in reproductive isolation, is probably one or more other clock gene(s). Thus, the series of studies may provide a novel factor concerning genetic quality control of mass-reared insect pests for SIT, which depends on successful mating of released males and wild females.

Reproductive isolation between *Anastrepha bistrigata* and *A. striata* (Diptera, Tephritidae)

Selivon, D; Morgante, J. 1997

The reproductive isolation between two closely related species, *Anastrepha bistrigata* and *A. striata*, was studied in the laboratory. Inter-specific copulation attempts were observed, but examination of the spermathecae showed that sperm transference did not occur, even after a prolonged period of contact between the mating pairs. These results indicate prezygotic isolation. The analysis of the hourly distribution of mating activities under laboratory conditions, here described for the first time for *A. bistrigata*, clearly showed differences for the two species, the activities being concentrated in the afternoon period for *A. striata* and in the morning for *A. bistrigata*.

Geographic and Ecological Dimensions of Host Plant-Associated Genetic Differentiation and Speciation in the *Rhagoletis cingulata* (Diptera: Tephritidae) Sibling Species Group

Doellman, MM; Schuler, H; Saint, GJ; Hood, GR; Egan, SP; Powell, THQ; Glover, MM; Bruzese, DJ; Smith, JJ; Yee, WL; Goughnour, RB; Rull, J; Aluja, M; Feder, JL. 2019

Ascertaining the causes of adaptive radiation is central to understanding how new species arise and come to vary with their resources. The ecological theory posits adaptive radiation via divergent natural selection associated with novel resource use; an alternative suggests character displacement following speciation in allopatry and then secondary contact of reproductively isolated but ecologically similar species. Discriminating between hypotheses, therefore, requires the establishment of a key role for ecological diversification in initiating speciation versus a secondary role in facilitating co-existence. Here, we characterize patterns of genetic variation and postzygotic reproductive isolation for tephritid fruit flies in the *Rhagoletis cingulata* sibling species group to assess the significance of ecology, geography, and non-adaptive processes for their divergence. Our results support the ecological theory: no evidence for intrinsic postzygotic reproductive isolation was found between two populations of allopatric species, while nuclear-encoded microsatellites implied strong ecologically based reproductive isolation among sympatric species infesting different host plants. Analysis of mitochondrial DNA suggested, however, that cytoplasmic-related reproductive isolation may also exist between two geographically isolated populations within *R. cingulata*. Thus, ecology associated with sympatric host shifts and cytoplasmic effects possibly associated with an endosymbiont may be the key initial drivers of the radiation of the *R. cingulata* group.

The clock gene cryptochrome of *Bactrocera cucurbitae* (Diptera: Tephritidae) in strains with different mating times

Fuchikawa, T; Sanada, S; Nishio, R; Matsumoto, A; Matsuyama, T; Yamagishi, M; Tomioka, K; Tanimura, T; Miyatake, T. 2010

Differences in mating time between populations can give rise to premating reproductive isolation. Tephritid fruit flies exhibit large variation in mating time among intra- or inter-specific populations. We previously cloned the clock gene period from two strains of melon fly, *Bactrocera cucurbitae*; in one the individuals mate early during the day, whereas in the other the individuals mate later. These strains were originally established by divergent artificial selection for developmental time, 'short' and 'long', with early and late mating times, respectively. The deduced amino acid sequences of PERIOD proteins for these two strains were reported to be identical. Here we cloned another clock gene cryptochrome (cry) from the two strains, and found two stable amino acid substitutions in the strains. In addition, the allele frequency at the two polymorphic sites of cry gene correlated with the circadian locomotor period (tau) across strains, whereas the expression pattern of cry mRNA in the heads of flies taken from the short strain significantly differed from that from the long strain. These findings suggest that variation in the cry gene is related to differences in the circadian behaviour in the two strains, thus implying that the cry gene may have an important role in reproductive isolation. *Heredity* (2010) 104, 387-392; doi: 10.1038/hdy.2009.167; published online 16 December 2009

The diapause response of *Rhagoletis pomonella* to varying environmental conditions and its significance for geographic and host plant-related adaptation

Feder, JL; Powell, THQ; Filchak, K; Leung, B. 2010

The recent shift of *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) from its ancestral host hawthorn to apple is a model for incipient sympatric speciation in action. Previous studies have shown that changes in the over-wintering pupal diapause are critical for differentially adapting *R. pomonella* flies to a difference in the fruiting times of apples vs. hawthorns, generating ecologically based reproductive isolation. Here, we exposed pupae of the hawthorn race to various combinations of pre- and over-wintering rearing conditions and analyzed their effects on eclosion time and genetics. We report certain unexpected results in regards to a combination of brief pre-winter and over-wintering periods indicative of gene*environment interactions requiring a reassessment of our current understanding of *R. pomonella* diapause. We present a hypothesis that involves physiological factors related to stored energy reserves in pupae that influences the depth and duration of *Rhagoletis* diapause. This 'pupal energy reserve' hypothesis can account for our findings and help clarify the role host plant-related life history adaptation plays in phytophage biodiversity.

Genetic correlations between life-history and behavioral traits can cause reproductive isolation

Miyatake, T; Shimizu, T. 1999

Reproductive isolation may often evolve as an indirect (pleiotropic) consequence of populations adapting to different environments or habitats. For example, niches that are temporally or seasonally offset can select for organisms with different developmental characteristics. These developmental differences can inadvertently cause reproductive isolation by a variety of means including shifts in mating activity patterns. Here, we show a genetic correlation between a life-history trait (developmental period) and a behavioral trait (time of mating) that causes significant premating isolation in the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). Fly lines selected for short and long developmental periods differ in their preferred times of mating during the evening. This difference translates into significant prezygotic isolation, as measured by mate choice tests. If the time of mating between two populations differed more than one hour, the isolation index was significantly higher than zero. These indicate that premating isolation can be established if the developmental period is divergently selected for. If such genetic correlations are ubiquitous in many organisms, multifarious divergent selection for life-history traits would often accelerate the evolution of reproductive isolation. We speculate that reproductive isolation may have been evolved via genetic correlations among time-related traits, for example, developmental period and time of mating, as in other organisms.

Divergent preferences of *Rhagoletis pomonella* host races for olfactory and visual fruit cues

Forbes, AA; Feder, JL. 2006

The apple and hawthorn-infesting host races of *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) represent a model for incipient sympatric speciation. Recent studies have shown that apple and hawthorn flies differ in their responses to host-fruit odor. Each host race preferentially orients to the volatile chemicals emanating from the surface of its natal fruit and is antagonized by non-natal fruit volatiles. Fruit odor discrimination is evolutionarily important due to its contributory role in host-specific mating, generating partial premating reproductive isolation between the races in sympatry. Visual cues are also involved in *R. pomonella* host searching, yet the extent to which they are used to discriminate between hosts has not been fully resolved, nor has their interaction with olfactory cues. Here, we report results from a series of two-way choice experiments conducted in the field on resident apple and hawthorn flies at a site near Fennville, MI, USA. Four key findings emerged from the study: (1) both olfactory and (2) visual cues increased host discrimination of apple and hawthorn flies for their respective natal fruit, (3) the presence of a single appropriate volatile blend on a natal or non-natal visual model generally increased the ability of flies to discriminate for their natal fruit, and (4) the presence of both natal and non-natal olfactory cues on fruit models did not enhance host discrimination above levels observed for visual models alone. We discuss the implications of our results for interpreting the findings of two-way host choice experiments and for sympatric speciation in phytophagous insects.

Ecological divergence among morphologically and genetically related *Asphondylia* species (Diptera: Cecidomyiidae), with new life history data for three congeners including the *Alpinia* fruit gall midge

Yukawa, J; Tokuda, M; Uechi, N; Yasuda, K; Ganaha-Kikumura, T; Matsuo, K; Shimizu, Y; Yamaguchi, D. 2019

Ecological data is crucial for determining the degree of reproductive isolation among closely related species, and in identifying the factors that have produced this divergence. We studied life history traits for three *Asphondylia* (Diptera: Cecidomyiidae) species that induce fruit galls either on *Alpinia*, *Ligustrum* or *Aucuba*, and we compared the traits with those published for three other closely related Japanese *Asphondylia* species. We found that the six species were significantly differentiated in important life history traits, such as host range, voltinism, lower developmental threshold temperature, thermal constant and diapausing season. The data indicate that divergence in the assessed life history traits evolves before morphological divergence, and such ecological divergence could strengthen isolating barriers among the taxa. We present scenarios on how host range expansion, host plant shift and host organ shift for galling initiate the early stages of speciation. We also highlight the importance of ecological data in identifying cryptic species. Specifically, we confirm that *Alpinia intermedia* (Zingiberaceae) is not an autumn-spring host of the soybean pod gall midge *Asphondylia yushimai* based on many differences in the life history traits between the *Alpinia* fruit gall midge *Asphondylia* sp. and *A. yushimai*.

From 'cryptic species' to integrative taxonomy: an iterative process involving DNA sequences, morphology, and behaviour leads to the resurrection of *Sepsis pyrrhosoma* (Sepsidae: Diptera)

Tan, DSH; Ang, Y; Lim, GS; Bin Ismail, MR; Meier, R. 2010

Tan, D. S. H., Ang Y., Lim, G. S., Ismail, M. R. B. & Meier, R. (2010). Front 'cryptic species' to integrative taxonomy: an iterative process involving DNA sequences, morphology, and behaviour leads to the resurrection of *Sepsis pyrrhosoma* (Sepsidae: Diptera). - *Zoologica Scripta*, 39, 51-61. The increased availability of DNA sequences has led to a surge of 'cryptic Species in the literature. These units are usually proposed based on finding genetically distinct lineages within species that were initially defined based on morphological characters. However, few authors attempt to confirm whether these 'cryptic' units are species and even fewer authors are explicit about which species concept is applied. Here, we use an example from Sepsidae (Diptera) to demonstrate how cryptic species can be validated by all iterative process involving several data sources and an evaluation of the data under different species concepts. A phylogeographic analysis based on 50 specimens for five species of the flavimana group revealed deep mitochondrial splits within *Sepsis flavimana* which was suggestive of a cryptic species. We resolve the initial conflict between DNA sequences and morphology by adding new morphological data as well as behavioural evidence and tests for reproductive isolation. One cryptic species is confirmed and *Sepsis pyrrhosoma*, a former synonym of *S. flavimana*, is here shown to be a valid species under most species concepts. We call thus document that the same data can lead to similar conclusions under conflicting concepts once different kinds of data are integrated.

Distinct Adult Eclosion Traits of Sibling Species *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae) Under Laboratory Conditions

Yee, WL; Goughnour, RB; Feder, JL. 2021

Closely related phytophagous insects that specialize on different host plants may have divergent responses to environmental factors. *Rhagoletis pomonella* (Walsh) and *Rhagoletis zephyria* Snow (Diptera: Tephritidae) are sibling, sympatric fly species found in western North America that attack and mate on plants of Rosaceae (similar to 60 taxa) and Caprifoliaceae (three taxa), respectively, likely contributing to partial reproductive isolation. *Rhagoletis zephyria* evolved from *R. pomonella* and is native to western North America, whereas *R. pomonella* was introduced there. Given that key features of the flies' ecology, breeding compatibility, and evolution differ, we predicted that adult eclosion patterns of the two flies from Washington State, USA are also distinct. When puparia were chilled, eclosion of apple- and black hawthorn-origin *R. pomonella* was significantly more dispersed, with less pronounced peaks, than of snowberry-origin *R. zephyria* within sympatric and nonsympatric site comparisons. Percentages of chilled puparia that produced adults were $\geq 67\%$ for both species. However, when puparia were not chilled, from 13.5 to 21.9% of apple-origin *R. pomonella* versus only 1.2% to 1.9% of *R. zephyria* eclosed. The distinct differences in eclosion traits of *R. pomonella* and *R. zephyria* could be due to greater genetic variation in *R. pomonella*, associated with its use of a wider range of host plants than *R. zephyria*.

Cytogenetics of the *Simulium arcticum* complex (Diptera: Simuliidae)

Shields, GF. NA

Descriptions of chromosomal rearrangements, geographic distributions and frequencies of nine siblings and 28 cytotypes of the *Simulium arcticum* Malloch complex are presented. Findings are based on six data sets that include approximately 21,000 chromosomally analyzed larvae from throughout the known geographic range of *S. arcticum*. This is the largest chromosomal data set for any North American complex of black flies. This summary emphasizes the need to chromosomally analyze taxa of black flies since this type of analysis can result in, not only, a better understanding of the number of taxa in a complex and their relationships but also, it may help to understand the initial stages of reproductive isolation within otherwise morphologically identical groups. Geographically, the streams of eastern Alaska, the entire province of the Yukon and northern Mexico should be sampled. Taxonomically the many cytotypes should be tested for reproductive status when they occur in sympatry with other siblings and cytotypes of the complex. Finally, comparative multi-omic research would be useful.

Evidence for spatial clines and mixed geographic modes of speciation for North American cherry-infesting *Rhagoletis* (Diptera: Tephritidae) flies

Doellman, MM; Saint Jean, G; Egan, SP; Powell, THQ; Hood, GR; Schuler, H; Bruzese, DJ; Glover, MM; Smith, JJ; Yee, WL; Goughnour, R; Rull, J; Aluja, M; Feder, JL. 2020

An important criterion for understanding speciation is the geographic context of population divergence. Three major modes of allopatric, parapatric, and sympatric speciation define the extent of spatial overlap and gene flow between diverging populations. However, mixed modes of speciation are also possible, whereby populations experience periods of allopatry, parapatry, and/or sympatry at different times as they diverge. Here, we report clinal patterns of variation for 21 nuclear-encoded microsatellites and a wing spot phenotype for cherry-infesting *Rhagoletis* (Diptera: Tephritidae) across North America consistent with these flies having initially diverged in parapatry followed by a period of allopatric differentiation in the early Holocene. However, mitochondrial DNA (mtDNA) displays a different pattern; cherry flies at the ends of the clines in the eastern USA and Pacific Northwest share identical haplotypes, while centrally located populations in the southwestern USA and Mexico possess a different haplotype. We hypothesize that the mitochondrial difference could be due to lineage sorting but more likely reflects a selective sweep of a favorable mtDNA variant or the spread of an endosymbiont. The estimated divergence time for mtDNA suggests possible past allopatry, secondary contact, and subsequent isolation between USA and Mexican fly populations initiated before the Wisconsin glaciation. Thus, the current genetics of cherry flies may involve different mixed modes of divergence occurring in different portions of the fly's range. We discuss the need for additional DNA sequencing and quantification of prezygotic and postzygotic reproductive isolation to verify the multiple mixed-mode hypothesis for cherry flies and draw parallels from other systems to assess the generality that speciation may commonly involve complex biogeographies of varying combinations of allopatric, parapatric, and sympatric divergence.

A genetic-morphological characterization of two cryptic species of the *Anastrepha fraterculus* complex (Diptera : Tephritidae)

Selivon, D; Perondini, ALP; Morgante, JS. 2005

Biological characteristics of two entities of the *Anastrepha fraterculus* complex (Diptera: Tephritidae), referred to in previous publications as *A. sp.1 aff. fraterculus* and *A. sp.2 aff. fraterculus*, were further studied by a combined analysis of isozymes, karyotypes, morphometry, and crossings, in samples from 10 Brazilian populations. A survey of 16 enzymatic systems comprising 19 loci showed significant differences in the allele frequencies at four loci, FUM, ME, HEX, and LDH, allowing the recognition of two population clusters. These clusters also differ in their karyotypes, especially in the length of the sex chromosomes and in the size and location of heterochromatic regions. A morpho-metric analysis of wings and the aculeus in samples from five populations clearly showed a distinction between the two clusters but not between populations within each cluster. A phenetic analysis based on the Mahalanobis distance matrix also arranged the populations into the same two clusters. Crosses between populations of the same cluster showed no significant differences in egg hatching and in the adult sex ratio. However, a significant decrease in egg hatching was observed in the intercluster crosses. In crosses of cluster 1 males to cluster 2 females, a significant deviation in the sex ratio was observed according to Haldane's rule, but not in the reciprocal crosses, indicating that a certain degree of reproductive isolation occurs between populations of cluster 1 and cluster 2. The results indicate that the two population clusters actually represent two cryptic species of the nominal species *Anastrepha fraterculus*, corroborating previous studies on this complex. We propose that the denominations *A. sp.1 aff. fraterculus* and *A. sp.2 aff. fraterculus* should be maintained until an appropriate taxonomic revision is made.

Infections with *Wolbachia* and *Spiroplasma* in the Scathophagidae and other Muscoidea

Martin, OY; Gubler, A; Wimmer, C; Germann, C; Bernasconi, MV. 2012

The microbes *Wolbachia* and *Spiroplasma* are common reproductive parasites of arthropods and may strongly influence reproduction of infected hosts and also impact on reproductive isolation. Such infections could hence influence results of many studies assessing reproductive behaviour and fitness of possible hosts, as well as reproductive isolation. Previous work indicates that infections with the microbes *Wolbachia* and *Spiroplasma* are common in the *Drosophilidae*. However, extensive and targeted surveys of other Dipteran families are lacking. Here we survey the yellow dung fly *Scathophaga stercoraria* and a range of other species from the Muscoidea (families Scathophagidae, Anthomyiidae, Fanniidae and Muscidae) collected in the field or obtained from museum collections for infection with the widespread reproductive parasites *Wolbachia* and *Spiroplasma*. Both have been shown to be heritable symbionts and affect reproduction in other Diptera. *S. stercoraria* is a very important model for the study of sexual selection, and in particular of postcopulatory processes, as it has played a major role in the history of research on sperm competition and cryptic female choice. Infections with *Wolbachia* were found to be widespread across the Muscoidea, whereas infections with *Spiroplasma* were rarer. We discuss the consequences of these findings and directions for future research on the impact of reproductive parasites on host reproduction in the Scathophagidae. (C) 2011 Elsevier B.V. All rights reserved.

Taxonomy and phylogeny of the *Asphondylia* species (Diptera: Cecidomyiidae) of North American goldenrods: challenging morphology, complex host associations, and cryptic speciation

Dorchin, N; Joy, JB; Hilke, LK; Wise, MJ; Abrahamson, WG. 2015

Reproductive isolation and speciation in herbivorous insects may be accomplished via shifts between host-plant resources: either plant species or plant organs. The intimate association between gall-inducing insects and their host plants makes them particularly useful models in the study of speciation. North American goldenrods (Asteraceae: *Solidago* and *Euthamia*) support a rich fauna of gall-inducing insects. Although several of these insects have been the subject of studies focusing on speciation and tritrophic interactions, others remain unstudied and undescribed. Among the latter are at least seven species of the large, cosmopolitan gall midge genus *Asphondylia* Loew (Diptera: Cecidomyiidae), the taxonomy and biology of which are elucidated here for the first time using morphological, molecular, and life-history data. We describe *Asphondylia pseudorosasp.nov.*, *Asphondylia rosulasp.nov.*, and *Asphondylia silvasp.nov.*, and redescribe *Asphondylia monacha* Osten Sacken, 1869 and *Asphondylia solidaginis* Beutenmüller, 1907, using morphological characters of adults, immature stages, and galls, as well as sequence data from both nuclear and mitochondrial genes. A neotype is designated for *A. solidaginis*, the type series of which is considered lost. We also provide information on the life history of all species, including a description of two inquiline cecidomyiids commonly found in the galls, *Clinodiplosis comitissp.nov.* and *Youngomyia podophyllae* (Felt, 1907), and on parasitoid wasps associated with the gall midges. *Asphondylia johnsoni* Felt, 1908, which was described from an unknown gall on an unknown *Solidago* host, is assigned to *nomina dubia*. Our phylogenetic analyses show that some of the *Asphondylia* species associated with goldenrods induce two different types of galls during their life cycle, some exhibit host alterations, and some do both. In the absence of reliable morphological differences, recognising species boundaries and deciphering host associations of species must rely heavily on molecular data. Our analysis suggests that radiation in this group has been recent and occurred through shifts among host plants. (c) 2015 The Linnean Society of London

Incipient speciation in the *Anastrepha fraterculus* cryptic species complex: reproductive compatibility between *A. sp.1* aff. *fraterculus* and *A. sp.3* aff. *fraterculus*

Roriz, AKP; Japyassu, HF; Joachim-Bravo, IS. 2017

Reproductive isolation among biological strains can be detected by analyzing reproductive barriers between populations. The *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae) cryptic species complex comprises eight morphotypes defined by morphometric characteristics. The present study analyzed the existence of pre- and post-zygotic isolation among populations of *A. sp.1* aff. *fraterculus* (*A. sp.1*) and *A. sp.3* aff. *fraterculus* (*A. sp.3*) determined by tests of sexual compatibility, analyses of the temporal pattern of calling behavior, and the reproductive performance of the offspring of homotypic and heterotypic crosses. Pre-zygotic tests indicated asymmetric matings with preferences for homotypic crosses. The *A. sp.3* population demonstrated distinct characteristics, including low copulation percentages and a high proportion of homotypic copulations under crowded conditions. The females of the *A. sp.1* population demonstrated lower copulation latency. The analyzed morphotypes demonstrated two peaks in pheromone emission activity, at the beginning and at the end of the morning and were differentiated in the abundance of males. The calling behavior of the **hybrids** between *A. sp.1* male and *A. sp.3* female crosses had an intermediate pattern compared to either parental type. Post-zygotic compatibility tests indicated low viability of the cross between *A. sp.3* males and *A. sp.1* females. Pronounced asymmetries were found in the sex ratios of the offspring of heterotypic crosses, and only **hybrids** from *A. sp.1* male vs. *A. sp.3* female crosses produced descendants. The observed pre- and post-zygotic incompatibilities reflect the probable occurrence of incipient speciation between *A. sp.1* and *A. sp.3*. A more adequate taxonomic classification of this species complex that considers the distinct characteristics of each morphotype will be needed to improve environmentally wise control methods against this insect pest.

Ongoing speciation within the *Anastrepha fraterculus* cryptic species complex: the case of the Andean morphotype

Devescovi, F; Abraham, S; Roriz, AKP; Nolasco, N; Castaneda, R; Tadeo, E; Caceres, C; Segura, DF; Vera, MT; Joachim-Bravo, I; Canal, N; Rull, J. 2014

The *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) cryptic species complex is currently composed of seven taxonomically recognized morphotypes. Both, pre- and post-zygotic isolation has been documented among four of these morphotypes, revealing that in fact they appear to be distinct biological entities. In order to progress in the full delimitation of species within the complex, we examined reproductive isolation between a Colombian population of the Andean morphotype and populations belonging to four other morphotypes spanning from Mexico to Argentina. Flies from the Andean morphotype exhibited strong pre-zygotic mating isolation through temporal partitioning of mating activity. Post-zygotic isolation was observed for crosses of males of all morphotypes and Andean morphotype females, yet most of the F1 **hybrid** male F1 **hybrid** female self-crosses showed normal levels of fertility, a finding suggesting a nuclear-cytoplasmic interaction according to previous studies. Overall, the Andean morphotype within the complex also appears to be a distinct biological entity. We discuss the implications of these findings for the understanding of speciation mechanisms in the Neotropical genus *Anastrepha*.

Sexual conflict and reproductive isolation in flies

Hosken, DJ; Martin, OY; Wigby, S; Chapman, T; Hodgson, DJ. 2009

Sexual conflict is predicted to generate more rapid reproductive isolation between larger populations. While there is some empirical support for this, the data are inconsistent and, additionally, there has been criticism of some of the evidence. Here we reanalyse two experimental-evolution datasets using an isolation index widely applied in the speciation literature. We find evidence for reproductive isolation through sexual conflict in *Sepsis cynipsea*, but not in *Drosophila melanogaster*, and this occurred to a greater degree in larger populations, which is consistent with previous findings.

Chromosomal Translocations in Black Flies (Diptera: Simuliidae)-Facilitators of Adaptive Radiation?

Adler, PH; Yadamsuren, O; Procnier, WS. 2016

A macrogenomic investigation of a Holarctic clade of black flies-the *Simulium cholodkovskii* lineage-provided a platform to explore the implications of a unique, synapomorphic whole-arm interchange in the evolution of black flies. Nearly 60 structural rearrangements were discovered in the polytene complement of the lineage,

including 15 common to all 138 analyzed individuals, relative to the central sequence for the entire subgenus *Simulium*. Three species were represented, of which two Palearctic entities (*Simulium cholodkovskii* and *S. decimatum*) were sympatric; an absence of **hybrids** confirmed their reproductive isolation. A third (Nearctic) entity had nonhomologous sex chromosomes, relative to the other species, and is considered a separate species, for which the name *Simulium nigricoxum* is revalidated. A cytophylogeny is inferred and indicates that the two Palearctic taxa are sister species and these, in turn, are the sister group of the Nearctic species. The rise of the *S. cholodkovskii* lineage encompassed complex chromosomal and genomic restructuring phenomena associated with speciation in black flies, viz. expression of one and the same rearrangement as polymorphic, fixed, or sex linked in different species; taxon-specific differentiation of sex chromosomes; and reciprocal translocation of chromosome arms. The translocation is hypothesized to have occurred early in male spermatogonia, with the translocated chromosomal complement being transmitted to the X- and Y-bearing sperm during spermatogenesis, resulting in alternate disjunction of viable F1 translocation heterozygotes and the eventual formation of more viable and selectable F2 translocation homozygous progeny. Of 11 or 12 independently derived whole-arm interchanges known in the family Simuliidae, at least six are associated with subsequent speciation events, suggesting a facilitating role of translocations in adaptive radiations. The findings are discussed in the context of potential structural and functional interactions for future genomic research.

Evolutionary neuroecology of olfactory-mediated sexual communication and host specialization in *Drosophila* - a review

Khallaf, MA; Knaden, M. 2022

In humans, finding a partner is quite a difficult task because there are many criteria that one needs to consider. However, in comparison to many animals, when choosing a partner, we easily discriminate between ourselves and members of other species through various communication systems. On the contrary, many fly species (Diptera) are morphologically similar and overlap in their geographical distributions and ecological habitats. Sexual interactions of most drosophilid flies occur on their hosts. Therefore, flies rely on olfactory sex pheromones, as well as on non-pheromonal chemicals such as host volatiles - which guide and restrict the search for conspecifics within limited locations - as honest signals for pre-mating reproductive isolation. A subtle divergence in the perception of these signals can lead to accumulated changes among populations of the same species, and ultimately to a reduction in gene flow and reproductive isolation. In recent years, we have seen an increased interest in how olfactory systems diverge to drive host adaptation and speciation. In this review, we discuss the evolutionary changes of the neural circuits that underlie mate recognition. We shed light onto sex pheromone communication systems, the construction of olfactory nervous systems, and the role of host specialization in reproductive isolation. Finally, leveraging the incipient speciation of *Drosophila mojavensis* Patterson populations, we highlight the underlying sensory mechanisms of the reproductive isolation barriers. In the end, we propose future research topics of the evolutionary neuroecology field of sexual communication.

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

Hagele, K; Kasper-Sonnenberg, M. 2000

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

Flight tone of field-collected populations of *Anopheles gambiae* and *An-arabiensis* (Diptera : Culicidae)

Wekesa, JW; Brogdon, WG; Hawley, WA; Besansky, NJ. 1998

Laboratory colonies of the human malaria vectors *Anopheles gambiae* Giles and *An. arabiensis* Patton have distinct flight tones. If flight tone similarly distinguishes natural populations of these sympatric sibling species, it may play a role in reproductive isolation of swarms that are otherwise behaviourally identical. To assess the fidelity of flight tone differences in natural populations, flight tone was measured in the F1 progeny of mosquitoes of both species captured in western Kenya. Flight tone distributions of wild *An. gambiae* and *An. arabiensis* were similar to their laboratory conspecifics. However, interspecies comparisons of flight tone of wild mosquitoes revealed significantly different but overlapping distributions for both sexes. Furthermore, when the effect of body size on flight tone was determined, there was a positive correlation between wing length and flight tone for both sexes of *An. gambiae* and *An. arabiensis*, suggesting that mosquito size is a significant variable affecting flight tone. Although these findings diminish any practical benefit of flight tone as a diagnostic tool in species identification, its potential role in pre-mating species recognition needs further investigation.

Phylogeography of the parasitic fly *Batrachomyia* in the Wet Tropics of north-east Australia, and susceptibility of host frog lineages in a mosaic contact zone

Hoskin, CJ; Mccallum, H. 2007

Parasites could differentially impact intraspecific host lineages due to genetic, phenotypic, ecological, or behavioural differences between the lineages, or the development of reproductive isolation between them. *Batrachomyia* (Diptera: Chloropidae) are flies that exclusively parasitize Australian frogs, and in the Wet Tropics rainforest of north-east Australia larvae are largely restricted to the green-eyed tree frog *Litoria genimaculata* (Anura: Hylidae). This frog species consists of two highly divergent genetic lineages that overlap in two nearby, but independent, contact zones. At one contact zone there has been extensive phenotypic divergence and speciation between the lineages whereas, at the other contact relatively lower levels of phenotypic divergence and reproductive isolation suggest that speciation has not occurred. In the present study we tested: (1) whether the deep phylogeographic divergence between northern and southern host populations is mirrored by congruent genetic structuring in the parasite populations and (2) whether the host lineages are differentially impacted by parasitism. We found that the two divergent frog lineages are parasitized by a single lineage of *Batrachomyia*, which exhibits strikingly little phylogeographic structuring. We found a significant difference in *Batrachomyia* prevalence between the host lineages at mixed lineage sites in both contact zones, with the magnitude and direction of this effect being consistent in both. The pattern did not differ between the two contacts even though recent phenotypic divergence and speciation has occurred between the lineages at one contact but not the other. Taken together, this suggests a fundamental difference in susceptibility between the genetically divergent host lineages. Using weight relative to body length as a measure of body condition, we found no differential impact of parasitism on the body condition of each host lineage, and no evidence that parasitism impacts the body condition of the host in general.

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

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

The cactophilic species *Drosophila serido* Vilela and Sene 1977 and *Drosophila antonietae* Tidon-Sklorz and Sene 2001 are two closely related species that have partially overlapping distributions in the Florianopolis-SC locality on the southern Brazilian coast. To characterize this contact zone with respect to possible introgression events and the differential use of cacti hosts, we analyzed male individuals that were collected in their natural environment or from the necrotic cacti in which they were reared, by using aedeagus morphology, isocitrate dehydrogenase isozymes, and the mitochondrial gene cytochrome oxidase I as diagnostic markers. Morphological and molecular markers showed a bimodal distribution, although there was some incongruence, suggesting the presence of asymmetric introgression events. These results suggest that gene flow occurred in the past but then ceased, possibly due to reinforcement of prezygotic reproductive isolation. Analysis of individuals reared in necrotic cacti of two sympatric species in this region showed that *D. antonietae*, but not *D. serido*, preferentially uses *Cereus hildmannianus* Mill. as a cactus host, which may represent an important influence on ecological differentiation by affecting the dynamics of these species in this area of sympatry.

Hybridization and reproductive isolation between diploid *Erythronium mesochoreum* and its tetraploid congener *E-albidum* (Liliaceae)

Roccaforte, K; Russo, SE; Pilson, D. 2015

Polyploidy has played an important role in angiosperm diversification, but how polyploidy contributes to reproductive isolation remains poorly understood. Most work has focused on postzygotic reproductive barriers, and the influence of ploidy differences on prezygotic barriers is understudied. To address these gaps, we quantified **hybrid** occurrence, interspecific self-compatibility differences, and the contributions of multiple pre- and postzygotic barriers to reproductive isolation between diploid *Erythronium mesochoreum* (Liliaceae) and its tetraploid congener *Erythronium albidum*. Reproductive isolation between the study species was nearly complete, and naturally occurring **hybrids** were infrequent and largely sterile. Although postzygotic barriers effected substantial reproductive isolation when considered in isolation, the study species' spatial distributions and pollinator assemblages overlapped little, such that interspecific pollen transfer is likely uncommon. We did not find evidence that *E. albidum* and *E. mesochoreum* differed in mating systems, indicating that self-incompatibility release may not have fostered speciation in this system. Ultimately, we demonstrate that *E. albidum* and *E. mesochoreum* are reproductively isolated by multiple, hierarchically-operating barriers, and we add to the currently limited number of studies demonstrating that early acting barriers such as pollinator-mediated isolation can be important for effecting and sustaining reproductive isolation in diploid-polyploid systems.

Cuticular hydrocarbon variation among *Rhagoletis* fruit flies (Diptera: Tephritidae): implications for premating reproductive isolation and ecological speciation

Hood, GR; Jennings, JH; Bruzese, DJ; Beehler, M; Schmitt, T; Feder, JL; Etges, WJ. 2022

1. Fruit flies in the genus *Rhagoletis* are a model for ecological speciation via sympatric host plant shifting. *Rhagoletis* mate on or near the fruit of their respective host plants, generating premating reproductive isolation between taxa specialised on different hosts. However, non-host-related premating isolation has been observed between some *Rhagoletis* species associated with morphological differences in body colour and wing patterns. 2. Here, the extent of epicuticular hydrocarbon (CHC) variation as a possible, additional determinant of mate choice in adults of six *Rhagoletis* taxa, including the apple and hawthorn-infesting host races of *R. pomonella* is investigated. 3. Gas-chromatography-mass-spectrometry revealed 36 repeatable and quantifiable hydrocarbon components present on the epicuticles of all six fly taxa, comprised of at least 53 different CHC compounds, with chain lengths varying from 27 to 34 carbon atoms, consisting of n-alkanes, mono-, dimethyl-, and trimethyl-alkanes, alkenes, and alkenadienes. There were significant differences in the relative proportions of CHCs between adult *R. cingulata*, *R. cornivora*, *R. zephyria*, *R. mendax*, and *R. pomonella*, as well as between the apple- and hawthorn-infesting host races of *R. pomonella*. Furthermore, within the *R. pomonella* host races and *R. mendax*, significant CHC differences were observed between the sexes and across collecting sites. 4. The results are consistent with variation in CHCs potentially contributing to observed patterns of premating isolation between *Rhagoletis* taxa, possibly due to combination of sexual and host-related selection, which will necessitate further, in depth chemical analyses and future mating trials to substantiate.

SPATIAL-DISTRIBUTION OF HOVERING MALE *TABANUS-NIGROVITTATUS* (DIPTERA, TABANIDAE)

MCAHON, MJ; GAUGLER, R. 1993

Male *Tabanus nigrovittatus* Macquart hover as a premating strategy in the early morning over Atlantic coastal salt marshes. These males are distributed with peak densities near mosquito ditches and with fewer hovering males farther from the ditch. Multivariate analysis indicated that male density or the distance between individuals is an important factor in structuring hovering male spatial distribution. When compared with previously published data, male densities match peak emergence of females. *T. nigrovittatus* numbers are lowest directly adjacent to mosquito ditches, where *Tabanus conterminus* (Walker) tends to hover, suggesting that spatial separation may contribute to reproductive isolation in these two sibling species.

Acoustic signals in the sand fly *Lutzomyia* (*Nyssomyia*) *intermedia* (Diptera: Psychodidae)

Vigoder, FM; Souza, NA; Peixoto, AA. 2011

Background: Acoustic signals are part of the courtship of many insects and they often act as species-specific signals that are important in the reproductive isolation of closely related species. Here we report the courtship songs of the sand fly *Lutzomyia* (*Nyssomyia*) *intermedia*, one of the main vectors of cutaneous leishmaniasis in Brazil. Findings: Recordings were performed using insects from three localities from Eastern Brazil: Posse and Jacarepagua in Rio de Janeiro State and Corte de Pedra in Bahia State. The three areas have remnants of the Brazilian Atlantic forest, they are endemic for cutaneous leishmaniasis and *L. intermedia* is the predominant sand fly species. We observed that during courtship *L. intermedia* males from all populations produced pulse songs consisting of short trains. No significant differences in song parameters were observed between the males of the three localities. Conclusions: *L. intermedia* males produce acoustic signals as reported for some other sand flies such as the sibling species of the *Lutzomyia longipalpis* complex. The lack of differences between the males from the three localities is consistent with previous molecular studies of the period gene carried out in the same populations, reinforcing the idea that *L. intermedia* is not a species complex in the studied areas and that the three populations are likely to have similar vectorial capacities.

Shared microsatellite loci in *Glossina morsitans sensu lato* (Diptera : Glossinidae)

Krafsur, ES; Endsley, MA. 2006

Estimation of allelic frequencies at three microsatellite loci among 20 populations of *Glossina morsitans morsitans* Westwood, *Glossina morsitans submorsitans* Newstead, and *Glossina morsitans centralis* Machado indicated only two of 99 alleles were shared between three subspecies and 18 between any two subspecies; 81 alleles were unshared. The conserved flanking regions of each locus were completely shared. Genetic differentiation among subspecies, based on allele size, was $R_{ST} = 0.87$, close to the theoretic maximum value. All evidence suggests longstanding and complete reproductive isolation in nature among the sibling species. They

should be elevated to specific rank.

Uncovering tropical diversity: six sympatric cryptic species of *Blepharoneura* (Diptera : Tephritidae) in flowers of *Gurania spinulosa* (Cucurbitaceae) in eastern Ecuador

Condon, M; Adams, DC; Bann, D; Flaherty, K; Gammons, J; Johnson, J; Lewis, ML; Marsteller, S; Scheffer, SJ; Serna, F; Swensen, S. 2008

Diversification of phytophagous insects is often associated with changes in the use of host taxa and host parts. We focus on a group of newly discovered Neotropical tephritids in the genus *Blepharoneura*, and report the discovery of an extraordinary number of sympatric, morphologically cryptic species, all feeding as larvae on calyces of flowers of a single functionally dioecious and highly sexually dimorphic host species (*Gurania spinulosa*) in eastern Ecuador. Molecular analyses of the mitochondrial cytochrome oxidase-I gene from flies reared from flowers of *G. spinulosa* reveal six distinct haplotype groups that differ by 7.2-10.1% bp (uncorrected pairwise distances; N = 624 bp). Haplotype groups correspond to six distinct and well-supported clades. Members of five clades specialize on the calyces of flowers of a particular sex: three clades comprise male flower specialists; two clades comprise female flower specialists; the sixth clade comprises generalists reared from male and female flowers. The six clades occupy significantly different morphological spaces defined by wing pigmentation patterns; however, diagnostic morphological characters were not discovered. Behavioural observations suggest specific courtship behaviours may play a role in maintaining reproductive isolation among sympatric species. Journal compilation (C) 2008 The Linnean Society of London.

Behavioural mechanisms of reproductive isolation between two hybridizing dung fly species

Giesen, A; Blanckenhorn, WU; Schafer, MA. 2017

Characterization of the phenotypic differentiation and genetic basis of traits that can contribute to reproductive isolation is an important avenue to understand the mechanisms of speciation. We quantified the degree of prezygotic isolation and geographical variation in mating behaviour among four populations of *Sepsis neocynipsea* that occur in allopatry, parapatry or sympatry with four populations of its sister species *Sepsis cynipsea*. To obtain insights into the quantitative genetic basis and the role of selection against **hybrid** phenotypes we also investigated mating behaviour of F-1 **hybrid** offspring and corresponding backcrosses with the parental populations. Our study documents successful **hybridization** under laboratory conditions, with low copulation frequencies in heterospecific pairings but higher frequencies in pairings of F-1 **hybrids** signifying **hybrid** vigour. Analyses of F-1 offspring and their parental backcrosses provided little evidence for sexual selection against **hybrids**. Longer copulation latencies in heterospecific pairings indicate species recognition, probably due to surface or volatile chemicals. The frequency of male mating attempts did not differ greatly between species or **hybrid** pairings, suggesting no male discrimination of mating partners. Female shaking duration, signifying female choice and/or reluctance to mate, differed strongly between the species and appears to contribute to avoiding heterospecific males; this trait is partially maternally inherited. Importantly, females of both species discriminated more strongly against males in areas of sympatry than allopatry indicating reinforcement. Shorter copulations in heterospecific parental pairings and longer copulations in F-1 **hybrids** suggest mechanistic difficulties with sperm transfer. Overall, our study highlights an important role of character displacement affecting mating behaviour of **hybridizing** sepsid species in geographical areas of coexistence. (C) 2017 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Allozyme differentiation among allopatric populations of *Anopheles nuneztovari* (Diptera: Culicidae)

Scarpassa, VM; Tadei, WP; Suarez, MF. 1996

Four enzymes in six geographic populations, four Brazilian and two Colombian, of *Anopheles nuneztovari* were studied. There were differences among the most frequent alleles in the EST5, ACON and MDH loci in the populations from Brazil and Colombia. The alpha-GPDC *allele was encountered very frequently in the Palo/Sit population in Colombia and the alpha-GPDB allele was found to be fixed in most of the Brazilian populations.* An additional alpha-GPD band was found only in Palo/Sit. The considerable genetic differentiation between populations in western Colombia vs. Brazil suggests that a certain degree of reproductive isolation exists between them.

Host-associated differentiation in reproductive behaviour of cecidomyiid midges on cranberry and blueberry

Cook, MA; Ozeroff, SN; Fitzpatrick, SM; Roitberg, BD. 2011

In British Columbia, Canada, *Dasineura oxycoccana* Johnson (Diptera: Cecidomyiidae) was initially found on highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae) and has recently become a pest of cranberry, *Vaccinium macrocarpon* Aiton, a crop that is often found in close proximity with blueberry. Previous work has shown no temporal isolation and a potential for gene flow between these two *D. oxycoccana* populations. However, important behavioural differences were observed suggesting the potential for host races or cryptic species. Host races and cryptic species differ in their degree of assortative mating and reproductive isolation from partial to complete. We assessed whether populations of adult *D. oxycoccana* on these two crops would discriminate against mates from different natal hosts. Mating experiments were conducted within the greenhouse in 2010 using small glass vials without host plants present. Our results show *D. oxycoccana* from cranberry and blueberry hosts displayed complete assortative mating in the absence of their host plants. Behavioural data collected from the different crosses suggest these two *D. oxycoccana* populations differ in sex pheromones and close-range 'courtship pheromones'. We conclude that *D. oxycoccana* populations on cranberry and blueberry in British Columbia are reproductively isolated and probably represent cryptic species.

Mating time of the west Indian fruit fly *Anastrepha obliqua* (Macquart) (Diptera : tephritidae) under laboratory conditions

Henning, F; Matioli, SR. 2006

Allochronic reproductive isolation seems to be an important factor in speciation processes in Tephritidae since specific mating times are a widespread feature of its species. The timing of matings of the West Indian fruit fly, *Anastrepha obliqua* (Macquart) was investigated through group-focal observations, during ten days, under laboratory conditions. The number of observed matings and males exhibiting calling behavior varied significantly according to time of day. Sexual activities seemed to be concentrated in the afternoon period, with the male calling behavior reaching a peak between 3:30p.m. and 4:30p.m., and mating occurred most frequently from 2:00p.m. to 4:30p.m.

A Taxonomic Reassessment of the *Phlebotomus argentipes* Species Complex (Diptera: Psychodidae: Phlebotominae)

Ilango, K. 2010

The taxonomic status of the sand fly *Phlebotomus* (*Euphlebotomus*) *argentipes* Annandale & Brunette 1908, which transmits *Leishmania* (*Leshmania*) *donovani* (Laveran & Mesnil 1903) in southern Asia, is reassessed, because variation in morphology, behavior, and distribution suggests it to be a complex of sibling species. The putative complex is composed of the nominotypical member, *p Phlebotomus argentipes sensu Stricto*, *Phlebotomus annandalei* Sinton 1923 status revived and

Phlebotomus glaucus Mitra & Roy 1953 new status. An allolectotype is designated for the female of *P. argentipes* as well as neotypes for the males of *P. annandalei* and *P. glaucus*. Morphological descriptions, illustrations, and keys are presented to allow identification of adult males and females. Based on female morphological characters, *P. argentipes* s.s., *P. annandalei* and *P. glaucus* can be distinguished from each other using principal component analysis. *P. glaucus* is widespread in India, occurring sympatrically with *P. argentipes* s.s. in *L. donovani*-endemic foci, whereas *P. annandalei* is peripatric to the type species in Chennai, Southern India. "In copula" is a process of postcopulatory sexual selection occurring as correlated coevolution of male and female reproductive traits, which drives species isolation. This pattern has been implicated in reproductive isolation among the members of the *P. argentipes* complex. Further research on adult behavior and larval biology of the *P. argentipes* complex is needed to understand the epidemiology and control of visceral leishmaniasis.

Geographic and behavioural isolation promote the differentiation of parapatric host-associated forms in bud-galling midges (Diptera: Cecidomyiidae)

Danon, G; Ben-Shlomo, R; Keidar, N; Dorchin, N. 2017

Host-associated differentiation (HAD) is widely recognized as a step towards speciation in phytophagous insects because adaptations to different host plants often lead to reproductive isolation. Various pre-mating and post-mating barriers can reduce gene flow between host-associated populations (HAPs), but establishing their relative contribution and the order in which they took effect during speciation is difficult, because thorough information is needed on past and present distribution ranges, behaviour and life history of the taxa involved. We investigated evidence for HAD in the gall-midge *Dasyneuriola* sp., which induces bud galls on the parapatric desert shrubs *Suaeda fruticosa* and *Suaeda asphaltica* in Israel. Based on mitochondrial sequence and amplified fragment length polymorphism analyses, we show that the populations from the two host plants are at an early stage of separation resulting from geographic and behavioural barriers between them. There was no assortative mating between the HAPs, but asymmetry between them was found in host fidelity by ovipositing females and in larval performance on the host plants. Together with findings on attack levels by natural enemies, these findings suggest that the original host plant in this system was *S. asphaltica*. We conclude that genetic differentiation in *Dasyneuriola* sp. was probably triggered by geographic separation, manifested by altitudinal rather than simple geographic distances, and that ecological barriers currently promote further divergence between the HAPs.

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

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

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

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

Hirai, Y; Kimura, MT. 1997

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

Cytotaxonomy of the *Prosimulium* (Diptera: Simuliidae) of Western Asia

Adler, PH; Sirin, U. 2014

Chromosomal analysis of more than 500 larval black flies from 19 sites in Armenia and Turkey revealed five taxa in the *Prosimulium hirtipes* group: *Prosimulium frontatum* Terteryan, *Prosimulium rachiliense* Djafarov cytoform 'A,' *P. rachiliense* Djafarov cytoform 'B,' *Prosimulium tomosvaryi* (Enderlein), and a new species of *Prosimulium*. To associate the names *rufipes* (Meigen) and *tomosvaryi* with cytological entities, larvae from the respective type localities in Germany were characterized chromosomally. *Prosimulium frontatum* is restricted to the Caucasus Mountains where studied populations have five unique inversions. It is most closely related to cytoforms 'A' and 'B' of *P. rachiliense*, the three taxa sharing one unique inversion. The two cytoforms of *P. rachiliense* are separated by about 1200 km, obscuring decisions about their reproductive isolation. The names *rachiliense* and possibly *pronevitschae* Rubtsov apply to cytoform 'A' in Armenia, whereas the name *fulvipes* (Edwards) might apply to cytoform 'B' in Turkey and to material formerly identified in Turkey as *P. rufipes*. Populations of *P. tomosvaryi* in Armenia are chromosomally distinct from previously studied populations in Europe and Morocco, although tied to European and Turkish populations by a shared X-linked inversion. We conservatively view Armenian, European, and Turkish populations of *P. tomosvaryi* as a single, polymorphic species. A new species, chromosomally related to *P. hirtipes* (Fries) and *P. tomosvaryi* by two uniquely shared inversions, was discovered in Turkey. (C) 2014 The Linnean Society of London

Cytogenetics of the Holarctic black fly *Simulium noelleri* (Diptera : Simuliidae)

Adler, PH; Kachvorian, EA. 2001

The polytene chromosomes of *Simulium noelleri* from 16 sites in Armenia, Canada, England, Germany, Russia, and Sweden were analyzed. A standard map is proposed for the *S. noelleri* species-group that is 11 fixed inversions removed from the original standard map of the subgenus *Simulium*. Based on one shared subterminal inversion in the III L arm, the *S. noelleri* species-group is in a trichotomy with the *S. bezzi* and *S. ornatum* species-groups. Reproductive isolation of *S. noelleri* and *S. decorum* is demonstrated chromosomally at sites where both occur together. All analyzed populations of *S. noelleri* are considered conservatively to represent a single species, although at least three cytotypes are recognized, based primarily on different sex-chromosome systems. Cytotype A, representing the type species of *S. noelleri*, occurs in Germany, England, and western Russia. Cytotype B occupies Sweden and western Canada, emphasizing the intimate connection between the northern Nearctic and Palearctic simuliid faunas. Cytotype C is known only from Armenia.

Evolution of pre-zygotic and post-zygotic barriers to gene flow among three cryptic species within the *Anastrepha fraterculus* complex

Rull, J; Abraham, S; Kovaleski, A; Segura, DF; Mendoza, M; Liendo, MC; Vera, MT. 2013

Tropical tephritids are ideally suited for studies on population divergence and speciation because they include species groups undergoing rapid radiation, in which morphologically cryptic species and sister species are abundant. The *fraterculus* species group in the Neotropical genus *Anastrepha* is a case in point, as it is composed of a complex of up to seven *A. fraterculus* morphotypes proposed to be cryptic species. Here, we document pre- and post-zygotic barriers to gene flow among adults of the Mexican *A. fraterculus* morphotype and three populations (Argentina, Brazil, and Peru) belonging to two separate morphotypes (Brazilian 1 and Peruvian). We unveiled three forms of pre-zygotic reproductive isolation resulting in strong assortative mating. In field cages, free-ranging male and female *A. fraterculus* displayed a strong tendency to form couples with members of the opposite sex belonging to their own morphotype, suggesting that male pheromone emission, courtship displays, or both intervene in shaping female choice before actual contact and coupling. In addition, males and females of the Peruvian morphotype became receptive and mated significantly later than adults of the Mexican and Brazilian 1 morphotypes. After contact, Mexican females exhibited greater mating discrimination than males when facing adults of the opposite sex belonging to either the Peruvian or the Brazilian 1 morphotype as evidenced by vigorous resistance to penetration once they had been forcefully mounted by heterotypic males. Forced copulations resulted in production of F1 **hybrids** that were either less viable (and partially fertile) than parental crosses or even sterile. Our results suggest that the Mexican morphotype is a distinct biological entity and that pre-zygotic reproductive isolation through divergence in courtship or male-produced pheromone and other mechanisms appear to evolve faster than post-zygotic isolation in the *fraterculus* species group.

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

Pike, N; Meats, A. 2002

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

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

Estrada, AP; Prieto, R. 2011

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

An integrative multidisciplinary approach to understanding cryptic divergence in Brazilian species of the *Anastrepha fraterculus* complex (Diptera: Tephritidae)

Dias, VS; Silva, JG; Lima, KM; Petitinga, CSCD; Hernandez-Ortiz, V; Laumann, RA; Paranhos, BJ; Uramoto, K; Zucchi, RA; Joachim-Bravo, IS. 2016

An integrative multidisciplinary approach was used to delimit boundaries among cryptic species within the *Anastrepha fraterculus* complex in Brazil. Sexual compatibility, courtship and sexual acoustic behaviour, female morphometric variability, variation for the mitochondrial gene COI, and the presence of *Wolbachia* were compared among *A. fraterculus* populations from the Southern (Vacaria, Pelotas, Bento Goncalves, SAo Joaquim) and Southeastern (Piracicaba) regions of Brazil. Our results suggest full mating compatibility among *A. fraterculus* populations from the Southern region and partial pre-zygotic reproductive isolation of these populations when compared with the population from the Southeastern region. *A. fraterculus* populations from both regions differed in the frequency of courtship displays and aspects of the calling phase and mounting acoustic signal. Morphometric analysis showed differences between Southern region and Southeastern region samples. All populations analyzed were infected with *Wolbachia*. The trees generated from the COI sequencing data are broadly congruent with the behavioural and morphometric data with the exception of one Southern population. The likely mechanisms by which *A. fraterculus* populations might have diverged are discussed in detail based on behavioural, morphometric, molecular genetics, and biogeographical studies.

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.

Population cytogenetic evidence for sibling species in *Anopheles annularis* (Diptera : Culicidae)

Atrie, B; Subbarao, SK; Pillai, MKK; Rao, SRV; Sharma, VP. 1999

Anopheles annularis van der Wulp populations from 6 districts in India were cytologically examined. An ovarian nurse cell polytene chromosome map for 5 arms is

presented. Nine inversions-w,i(1),j(1), and k(1) on chromosome arm 2; j(1) and z in arm 3; h(1) and s(1) on arm 4; and k on arm 5-were polymorphic in these populations. In districts Shahjahanpur and Ghaziabad populations, for j(1) inversion on arm 2, there were no heterozygotes. This was taken as evidence for reproductive isolation between 2 populations. The 2 were provisionally designated as species A and B, characterized respectively by +j(1) and j(1) arrangements in chromosome arm 2. The other 4 populations were identified as species A.

INCIPIENT SEXUAL ISOLATION IN DROSOPHILA-PSEUDOOBSCURA BOGOTANA AYALA AND DOBZHANSKY (DIPTERA, DROSOPHILIDAE)

NOOR, MA. 1995

Incipient sexual isolation was found between the subspecies *Drosophila pseudoobscura bogotana* (Ayala and Dobzhansky) and *D. p. pseudoobscura* (Frolova). Males took longer to initiate courtship with heterosubspecifics than consubspecifics. This divergence could represent an early step in the speciation of these taxa. The subspecies' pheromones do not appear to have diverged, so the difference is attributed to female behavior.

Evolution of male genitalia in the *Drosophila repleta* species group (Diptera: Drosophilidae)

Stefanini, MI; Gottschalk, MS; Calvo, NS; Soto, IM. 2021

The *Drosophila repleta* group comprises more than one hundred species that inhabit several environments in the Neotropics and use different hosts as rearing and feeding resources. Rather homogeneous in their external morphology, they are generally distinguished by the male genitalia, seemingly their fastest evolving morphological trait, constituting an excellent model to study patterns of genital evolution in the context of a continental adaptive radiation. Although much is known about the evolution of animal genitalia at population level, surveys on macroevolutionary scale of this phenomenon are scarce. This study used a suite of phylogenetic comparative methods to elucidate the macroevolutionary patterns of genital evolution through deep time and large continental scales. Our results indicate that male genital size and some aspects of shape have been evolving by speciation evolution, probably due to the microevolutionary processes involved in species mate recognition. In contrast, several features of the aedeagus shape seemed to have evolved in a gradual fashion, with heterogeneous evolutionary phenotypic rates among clades. In general, the tempo of the evolution of aedeagus morphology was constant from the origin of the group until the Pliocene, when it accelerated in some clades that diversified mainly in this period. The incidence of novel ecological conditions in the tempo of aedeagus evolution and the relationship between species mate recognition and speciation in the *Drosophila repleta* group are discussed.

Evolution of phenologically distinct populations of *Rhagoletis mendax* (Diptera : Tephritidae) in highbush blueberry fields

Teixeira, LAF; Polavarapu, S. 2003

Populations of *Rhagoletis mendax* with novel late flight period, in August-September, have been found in two commercial highbush blueberry fields in New Jersey, thriving in fruit left behind after the harvest of a late blueberry cultivar. A small number of flies with similar late flight period have also been captured in several locations where blueberry maggot infests wild hosts. Using allozyme markers, we found that allele frequencies in wild blueberry maggot populations are constant from June to September, and distinct from those found in populations infesting the two commercial fields. The populations in the commercial fields present a pattern of decreased genetic diversity, including lower heterozygosity and distortions in allele frequency distribution. These data suggest that a founder effect, the loss of genetic diversity caused by the establishment of a small sample of individuals as a newly isolated entity, is responsible for the genetic differences between the populations in the commercial fields and in wild areas. The founder effect most likely occurred because only a small proportion of the flies present in wild areas possesses the late phenology required for the colonization of the commercial fields. The seasonal asynchrony between the populations in commercial fields and those in wild areas may also have contributed to maintain the founder effect by limiting gene flow. This study suggests that early in a phenological transition, if the onset of prezygotic reproductive isolation is relatively fast, evolution at the genetic level might be caused mostly by genetic drift. This study further confirms that the flight period is plastic in the blueberry maggot.

Pleiotropic effect, clock genes, and reproductive isolation

Miyatake, T. 2002

The mechanism by which a clock gene pleiotropically controlling life history and behavioral traits causes reproductive isolation is explained using a model species, the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Melon flies mate once a day, at dusk. The population selected for life history traits exhibits correlated responses in the time of mating during the day. For example, the fly populations selected for faster (slower) development have an earlier (later) time of mating. A circadian rhythm controls the time of mating. The circadian periods in constant darkness were about 22h in lines selected for a short developmental period and about 31 h in lines selected for a long developmental period. The data on crosses between the selected lines indicated that the developmental period is controlled by a polygene, whereas the circadian period may be controlled by a single clock gene. These results suggest a clock gene pleiotropically controls developmental and circadian periods in the melon fly. Reproductive isolation may often evolve as an indirect (pleiotropic) consequence of adaptation to different environments or habitats. For example, niches that are temporally or seasonally offset can select organisms with different developmental characteristics. These developmental differences can inadvertently cause reproductive isolation by a variety of means including shifts in mating activity patterns. The difference in time of mating between populations selected for developmental period translated into significant prezygotic isolation, as measured by mate choice tests. If the mating time between populations differed more than 1 h, the isolation index was significantly higher than zero. These findings indicate that premating isolation can be established by a pleiotropic effect of a clock gene. There are many examples in which the difference in timing of reproduction prevents gene flow between populations, such as the egg spawning time in marine organisms, the flowering time in angiosperms, and the time of mating in insects. In, such, organisms; if genetic correlations between circadian rhythm and reproductive traits exist, multifarious divergent selection for life history traits would often accelerate the evolution of reproductive isolation through clock genes. Natural populations may diverge in reproduction time through drift, direct natural selection for time of reproduction, or as a by-product effect of genetic correlations. In any case, clock genes are keys in reproductive isolation.

High-Elevation Chromosomal Diversity of Black Flies (Diptera: Simuliidae) in Thailand

Adler, PH; Srisuka, W; Van Lun, LL; Takaoka, H; Saeung, A. 2019

The highest elevations of tropical mountains, much like oceanic islands, often support disproportionately high levels of endemism, some of which is concealed as cryptic species. In a search for additional species, we explored the chromosomal diversity of three morphologically defined species of black flies known only from the upper elevations of Doi Inthanon, Thailand's highest mountain. Analysis of the polytene chromosomes facilitated the discovery of the previously unknown larva of *Simulium kiewmaepanense* Takaoka & Srisuka, permitted linkage of the formal name of *S. undecimum* Takaoka, Srisuka & Saeung with its previous cytological identity, and revealed cryptic species, designated A and B, within *S. tenebrosus* Takaoka, Srisuka & Atiporn. A probable new species of microsporidium was discovered in the larval fat body of *S. kiewmaepanense*, suggesting that at least some symbiotic organisms of endemic hosts are also restricted to Doi Inthanon. A mitochondrial DNA analysis did not distinguish the two cryptic species of *S. tenebrosus* despite complete reproductive isolation chromosomally inferred from an absence of **hybrids**, emphasizing the need for integrated approaches to species problems. The chromosomes of all four analyzed species were monomorphic, or

nearly so, perhaps reflecting the suppression of polymorphic inversions that otherwise would restrict recombination needed to generate adequate variation in the peripheral environment of the mountain summit.

Geographic patterns of postzygotic isolation between two closely related widespread dung fly species (*Sepsis cynipsea* and *Sepsis neocynipsea*; Diptera: Sepsidae)

Giesen, A; Schafer, MA; Blanckenhorn, WU. 2019

Identifying the contribution of pre- and postzygotic barriers to gene flow is a key goal of speciation research. The widespread dung fly species *Sepsis cynipsea* and *Sepsis neocynipsea* offer great potential for studying the speciation process over a range of opportunities for gene exchange within and across sister species (cross-continental allopatry, continental parapatry and sympatry). We examined the role of postcopulatory isolating barriers by comparing female fecundity and egg-to-adult viability of F-1 and F-2 **hybrids**, as well as backcrosses of F-1 **hybrids** with the parental species, via replicated crosses of sym-, para- and allopatric populations. Egg-to-adult viability was strongly but not totally suppressed in **hybrids**, and offspring production approached nil in the F-2 generation (**hybrid** breakdown), indicating yet unspecified intrinsic incompatibilities. Viable F-1 **hybrid** offspring showed almost absolute male (the heterogametic sex) sterility while females remained largely fertile, in accordance with Haldane's rule. **Hybridization** between the two species in European areas of sympatry (Swiss Alps) indicated only minor reinforcement based on fecundity traits. Crossing geographically isolated European and North American *S. neocynipsea* showed similar albeit weaker isolating barriers that are most easily explained by random genetic drift. We conclude that in this system with a biogeographic continuum of reproductive barriers, speciation is mediated primarily by genetic drift following dispersal of flies over a wide (allopatric) geographic range, with some role of natural or sexual selection in incidental or direct reinforcement of incompatibility mechanisms in areas of European sympatry. *S.*(*sub*)*species* status of continental *S. neocynipsea* appears warranted.

Recursive adaptation in action: allochronic isolation and divergence of host-associated populations of the apple maggot fly, *Rhagoletis pomonella*, following its recent introduction to the western USA

Mattsson, M; Hood, GR; Yee, WL; Doellman, MM; Bruzese, DJ; Goughnour, RB; Driscoll, AL; Van Dexter, S; Tait, C; Glover, MM; Meyers, P; Ruedas, LA; Feder, JL. 2022

An outstanding issue in the study of insect host races concerns the idea of 'recursive adaptive divergence', whereby adaptation can occur repeatedly across space and/or time, and the most recent adaptive episode is defined by one or more previously similar cases. The host plant shift of the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae, Carpomyini), from ancestral downy hawthorn [*Crataegus mollis* (Torr. & A. Gray) Scheele] to introduced, domesticated apple (*Malus domestica* Borkh.) in the eastern USA has long served as a model system for investigating ecologically driven host race formation in phytophagous insect specialists. Here, we report results from an annual geography survey of eclosion time demonstrating a similar ecological pattern among nascent host-associated populations of the fly recently introduced ca. 40 years ago from its native range in the east into the Pacific Northwest (PNW) region of the USA. Specifically, using data collected from 25 locations across 5 years, we show that apple-infesting fly populations in the PNW have rapidly and repeatedly shifted (and maintained differences in) their adult eclosion life-history timing to infest two novel hawthorn hosts with different fruiting phenologies - a native species (*Crataegus douglasii* Lindl.) and an introduced species (*Crataegus monogyna* Jacq.) - generating partial allochronic reproductive isolation in the process. The shifts in the PNW parallel the classic case of host race formation in the eastern USA, but have occurred bi-directionally to two hawthorn species with phenologies slightly earlier (black hawthorn) and significantly later (ornamental hawthorn) than apple. Our results imply that *R. pomonella* can both possess and retain extensive-standing variation (i.e., 'adaptive memory') in diapause traits, even following introductions, to rapidly and temporally track novel phenological host opportunities when they arise. Thus, 'specialized' host races may not constitute evolutionary dead ends. Rather, adaptive phenotypic and genetic memory may carry over from one host shift to the next, recursively facilitating host race formation in phytophagous insects.

REPRODUCTIVE ISOLATION BETWEEN *UROPHORA-AFFINIS* AND *UROPHORA-QUADRIFASCIATA* (DIPTERA, TEPHRTIDAE) IN BRITISH-COLUMBIA

BERUBE, DE; MYERS, JH. 1983

Black flies (Diptera: Simuliidae) of east-central Saskatchewan, with description of a new species and implications for pest management

Adler, PH; Mason, PG. 1997

A 5-year study of the black flies of east-central Saskatchewan revealed 21 species, including *Simulium incognitum* sp.nov. Chromosomal and ecological evidence for reproductive isolation is presented for this new species, formerly known as *S. venustum* Say CC4, and *S. venustum* Say CC. All 21 species in the study are associated with productive streams and rivers. As many as nine of these species might comprise the pest assemblage harassing livestock. The major pest, *S. luggeri* Nicholson and Mickel, is cytologically distinct from populations in the eastern United States on the basis of a two-step, Y-linked inversion in the IIS chromosomal arm. *Simulium luggeri* is the only livestock pest that breeds almost solely in large streams and rivers. The other eight probable pests breed entirely or partly in streams less than 10 m wide, often below beaver dams, suggesting that management efforts should specifically target these sites.

Molecular identification of two *Culex* (Culex) species of the neotropical region (Diptera: Culicidae)

Laurito, M; Ayala, AM; Almiron, WR; Gardenal, CN. 2017

Culex bidens and *C. interfor*, implicated in arbovirus transmission in Argentina, are sister species, only distinguishable by feature of the male genitalia; however, intermediate specimens of the species in sympatry have been found. Fourth-instar larvae and females of both species share apomorphic features, and this lack of clear distinction creates problems for specific identification. Geometric morphometric traits of these life stages also do not distinguish the species. The aim of the present study was to assess the taxonomic status of *C. bidens* and *C. interfor* using two mitochondrial genes and to determine the degree of their reproductive isolation using microsatellite loci. Sequences of the ND4 and COI genes were concatenated in a matrix of 993 nucleotides and used for phylogenetic and distance analyses. Bayesian and maximum parsimony inferences showed a well resolved and supported topology, enclosing sequences of individuals of *C. bidens* (0.83 BPP, 73 BSV) and *C. interfor* (0.98 BPP, 97 BSV) in a strong sister relationship. The mean K2P distance within *C. bidens* and *C. interfor* was 0.3% and 0.2%, respectively, and the interspecific variation was 2.3%. Bayesian clustering also showed two distinct mitochondrial lineages. All sequenced mosquitoes were successfully identified in accordance with the best close match algorithm. The low genetic distance values obtained indicate that the species diverged quite recently. Most morphologically intermediate specimens of *C. bidens* from Co R doba were heterozygous for the microsatellite locus GT51; the significant heterozygote excess observed suggests incomplete reproductive isolation. However, *C. bidens* and *C. interfor* should be considered good species: the ventral arm of the phallosome of the male genitalia and the ND4 and COI sequences are diagnostic characters.

Effects of Female fruit-marking Pheromones on Oviposition, Mating, and Male Behavior in the Neotropical Species *Rhagoletis conversa* Brethes and *Rhagoletis brncici* Frias (Diptera: Tephritidae)

Frias-Lasserre, D. 2015

Sex pheromones produced by females of *Rhagoletis conversa* Br., and *Rhagoletis brncici* Frias are deposited on the surface of fruits after the eggs are laid. These pheromones repel other females, preventing repeated oviposition on the same fruit. They also attract males, thus assisting mating. Observations were made on wild populations, and cross-species behavioral tests were performed on males. The pheromone released by females was significantly more attractive for males of the same species. The two species showed remarkable differences in mating behavior, in the duration of oviposition, and in the number of circuits made around the fruit after eggs were laid. A morphological analysis of flies collected from their respective host plants indicated great host fidelity and the reproductive isolation of the two species. Possibly, the specific male-arresting effect of this pheromone was important for the sympatric speciation and evolution of these sibling species.

Genetic and phenotypic differentiation suggests incipient speciation within *Drosophila arizonae* (Diptera: Drosophilidae)

Rampasso, AS; Markow, TA; Richmond, MP. 2017

The cactophilic sister species *Drosophila arizonae* and *Drosophila mojavensis*, distributed across southwestern USA, Mexico and Guatemala, constitute a well-suited model to study ecology, genetic divergence and speciation. Although *D. arizonae* is the more broadly distributed of the two, its evolutionary biology has been less intensively investigated than *D. mojavensis*. Given the important role of genitalic variation in reproductive isolation, we explored the relationship between male aedeagus morphology and genetic differentiation of geographically distinct strains of *D. arizonae*. We used mitochondrial cytochrome c oxidase subunit I sequence data to establish the phylogenetic relationships among different *D. arizonae* strains and compared morphological variation, using elliptic Fourier descriptors, among five populations. Our results indicate that the Tuxtla Gutierrez, Chiapas, population may be at the early stages of lineage divergence. Tuxtla Gutierrez was the only locality sampled south of the Trans-Mexican Volcanic Belt and the Isthmus of Tehuantepec, both of which have been implicated as gene flow barriers in other organisms. Our results suggest that *D. arizonae* is an emerging model system for studies of incipient speciation.

On the Relationship between *Zaprionus spinipilus* Chassagnard & McEvey and *Z. vittiger* Coquillett, the Type Species of the Genus *Zaprionus* (Diptera: Drosophilidae)

Yassin, A; Amabis, JM; Da Lage, JL; Debiais-Thibaud, M; David, JR. 2010

Zaprionus vittiger Coquillett is the type species of the genus *Zaprionus* Coquillett. However, the species is only known from five old museum specimens collected from South Africa and Malawi. It has often been confused with many other *Zaprionus* species, especially with *Z. spinipilus* Chassagnard & McEvey, a widespread species in Africa known from Madagascar, Malawi, Ethiopia and Cameroon. We have recently collected flies from the type localities of both species (South Africa and Madagascar, respectively). This has prompted us to test the taxonomic boundaries of these two nominal species using molecular (the mitochondrial COII and the nuclear Amyrel genes), chromosomal, morphological (internal and external genitalia), and reproductive isolation analyses. The results suggest *Z. spinipilus* to be a junior synonym to *Z. vittiger*.

The effects of host race, gender, and host plant distribution on alighting behavior, mating, and oviposition in *Eurosta solidaginis*

Horner, JD; Craig, TP; Itami, JK. 2008

Eurosta solidaginis Fitch (Diptera: Tephritidae) has formed host races on *Solidago altissima* L. and *Solidago gigantea* Ait. (Asteraceae), and reproductive isolation between these host races is brought about in part by host-associated assortative mating. Any non-assortative mating creates the potential for gene flow between the populations, and we investigated the conditions that favored non-assortative mating. We hypothesized that the frequency of non-assortative mating would be influenced by differences in the behaviors of the host races and sexes and by the presence and pattern of distribution of the two host species. To test these hypotheses, we caged flies on four combinations of 32 potted host plants: all *S. altissima*, all *S. gigantea*, and cages with both host species arranged in either two pure species blocks or randomly dispersed. We recorded the number of flies of each host race that alighted on each host species and the frequency of mating within and between the host races. Males of both host races were observed on plants more frequently than females. Flies of the host race from *S. gigantea* (gig flies) were observed on plants in greater absolute numbers, and they mated more frequently than flies of the host race from *S. altissima* (alt flies). In all treatments, gig flies of both sexes were found on non-natal host plants significantly more frequently than alt flies, and gig females showed a weaker preference for their host species than did gig males or alt flies of either gender for their respective natal hosts. Assortative mating predominated in all treatments, and flies from each host race mated more frequently in cages containing their own host plant. The frequency of non-assortative mating varied among treatments, with the matings between alt female x gig male being more common in the pure *S. altissima* treatment and the gig female x alt male being more frequent in the pure *S. gigantea* and random treatments. Matings between gig male x alt female were more common overall than the reciprocal mating, because gig males were more active in pursuing matings and in alighting on the non-natal host plant than alt flies. Non-assortative matings were more frequent in the random than in the block treatments, but this difference was not significant. Because of strong selection against oviposition into the alternate host, we hypothesized that host plant distribution would not affect oviposition preference. We tested this hypothesis by examining the oviposition behavior of naive, mated females in two treatments in which both host species were present: either arranged in blocks or randomly dispersed. Females oviposited only into their natal host, regardless of host plant distribution.

Species delimitation in the *Drosophila aldrichi* subcluster (Diptera : Drosophilidae) using DNA sequences

Oliveira, DCSG; Leonidas, M; Etges, WJ; O'Grady, PM; Desalle, R. 2008

DNA sequence data has been successfully used to verify current species-level taxonomic hypotheses based on morphology and other characters. Setting species boundaries in the *Drosophila* repleta group has been challenging because this group contains several cryptic taxa and morphologically polymorphic populations. Mitochondrial (cox1 and nad2) and nuclear (sina and Marf) genes were employed to assess species limits for two traditionally recognized, closely related, and taxonomically problematical species, *D. aldrichi* and *D. wheeleri*. Both tree-based and character-based methods were used to show that *D. wheeleri* is indeed a distinct species; however, our data shows that *D. aldrichi* is a paraphyletic assemblage of two lineages as previously suggested based on patterns of reproductive isolation. One lineage is sister to *D. wheeleri* and includes populations originating from southern and western Mexico (western-aldrichi). The second, basal group also contains flies from southern Mexico, along with populations from the northern and eastern regions within the species boundaries traditionally described as *D. aldrichi* (eastern-aldrichi). The populations of *D. aldrichi* that were introduced into Australia were found to be included in the eastern-aldrichi group. Our results, particularly those based on the rapidly evolving mtDNA sequences, confirm the presence of at least two cryptic species previously referred to as "D. aldrichi".

ETHOLOGICAL MECHANISMS OF REPRODUCTIVE ISOLATION IN CULEX-PIPIENS AND AEDES-MARIAE COMPLEXES (DIPTERA, CULICIDAE)

BULLINI, L; COLUZZI, M. 1980

Specificity of accessory gland extracts in three *Delia* fly species (Diptera: Anthomyiidae)

Spencer, JL; Candolfi, MP; Keller, JE; Miller, JR. 1997

Extracts of testes and male accessory (paragonial) glands made from three species of *Delia* (onion fly (*D. antiqua*), seedcorn fly (*D. platura*), and cabbage fly (*D. radicum*)) were injected into conspecific virgin females. Extracts of paragonial glands, but not testes, from onion, seedcorn and cabbage fly males stimulated oviposition and suppressed mating when injected into conspecific virgin females. When extracts of paragonial glands from males of these species were injected into heterospecific virgin females, the extracts of *D. antiqua* and *D. platura* were fully cross-reactive with respect to oviposition; interspecific injection stimulated oviposition at the level of the conspecific mated controls. Injection of *D. radicum* extract fully activated the *D. antiqua* and *D. platura* ovipositional response. *D. antiqua* extract caused mating inhibition and partial oviposition in *D. radicum*; that of *D. platura* had no effect on either oviposition or mating inhibition in *D. radicum*. These results suggest that *D. antiqua* and *D. platura* are more closely related to one another than either is to *D. radicum*, and agree with published anatomically-based phylogenies and a genetic distance calculation based on eight enzyme loci. The occurrence of sex peptide cross-reactivity, though asymmetrical, between *D. radicum* versus *D. antiqua* and *D. platura* indicates that, functionally, sex peptides have changed little during the evolution of this genus. An emerging pattern of broad cross-reactivity within genera suggests that sex peptides are not an initiator of reproductive isolation.

GENETIC DIVERGENCE ALONG THE SPECIATION CONTINUUM: THE TRANSITION FROM HOST RACE TO SPECIES IN RHAGOLETIS (DIPTERA: TEPHRTIDAE)

Powell, THQ; Hood, GR; Murphy, MO; Heilveil, JS; Berlocher, SH; Nosil, P; Feder, JL. 2013

Studies of related populations varying in their degrees of reproductive isolation can provide insights into speciation. Here, the transition from partially isolated host races to more fully separated sibling species is investigated by comparing patterns of genetic differentiation between recently evolved (approximate to 150 generations) apple and ancestral hawthorn-infesting populations of *Rhagoletis pomonella* to their sister taxon, the undescribed flowering dogwood fly attacking *Cornus florida*. No fixed or diagnostic private alleles differentiating the three populations were found at any of 23 microsatellites and 10 allozymes scored. Nevertheless, allele frequency differences were sufficient across loci for flowering dogwood fly populations from multiple localities to form a diagnosable genotypic cluster distinct from apple and hawthorn flies, indicative of species status. Genome-wide patterns of differentiation were correlated between the host races and species pair comparisons along the majority of chromosomes, suggesting that similar disruptive selection pressures affect most loci. However, differentiation was more pronounced, with some additional regions showing elevated divergence, for the species pair comparison. Our results imply that *Rhagoletis* sibling species such as the flowering dogwood fly represent host races writ large, with the transition to species status primarily resulting from increased divergence of the same regions separating apple and hawthorn flies.

Isolation and characterization of microsatellite loci from the apple maggot fly *Rhagoletis pomonella* (Diptera : Tephritidae)

Velez, S; Taylor, MS; Noor, MAF; Lobo, NF; Feder, JL. 2006

We report the isolation and development of 81 novel primers for amplifying microsatellite loci in the *Rhagoletis pomonella* sibling species complex, and the sequencing, characterization and analysis of basic population genetic parameters for nine of these genes. We also report the successful cross-species amplification of several of these loci. The *R. pomonella* sibling species complex is a textbook example of genetic differentiation in sympatry via host-plant shifting. Microsatellite markers can be useful for mapping host-plant-associated adaptations in *Rhagoletis* that generate reproductive isolation and facilitate speciation, as well as for resolving the genetic structure and evolutionary history of fly populations.

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

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

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

Combining morphology and molecular data to improve *Drosophila paulistorum* (Diptera, Drosophilidae) taxonomic status

Zanini, R; Muller, MJ; Vieira, GC; Valiati, VH; Depra, M; Valente, VLD. 2018

The willistoni species subgroup has been the subject of several studies since the latter half of the past century and is considered a Neotropical model for evolutionary studies, given the many levels of reproductive isolation and different evolutionary stages occurring within them. Here we present for the first time a phylogenetic reconstruction combining morphological characters and molecular data obtained from 8 gene fragments (COI, COII, Cytb, Adh, Ddc, Hb, kl-3 and per). Some relationships were incongruent when comparing morphological and molecular data. Also, morphological data presented some unresolved polytomies, which could reflect the very recent divergence of the subgroup. The total evidence phylogenetic reconstruction presented well-supported relationships and summarized the results of all analyses. The diversification of the willistoni subgroup began about 7.3 Ma with the split of *D. insularis* while *D. paulistorum* complex has a much more recent diversification history, which began about 2.1 Ma and apparently has not completed the speciation process, since the average time to sister species separation is one million years, and some entities of the *D. paulistorum* complex diverge between 0.3 and 1 Ma. Based on the obtained data, we propose the categorization of the former semispecies of *D. paulistorum* as a subspecies and describe the subspecies *D. paulistorum amazonian*, *D. paulistorum andeanbrazilian*, *D. paulistorum centroamerican*, *D. paulistorum interior*, *D. paulistorum orinocan* and *D. paulistorum transitional*.

Morphological comparison among *Drosophila lini* and its two new sibling species (Diptera : Drosophilidae)

Zannat, MT; Toda, MJ. 2002

Morphological differences are investigated using several culture strains of three sibling species collected from Taiwan and Guangdong in China and Pyinoolwin and Yangon in Myanmar. Careful examination of male terminalia reveals distinguishable differences in the paramere and the aedeagal basal process among the three species. In addition, a number of quantitative characters are compared. Kruskal-Wallis tests with Bonferroni correction, which are carried out separately for each sex,

detect significant differences in 15 characters, of which two are male-specific, among the three species. Canonical discriminant analysis using these characters reveals that the three species can be distinguished from each other with high confidence for both sexes. The results clearly show the presence of three good species, *Drosophila* (Sophophora) *lini* Bock & Wheeler, 1972 and its two new siblings. The new species are described as *Drosophila* (Sophophora) *ohnishii* sp. nov. from Pyinoolwin and *Drosophila* (Sophophora) *ogumai* sp. nov. from Yangon. The morphological differentiation among the three sibling species does not coincide with the degree of reproductive isolation (based on a previous study). The premating isolation pattern suggests two possibilities that premating isolation has been evolved or reinforced in sympatric populations between *D. ohnishii* and *D. lini* and between *D. ohnishii* and *D. ogumai* or that it has evolved in a very restricted local population of *D. ohnishii*, possibly by a few mutations.

Taxonomy and phenotypic relationships of the *Anastrepha fraterculus* complex in the Mesoamerican and Pacific Neotropical dominions (Diptera, Tephritidae)

Hernandez-Ortiz, V; Canal, NA; Salas, JOT; Ruiz-Hurtado, FM; Dzul-Cauich, JF. 2015

Previous morphometric studies based on linear measurements of female structures of the aculeus, mesonotum, and wing revealed the existence of seven morphotypes within the *Anastrepha fraterculus* cryptic species complex along the Neotropical Region. The current research followed linear and geometric morphometric approaches in 40 population samples of the nominal species *A. fraterculus* (Wiedemann) spread throughout the Meso-American and Pacific Neotropical dominions (including Mexico, Central America, Venezuela, Colombia, Ecuador, and Peru). The goals were to explore the phenotypic relationships of the morphotypes in these biogeographical areas; evaluate the reliability of procedures used for delimitation of morphotypes; and describe their current distribution. Findings determined that morphotypes previously recognized via the linear morphometrics were also supported by geometric morphometrics of the wing shape. In addition, we found an eighth morphotype inhabiting the highlands of Ecuador and Peru. Morphotypes are related into three natural phenotypic groups nominated as Mesoamerican-Caribbean lineage, Andean lineage, and Brazilian lineage. The hypothesis that lineages are not directly related to each other is discussed, supported by their large morphological divergence and endemism in these three well-defined biogeographic areas. In addition, this hypothesis of the non-monophyly of the *A. fraterculus* complex is also supported by evidence from other authors based on molecular studies and the strong reproductive isolation between morphs from different lineages.

An experimental assessment of reproductive isolation and its consequences for seasonal hybridization dynamics

Foucault, Q; Wieser, A; Heumann-Kiesler, C; Diogo, J; Cocchiara, B; Nowak, C; Waldvogel, AM; Pfenninger, M. 2019

While the spatial aspect of **hybridization** has been investigated in depth, the temporal aspect has not. We analysed the seasonal **hybridization** dynamics of a multivoltine non-biting midge sister species pair, *Chironomus riparius* and *C. piger*. We investigated the fertility and fitness of F-1 **hybrids**, and all possible first-generation backcross **hybrids**, under different temperature conditions. Based on microsatellite markers, we then inferred the presence of different **hybrid** classes in seasonal field samples (spring and autumn) from a site of co-occurrence over two consecutive years. All experimental **hybrids** showed reduced fertility albeit without a specific temperature effect. Fitness was zero in one F-1 direction, but not reduced in the other. No sex ratio distortion was observed. However, fitness was reduced in all backcrosses, indicating that **hybridization** should be rare in the field. In the seasonal samples, *C. piger* was found only in autumn individuals. All 359 genotyped individuals were attributed to either of the pure species. However, several individuals of the autumn samples carried signs of later generation introgression, indicating that actual **hybridization** must take place recurrently during summer. We thus present evidence that seasonal environmental changes can drive **hybridization** dynamics and that long-term recurrent **hybridization** need not affect species integrity.

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

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

The *Drosophila willistoni* subgroup represents a complex with varying taxonomic levels. It encompasses *D. willistoni* and its five sibling species: *D. equinoxialis*, *D. insularis*, *D. paulistorum*, *D. pavlovskiana* and *D. tropicalis*. Of these, *D. equinoxialis*, *D. tropicalis* and *D. willistoni* present differentiation at subspecific level, whereas *D. paulistorum* represents a superspecies, formed by six semispecies. Despite this taxonomic and evolutionary complexity, many of these semi and subspecific taxa have not yet had their phylogenetic status tested in an explicitly molecular study. Aiming to contribute to the understanding of the evolution of this challenging group, we analyzed nucleotide sequences from two mitochondrial and four nuclear datasets, both individually and simultaneously, through different phylogenetic methods. High levels of incongruence were detected among partitions, especially concerning the mitochondrial sequences. As this incongruence was found to be statistically significant and robust to the use of different models and approaches, and basically restricted to mitochondrial loci, we suggest that it may stem mainly from **hybridization**-mediated asymmetrical introgression. Despite this, our nuclear data finally led to a phylogenetic hypothesis which further refines several aspects related to the *willistoni* subgroup phylogeny. In this respect, *D. insularis*, *D. tropicalis*, *D. willistoni* and *D. equinoxialis* successively branched off from the *willistoni* subgroup main stem, which recently subdivided to produce *D. paulistorum* and *D. pavlovskiana*. As regards the semispecies evolution, we found evidence of a recent diversification, which highly influenced the obtained results due to the associated small levels of genetic differentiation, further worsened by the possibly associated incompletely sorted ancestral polymorphisms and by the possibility of introgression. This study also raises the question of whether these semispecies are monophyletic at all. This reasoning is particularly interesting when one considers that similar levels of reproductive isolation could be attained through infection with different *Wolbachia* strains.

Reproductive compatibility among Mexican populations of *Anastrepha obliqua*: theoretical and management implications

Rull, J; Tadeo, E; Lasa, R; Diaz-Fleischer, F; Arredondo, J; Aluja, M. 2018

The *fraterculus* species group, composed of 34 species in the genus *Anastrepha* (Diptera: Tephritidae), includes the *fraterculus* cryptic species complex formed by eight reproductively isolated morphotypes. A previous study revealed six genetic mitochondrial types of *Anastrepha obliqua*, suggesting the existence of a second cryptic species complex. However, marked discrepancies between nuclear and mitochondrial loci rather suggest incomplete lineage sorting or introgression between *A. obliqua* and *A. fraterculus*. Such **hybridization** could nevertheless result in reproductive isolation, an outcome that could affect efficacy of area-wide management for the most important pest of mangos in America. Two mitochondrial types occur in Mexico, and the limits of a third one, encompassing Central American populations, have not been clearly established. Here, we tested reproductive compatibility among three *A. obliqua* populations from the Pacific and a population from the Gulf of Mexico. No evidence of pre-zygotic isolation was found. Flies from the Atlantic mated randomly for equal duration with individuals from three Pacific populations. Homotypic and heterotypic crosses produced similar numbers of eggs, with heterotypic crosses of Pacific males and Atlantic females hatching in lower proportions. Larvae of all cross types developed equally in mangos and exhibited no sex ratio distortion of **hybrid** F1. The three mitochondrial types identified in Mexico and Central America do not appear to be cryptic species and can be managed using one single strain for the sterile insect technique.

Experimental hybridization and reproductive isolation between two sympatric species of tephritid fruit flies in the *Anastrepha fraterculus* species group

Rull, J; Tadeo, E; Lasa, R; Rodriguez, CL; Altuzar-Molina, A; Aluja, M. 2018

Among tephritid fruit flies, **hybridization** has been found to produce local adaptation and speciation, and in the case of pest species, induce behavioral and ecological

alterations that can adversely impact efficient pest management. The *fraterculus* species group within *Anastrepha* (Diptera: Tephritidae), is a rapidly radiating aggregate, which includes cryptic species complexes, numerous sister species, and several pest species. Molecular studies have highlighted the possibility of introgression between *A. fraterculus* and *A. obliqua*. Reproductive isolation has been studied among morphotypes of the *A. fraterculus* species complex as a tool for species delimitation. Here we examined the existence and strength of prezygotic and postzygotic isolation between sympatric populations of two closely related species within the highly derived *fraterculus* group (*A. fraterculus* and *A. obliqua*), coexisting in nature. Although adults of both species showed a strong tendency for assortative mating, a small proportion of **hybrid** pairings in both directions were observed. We also observed asymmetric postzygotic isolation, with one **hybrid** cross displaying a strong reduction in fecundity and F1 egg fertility. Survival was greater for the progeny of homotypic and **hybrid** crosses in the maternal host. There was a marked female biased sex ratio distortion for both F1 **hybrid** adults. **Hybridization** between *A. fraterculus* and *A. obliqua* in nature may be difficult but possible; these two species display stronger reproductive isolation than all pairs of species previously examined in the *A. fraterculus* species complex. Asymmetric postzygotic isolation is suggestive of Wolbachia mediated cytoplasmic incompatibilities that may be exploited in area-wide pest management.

Evidence for sexual isolation as a prezygotic barrier to gene flow between morphologically divergent species of *Rhagoletis* fruit flies

Hood, GR; Egan, SP; Feder, JL. 2012

1. Certain groups of fruit flies in the genus *Rhagoletis* (Diptera: Tephritidae) are exemplars for sympatric speciation via host plant shifting. Flies in these species groups are morphologically similar and overlap in their geographic ranges, yet attack different, non-overlapping sets of host plants. Ecological adaptations related to differences in host choice and preference have been shown to be important prezygotic barriers to gene flow between these taxa, as *Rhagoletis* flies mate on or near the fruit of their respective host plants. Non-host-related assortative mating is generally absent or present at low levels between these sympatrically diverging fly populations. 2. However, some *Rhagoletis* taxa occasionally migrate to non-natal plants that are the primary hosts of other, morphologically differentiated fly species in the genus. These observations raise the question of whether sexual isolation may reduce courtship and copulation between morphologically divergent species of *Rhagoletis* flies, contributing to their prezygotic isolation along with host-specific mating. 3. Using reciprocal multiple-choice mating trials, we measured sexual isolation among nine species pairs of morphologically differentiated *Rhagoletis* flies. Complete sexual isolation was observed in eight of the nine comparisons, while partial sexual isolation was observed in the remaining comparison. 4. We conclude that sexual isolation can be an effective prezygotic barrier to gene flow contributing to substantial reproductive isolation between many morphologically distinct *Rhagoletis* species, even in the absence of differential host plant choice and host-associated mating.

First record of *Culex* (*Culex*) *bidens* (Diptera: Culicidae) in Colombia: Taxonomic and epidemiological implications

Laurito, M; Hoyos-Lopez, R. 2018

Arbovirus transmission cycles must be studied locally since both vectors and hosts vary in different regions. Colombia has a highly diverse mosquito fauna. *Culex* (*Culex*) *bidens* is reported here for the first time in Colombia. Because *Cx. bidens* Dyar and Knab and *Cx. declarator* Dyar and Knab share a close taxonomic history and because it is difficult to differentiate between them, a morphological and molecular comparison was performed. The male genitalia of three specimens of *Cx. bidens* from Colombia were mounted on microscope slides and morphologically compared with the male genitalia of *Cx. declarator* also from Colombia. In *Cx. bidens*, the individual teeth of the lateral plate are long, straight, laterally directed and sharply pointed; in *Cx. declarator* these teeth are robust, curved, with convex margins and bluntly rounded. Moreover, DNA was extracted from the same specimens and a fragment of the cytochrome c oxidase subunit I mitochondrial gene was amplified and sequenced. Neither *Cx. bidens* nor *Cx. declarator* were clustered in the Neighbour-joining topology, with K2P interspecific divergence between 0.15-1.45%. The circulation of Eastern Equine Encephalitis Virus in Colombia was reported since 1957 and *Cx. bidens* was suspected to be the vector of this virus during an epizootic in Argentina in 1988. **Hybridization** between species of the subgenus *Culex* has been demonstrated, hence the degree of reproductive isolation between *Cx. bidens* and *Cx. declarator* should be investigated, as well as their taxonomic status, because they only can be discriminated by a single male genitalic feature and not by nuclear or mitochondrial markers.

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

Yassin, A. 2008

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

Integrative taxonomy and a new species description in the *sturtevanti* subgroup of the *Drosophila saltans* group (Diptera: Drosophilidae)

Madi-Ravazzi, L; Segala, LF; Roman, BE; Alevi, KCC; Prediger, C; Yassin, A; Hua-Van, A; Miller, WJ. 2021

Although the biological concept of species is well established in animals, sometimes the decision about the specific status of a new species is difficult and hence requires support of an integrative analysis of several character sets. To date, the species *Drosophila sturtevanti*, *D. magalhaesi*, *D. milleri* and *D. dacunhai*, belonging to the *sturtevanti* subgroup of the Neotropical *saltans* species group, are identified mainly by the aedeagus morphology, but also present some differences in spot coloration and patterning of the female sixth tergite and in the shape and size of the spermathecae, parallel to a pattern of reproductive isolation. In the present study, we describe a novel *saltans* group species from French Guiana belonging to the *sturtevanti* subgroup. Our species designation is based on an integrative approach covering (i) aedeagi and spermathecae morphology by scanning electron microscopy, (ii) analysis of female sixth-tergite color, (iii) morphometrical analysis of aedeagi and wings, (iv) analysis of partial sequence of the COI, COII and ND4 mitochondrial genes as well as (v) intercrosses for analysis of reproductive isolation. The comparative analysis of the results on these markers with those of *D. sturtevanti*, *D. milleri* and *D. dacunhai* supports that this line belongs to a new species of the *sturtevanti* subgroup that we name *Drosophila lehrmanae* sp. nov. in honor of Prof. Lee Ehrman 's 85th birthday.

Courtship in Two Morphotypes of the *Anastrepha fraterculus* (Diptera: Tephritidae) Cryptic Species Complex and their Implications for Understanding Mate Recognition

Roriz, AKP; Japyassu, HF; Joachim-Bravo, IS. 2018

Ritualized courtship behaviors are used to recognize potential mates and behavioral patterns are inevitably different among populations that demonstrate reproductive incompatibility. We characterized and compared the courtship behaviors of two morphotypes of the cryptic species complex *Anastrepha fraterculus*: Brazil-1 morphotype and Brazil-3 morphotype. Courtship behaviors were filmed to analyze the behavioral sequences of these two morphotypes during homotypic crossings. The behavioral units Alignment (AL) and Abdominal movements (AB and AB-call) were newly recognized in the courtship ethogram of *Anastrepha fraterculus* males. The two morphotypes show distinct behavioral sequences leading up to copulation. Some behaviors were repeated frequently during the courtship process, while others were more restricted to the final moments of courtship. The three behavioral units that contributed most to copulation success were Contact, Alignment, and Arrowhead 1 in the Brazil-1 morphotype and Alignment, Arrowhead 1, and Fanning in the Brazil-3 morphotype. Some behavioral routines differed across the two morphotypes. Significant differences were also noted between the frequencies of the behavioral units displayed during courtship in the two morphotypes. The relationships between the pre-zygotic incompatibilities of the Brazil-1 and Brazil-3 morphotypes and the differences between the courtship behaviors of their males are discussed. Our results indicate that behavioral isolation is involved in the process of pre-zygotic reproductive isolation of Brazil-1 and Brazil-3 morphotypes.

MODIFICATION OF FEMALE ONION FLY, *DELIA-ANTIQUA* (MEIGEN), REPRODUCTIVE-BEHAVIOR BY MALE PARAGONIAL GLAND EXTRACTS (DIPTERA, ANTHOMYIIDAE)

SPENCER, JL; BUSH, GL; KELLER, JE; MILLER, JR. 1992

Egg depositional rates of onion flies, *Delia antiqua* (Meigen), injected thoracically with extracts of male paragonial glands were identical (14.5 eggs/female/day) to those of normally mated females. Moreover, when continuously exposed to males, extract-injected females refused to mate and produced unfertilized eggs for the duration of the > 15-day experiment. For this normally monocoitic dipteran, < 1 male equiv of paragonial secretion completely reproduced the ovipositional responses characteristic of normal mating, and this effect required no involvement of the genitalia or genital chamber. We suggest that the receptor for the active chemical(s) (sex peptide ?) would be an excellent target for biorational insect control by sterilization. Moreover, these primer sex pheromones might play an important role in insect reproductive isolation and evolution.

Host-associated differences in emergence pattern, reproductive behavior and life history of *Asteralobia sasakii* (Monzen) (Diptera : Cecidomyiidae) between populations on *Ilex crenata* and *I. integra* (Aquifoliaceae)

Tabuchi, K; Amano, H. 2003

We assessed potential adult longevity and male choice for a mate in two populations of *Asteralobia sasakii* associated with *Ilex crenata* and *I. integra* to test the hypothesis that interbreeding between these populations is prevented by the difference in emergence timing in the field. A previous study showed that the last day of adult emergence on *I. integra* and the first day of adult emergence on *I. crenata* were separated by six to 11 days. When adults were maintained in the laboratory with restricted flying, mating and oviposition, adults from both populations survived for four to five days, suggesting that the period when adults were active did not overlap between the two populations in the field. The mate choice test showed that males clearly approached females of the same host origin. These results suggest that the two populations have little chance of interbreeding even when they occur sympatrically. Other life history parameters such as the number of eggs in the ovary, daily emergence pattern, sex ratio and the duration of copulation also varied between the two populations. Our overall results strongly suggest that the two populations on different host plants can be referred to as distinct species.

Biology and adaptive radiation in the gall-inducing Cecidomyiidae (Insecta Diptera) and Calophyidae (Insecta Hemiptera) on *Mangifera indica* (Anacardiaceae) in the Indian subcontinent

Raman, A; Burckhardt, D; Harris, KM. 2009

Out of the nearly 250 species of plant-feeding arthropods that utilize *Mangifera indica*, about 25 gall midge species (Diptera Cecidomyiidae) (mostly species of *Procontarinia* Kieffer & Cecconi 1906) and one psyllid species (*Apsylla cistellata* (Buckton 1893), Hemiptera Calophyidae) are the known gall-inducing taxa. Almost all of these gall midges induce galls on leaves, whereas the psyllid induces galls on axillary vegetative buds. The *M. indica*-associated species of *Procontarinia* do not show any striking level of shifts either between organs within *M. indica* or between other Indian anacardiaceous taxa such as *Anacardium*, *Buchanania*, *Lannea*, *Holigarna*, *Pistacia*, and *Semecarpus*, which, in fact, host several other species of Cecidomyiidae and Calophyidae. Given that galls induced by a suspected gall-midge species on the fossil leaves of an ancestral taxon of *Mangifera indica* from the Upper Palaeocene sediments of north-eastern India exist, the lack of radiation from leaves either to other organs of *M. indica* or to other anacardiaceous extant taxa suggests that neither the feeding behaviour nor the larval salivary physiology of species of *Procontarinia* has changed over time. The reason for such a conservative behaviour could be either the lack of resistance-breaking genes in the *M. indica*-associated *Procontarinia* complex or the abundance of populations of *M. indica*. A provisional reconstruction of the relationships among the extant *M. indica*-infesting gall-inducing Cecidomyiidae reveals that an extremely modest level of radiation in the leaf-gall-inducing species has occurred from those species that induce structurally simple galls to those that induce structurally complex galls. Because *M. indica* is an evergreen, polyaxial species, with new leaf flushes available almost throughout the year, and thus offering a continuous nutritional supply, the radiation and diversification of gall midges could have been restricted. In addition, the diverse, geographically localized varieties of *M. indica* with different types of secondary chemicals (e.g., xanthonenes and flavanols) may have also imposed a selection pressure on the radiation of gall midges; for instance, mangiferin, the principal secondary chemical present in *M. indica*, possibly acts as an attractant to the Cecidomyiidae and *Apsylla cistellata* and has, consequently, restricted their diversification. We speculate that the gall midges may have progressed conservatively specializing along the host-plant lines, because of the need for precise timing and for minimizing competitive interactions with other parasitic, predatory, and inquiline arthropods, from the time of original host colonization. Available data on the gall-inducing species of the *Procontarinia* complex on the leaves of *M. indica* indicates that both the location and structure of galls generally agree with the patterns evident in other groups of gall-inducing insects, which induce galls of similar morphology, suggesting pathways of *Procontarinia* phylogeny. Galls of different morphologies occur concurrently, mostly on the leaves of *M. indica*, and such an occurrence needs to be explored to establish the origin of multiple species of *Procontarinia*. In the context of *M. indica*-associated gall midges, only *Procontarinia mangiferae* lives both on stems and leaves of *M. indica*, indicating a critical step in *Procontarinia* speciation, through reproductive isolation involving phenological separation. Different species of *Procontarinia* occur on the same host tree and on the same leaf, but how different populations of gall-midge taxa partition their resources effectively and reduce inter-specific competition remain as questions. Effective utilization of the host plant and consequent speciation in the *Procontarinia* complex have resulted from the condition that continuously blossoming individual trees of *M. indica* are available throughout extensive landscapes. But apart from these speculative propositions, a need for empirical studies on the phylogeny and interactions between *Procontarinia* (and related genera) and *M. indica* exists. In Calophyidae, *Apsylla* Crawford 1912 did not speciate on *M. indica*; this may also be true for the three species of Calophyidae Lbw 1879 associated with *M. indica*. Due to insufficient information this proposition remains unverified.

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 pluriseta, nigriseta and, especially, athletica 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.

Distribution, host plant affiliation, phenology, and phylogeny of walnut-infesting *Rhagoletis* flies (Diptera: Tephritidae) in Mexico

Rull, J; Aluja, M; Tadeo, E; Guillen, L; Egan, S; Glover, M; Feder, JL. 2013

Many taxa of Nearctic origin have diversified in the subtropical highlands of Mexico. In particular, flies in the genus *Rhagoletis* have undergone episodes of isolation and gene flow during Pleistocene glaciations and post-glacial times that have produced lineage differentiation and reproductive isolation. To reach a better understanding of the phylogeography of the genus *Rhagoletis*, a host plant survey of the walnut-infesting *Rhagoletis* *suavis* species group was conducted across sixteen states comprising 34 different collecting sites in Mexico over a 9-year period. Five species of *Juglans* were found to be infested by three species of walnut-attacking *Rhagoletis* flies. Several species of parasitoids were also recovered from collections, but in contrast to their walnut fly hosts, they revealed little evidence for host or geographic subdivision. There was no consistent difference in mean eclosion time between walnut fly species or populations associated with different host walnuts in Mexico, unlike the case for other *Rhagoletis* species, in which allochronic isolation arising from variation in diapause timing is a major ecological adaptation, reproductively isolating flies. We compare the distribution of *R. suavis* flies in Mexico with those of other *Rhagoletis* species attacking hawthorns and cherries, and discuss its implications for population divergence and speciation.(c) 2013 The Linnean Society of London.

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

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

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

Gene and species trees reveal mitochondrial and nuclear discordance in the *Drosophila cardini* group (Diptera: Drosophilidae)

De Re, FC; Loreto, ELS; Robe, LJ. 2010

The *Drosophila cardini* group includes 15 species, which are subdivided into the *D. cardini* and *D. dunni* subgroups. Although many phylogenetic hypotheses have been proposed for this group during the last five decades (based on patterns of reproductive isolation, morphology of male genitalia, chromosomal inversions, isozyme variation, or molecular sequence data), these are mostly discordant with each other. We aimed to clarify some of the evolutionary patterns related to the origin of this incongruence, while also attempting to provide a better-supported phylogenetic hypothesis for the *D. cardini* subgroup. For this purpose, sequences from three mitochondrial and three nuclear loci were gathered for at least eight species, and both individual gene trees and joint species tree estimates were evaluated. Although there was concordance among gene trees within each of the nuclear and mitochondrial sets, considerable incongruence was revealed in the comparisons between these two data sets. The branching position of *D. neocardini* was the main source of incongruence, and species trees reconstructed using different approaches with and without this species were particularly incongruent. In addition to providing a better approximation of the evolutionary history of the *D. cardini* group, this study suggests that incomplete lineage sorting or introgression may be biasing previous species tree estimates. More generally, the results also suggest that the use of supermatrix methods can lead to an overestimation of support for the inferred relationships, and highlight the potential effects of different taxon sampling strategies in phylogenetic reconstruction.

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

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

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

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

Pike, N. 2004

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

Characterisation of the chemical profiles of Brazilian and Andean morphotypes belonging to the *Anastrepha fraterculus* complex (Diptera, Tephritidae)

Vanickova, L; Brizova, R; Pompeiano, A; Ferreira, LL; de Aquino, NC; Tavares, RD; Rodriguez, LD; Mendonca, AD; Canal, NA; do Nascimento, RR. 2015

Fruit fly sexual behaviour is directly influenced by chemical and non-chemical cues that play important roles in reproductive isolation. The chemical profiles of pheromones and cuticular hydrocarbons (CHs) of eight fruit fly populations of the Andean, Brazilian-1 and Brazilian-3 morphotypes of the *Anastrepha fraterculus* cryptic species complex originating from Colombia (four populations) and Brazil (four populations) were analysed using two-dimensional gas chromatography with mass spectrometric detection. The resulting chemical diversity data were studied using principal component analyses. Andean morphotypes could be discriminated from the Brazilian-1 and Brazilian-3 morphotypes by means of male-borne pheromones and/or male and female CH profiles. The Brazilian-1 and Brazilian-3 morphotypes were found to be monophyletic. The use of chemical profiles as species- and sex-specific signatures for cryptic species separations is discussed.

Gene flow and genetic structure of *Bactrocera carambolae* (Diptera, Tephritidae) among geographical differences and sister species, *B. dorsalis*, inferred from microsatellite DNA data

Aketarawong, N; Isasawin, S; Sojikul, P; Thanaphum, S. 2015

The Carambola fruit fly, *Bactrocera carambolae*, is an invasive pest in Southeast Asia. It has been introduced into areas in South America such as Suriname and Brazil. *Bactrocera carambolae* belongs to the *B. dorsalis* species complex, and seems to be separated from *B. dorsalis* based on morphological and multilocus phylogenetic studies. Even though the Carambola fruit fly is an important quarantine species and has an impact on international trade, knowledge of the molecular ecology of *B. carambolae*, concerning species status and pest management aspects, is lacking. Seven populations sampled from the known geographical areas of *B. carambolae* including Southeast Asia (i.e., Indonesia, Malaysia, Thailand) and South America (i.e., Suriname), were genotyped using eight microsatellite DNA markers. Genetic variation, genetic structure, and genetic network among populations illustrated that the Suriname samples were genetically differentiated from Southeast Asian populations. The genetic network revealed that samples from West Sumatra (Pekanbaru, PK) and Java (Jakarta, JK) were presumably the source populations of *B. carambolae* in Suriname, which was congruent with human migration records between the two continents. Additionally, three populations of *B. dorsalis* were included to better understand the species boundary. The genetic structure between the two species was significantly separated and approximately 11% of total individuals were detected as admixed ($0.100 \leq Q \leq 0.900$). The genetic network showed connections between *B. carambolae* and *B. dorsalis* groups throughout Depok (DP), JK, and Nakhon Sri Thammarat (NT) populations. These data supported the hypothesis that the reproductive isolation between the two species may be leaky. Although the morphology and monophyly of nuclear and mitochondrial DNA sequences in previous studies showed discrete entities, the hypothesis of semipermeable boundaries may not be rejected. Alleles at microsatellite loci could be introgressed rather than other nuclear and mitochondrial DNA. *Bactrocera carambolae* may be an incipient rather than a distinct species of *B. dorsalis*. Regarding the pest management aspect, the genetic sexing Salaya5 strain (SY5) was included for comparison with wild populations. The SY5 strain was genetically assigned to the *B. carambolae* cluster. Likewise, the genetic network showed that the strain shared greatest genetic similarity to JK, suggesting that SY5 did not divert away from its original genetic makeup. Under laboratory conditions, at least 12 generations apart, selection did not strongly affect genetic compatibility between the strain and wild populations. This knowledge further confirms the potential utilization of the Salaya5 strain in regional programs of area-wide integrated pest management using SIT.

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

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

Seventeen isolines of *Anopheles barbirostris* derived from animal-biting female mosquitoes showed three karyotypic forms: Form A (X-2, Y-1) in five isolines from Phetchaburi province; Form B (X-1, X-3, Y-2) in three and eight isolines from Chiang Mai and Ubon Ratchathani provinces, respectively; Form C (X-2, Y-3) in one isolate from Phetchaburi province. All 17 isolines exhibited an average branch summation of seta 2-VI pupal skins ranging from 12.1-13.0 branches, which was in the limit of *A. barbirostris* (6-18 branches). Of the 12 human-biting isolines from Chiang Mai province, five isolines showed Form B (X-2, Y-2), and seven isolines exhibited a new karyotypic form designated as Form E (X-2, Y-5). All of 12 isolines had an average branch summation of seta 2-VI pupal skins ranging from 22.4-24.5 branches, which was in the limit of *Anopheles campestris* (17-58 branches). Thus, they were tentatively designated as *A. campestris*-like Forms B and E. Hybridization between *A. campestris*-like Forms B and E showed that they were genetically compatible, yielding viable progeny for several generations suggesting conspecific relationships of these two karyotypic forms. Reproductive isolation among crosses between *A. campestris*-like Form B and *A. barbirostris* Forms A, B, and C strongly suggested the existence of these two species. In addition, the very low intraspecific variation (genetic distance < 0.005) of the nucleotide sequence of ITS2 of the rDNA and COI and COII of mitochondrial DNA of the seven isolines of *A. campestris*-like Forms B and E supported their conspecific relationship. The large sequence divergence of ITS2 (0.203-0.268), COI (0.026-0.032), and COII (0.030-0.038) from genomic DNA of *A. campestris*-like Forms B and E and the *A. barbirostris* Forms A, B, and C clearly supported cytogenetic and morphological evidence.

Analysis of the activity patterns of two sympatric sandfly siblings of the *Lutzomyia longipalpis* species complex from Brazil

Rivas, GBS; Souza, NA; Peixoto, AA. 2008

Lutzomyia longipalpis s.l. (Lutz & Neiva) (Diptera: Psychodidae) is the main vector of visceral leishmaniasis in Latin America. Differences in copulation songs, pheromones and molecular markers show that *L. longipalpis* is a species complex in Brazil. The patterns of activity of insect vectors are important in disease transmission. In addition, differences in activity rhythms have a potential role as a temporal reproductive isolation mechanism in closely related species. We compared the activity patterns of males and females of two sympatric species of the *Longipalpis* complex from Sobral (Ceara State, Brazil) in controlled laboratory conditions. We observed small but significant differences between the two species in the activity phase in both males and females.

Population cytogenetic and molecular evidence for existence of a new species in *Anopheles fluviatilis* complex (Diptera: Culicidae)

Nanda, N; Singh, OP; Dua, VK; Pandey, AC; Nagpal, BN; Adak, T; Dash, AP; Subbarao, SK. 2013

Anopheles fluviatilis James, an important malaria vector in the Oriental region has been established as a complex of at least three cryptic species which vary in their biological characteristics and malaria transmission potential. The sibling species S, T and U of *Fluviatilis* Complex can be identified by examination of species-specific fixed inversions in the polytene chromosomes and can also be differentiated by an allele-specific PCR assay based on differences in the D3 region of 28S ribosomal DNA (rDNA) of these species. Here we report a new *An. fluviatilis* population from villages under Laksar Community Health Centre, District Haridwar (Uttarakhand state), India which differs from the three sibling species of *Fluviatilis* Complex by two fixed paracentric inversions, s(1) and S in polytene chromosome arms 2 and 3 respectively. Longitudinal study carried out in study villages showed that the new cytotype was sympatric with species T and U in all the collections and no inversion heterozygotes were observed between them. Thus presence of two fixed paracentric inversions in polytene chromosomes with total absence of inversion heterozygotes demonstrates reproductive isolation which unequivocally establishes this cytological variant as a new species, provisionally designated as species V in the *Fluviatilis* Complex. Analysis of DNA sequences of 133 domain of 28S rDNA and ITS 2 region has also shown that species V is distinctly different from species S, T and U. With the discovery of new species in the *Fluviatilis* Complex, in-depth studies are required to know its distribution pattern and biological characteristics and to ascertain its role in malaria transmission. (C) 2012 Elsevier B.V. All rights reserved.

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

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

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

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

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

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

Behavioural barriers to reproduction may evolve faster than sexual morphology among populations of a dung fly (Sepsidae)

Puniamoorthy, N. 2014

Reproductive traits often evolve rapidly, and some suggest that behavioural traits, in particular, can diverge faster than morphology, resulting in sexual isolation between populations/species. An earlier study of a Neotropical dung fly, *Archiseptis diversiformis* (Diptera: Sepsidae), reported anecdotally that two central American populations that were approximately 500 km apart (Costa Rica and Panama) differed in male courtship behaviour despite being morphologically similar. Here, I present results of an in-depth study designed (1) to test whether these two populations show qualitative and/or quantitative differences in mating behaviour and morphology, (2) to test whether individuals from either population show some degree of reproductive isolation and (3) to characterize population variation in a particularly fast evolving mitochondrial gene fragment, cytochrome oxidase c subunit I (COI), to estimate genetic differences between the two populations. Despite similarities in overall courtship, I identified behaviours that were clearly population specific, and report that both populations showed strong premating isolation in one-on-one crosses. However, after extended exposure in mass-container group crosses, individuals did produce adult F1 offspring, suggesting that isolation is incomplete. Surprisingly, morphometric analyses indicated that these two populations differed significantly in sexually monomorphic adult wing shape but differed only moderately in sexually dimorphic male forefemur shape, and not at all in male genital clasper shape. Finally, both populations were genetically similar, forming a single, monophyletic cluster with low uncorrected pairwise distances (COI threshold < 1 %), suggesting that marked behavioural differences between both populations have evolved quickly but without complete isolation. (C) 2014 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

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

Schwarz, D; McPherson, BA. 2007

Question: Environmental disturbance can disrupt habitat choice as an ecological barrier to **hybridization** between host-specific parasites that mate on their host. Is environment-independent mate choice a sufficient barrier to prevent **hybridization** when ecological isolation breaks down? Hypothesis: Males and females will not discriminate between conspecific and heterospecific mating partners in the absence of host cues. Study system: *Rhagoletis mendax* and *R. zephyria* (Diptera: Tephritidae). **Hybridization** between these two taxa resulted in the Lonicera fly, an example of **hybrid** speciation in animals. The Lonicera fly is found only on non-native honeysuckle. *Rhagoletis mendax* and *R. zephyria* discriminate against each other's host but not honeysuckle. This suggests the local breakdown of reproductive isolation via host choice following the introduction of an invasive plant. Methods: We combined males and females of both species in a multi-choice experiment in the laboratory and recorded mating events. Conclusion: Without host plant cues, mate choice is an incomplete barrier to **hybridization**. Reproductive isolation between host-specific parasites can be influenced by environmental disturbance because a non-ecological barrier (mate choice) alone is too weak to maintain reproductive isolation.

Rapid and repeatable shifts in life-history timing of *Rhagoletis pomonella* (Diptera: Tephritidae) following colonization of novel host plants in the Pacific Northwestern United States

Mattsson, M; Hood, GR; Feder, JL; Ruedas, LA. 2015

Host shifts of phytophagous insect specialists to novel plants can result in divergent ecological adaptation, generating reproductive isolation and potentially new species. *Rhagoletis pomonella* fruit flies in eastern North America underwent a host shift similar to 160 ya from native downy hawthorn (*Crataegus mollis*) to introduced, domesticated apple (*Malus domestica*). Divergent selection on diapause phenology related to the earlier fruiting time of apples versus downy hawthorns resulted in partial allochronic reproductive isolation between the fly races. Here, we test for how rapid and repeatable shifts in life-history timing are driving ecological divergence of *R. pomonella* in the Pacific Northwestern USA. The fly was introduced into the region via larval-infested apples 40-65 ya and now attacks native black hawthorn (*Crataegus douglasii*) and introduced ornamental hawthorn (*Crataegus monogyna*), in addition to early- and late-maturing apple varieties in the region. To investigate the life-history timing hypothesis, we used a field-based experiment to characterize the host-associated eclosion and flight activity patterns of adults, and the feeding times of larvae at a field site in Vancouver, Washington. We also assessed the degree to which differences in host-fruiting time generate allochronic isolation among apple-, black hawthorn-, and ornamental hawthorn-associated fly populations. We conclude that host-associated fly populations are temporally offset 24.4% to 92.6% in their seasonal distributions. Our results imply that *R. pomonella* possesses the capacity for rapid and repeatable shifts in diapause life history to match host-fruiting phenology, which can generate ecologically based reproductive isolation, and potentially biodiversity in the process.

SIMULIIDAE) IN ECUADOR

CHARALAMBOUS, M; READY, PD; SHELLEY, AJ; ARZUBE, M; LOWRY, CA. 1993

Four cytotypes of *Simulium exiguum* occur in Ecuador, where this morphospecies is the primary vector of onchocerciasis. In this paper, we give the first full description of the banding pattern of the larval polytene chromosomes of the Quevedo cytotype and assess its degree of genetic separation from the Bucay cytotype. Both cytotypes differ from the chromosomal standard sequence (of the Cayapa cytotype) by the fixed inversions IIL-5 and IIL-6. The Quevedo cytotype additionally differs from the standard and Bucay cytotypes by possessing a differentiated X chromosome, which is indicated by the inversion IIS-A. As the degree of reproductive isolation between the Bucay and Quevedo cytotypes has not yet been established, they must be regarded as intraspecific variants of the same species. In fact, isoenzyme characterizations showed that the Bucay and Quevedo cytotypes are differentiated only to the extent expected of incipient species or geographical populations. Moreover, the sibling species status previously given to the Bucay cytotype needs to be reassessed, there being inadequate analysis from areas in Ecuador where Bucay occurs in sympatry with the standard Cayapa cytotype. No isoenzyme electromorphs were discovered that identified all or most adult females of any one (cytotype-pure) collection.

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

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

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

Spatial swarm segregation and reproductive isolation between the molecular forms of *Anopheles gambiae*

Diabate, A; Dao, A; Yaro, AS; Adamou, A; Gonzalez, R; Manoukis, NC; Traore, SF; Gwadz, RW; Lehmann, T. 2009

Anopheles gambiae, the major malaria vector in Africa, can be divided into two subgroups based on genetic and ecological criteria. These two subgroups, termed the M and S molecular forms, are believed to be incipient species. Although they display differences in the ecological niches they occupy in the field, they are often sympatric and readily **hybridize** in the laboratory to produce viable and fertile offspring. Evidence for assortative mating in the field was recently reported, but the underlying mechanisms awaited discovery. We studied swarming behaviour of the molecular forms and investigated the role of swarm segregation in mediating assortative mating. Molecular identification of 1145 males collected from 68 swarms in Donéguebouyou, Mali, over 2 years revealed a strict pattern of spatial segregation, resulting in almost exclusively monotypic swarms with respect to molecular form. We found evidence of clustering of swarms composed of individuals of a single molecular form within the village. Tethered M and S females were introduced into natural swarms of the M form to verify the existence of possible mate recognition operating within-swarm. Both M and S females were inseminated regardless of their form under these conditions, suggesting no within-mate recognition. We argue that our results provide evidence that swarm spatial segregation strongly contributes to reproductive isolation between the molecular forms in Mali. However this does not exclude the possibility of additional mate recognition operating across the range distribution of the forms. We discuss the importance of spatial segregation in the context of possible geographic variation in mechanisms of reproductive isolation.

Asymmetric reproductive isolation and interference in neriid flies: the roles of genital morphology and behaviour

Bath, E; Tatarnic, N; Bonduriansky, R. 2012

The processes underlying reproductive isolation, and the traits involved, are the subject of considerable debate in evolutionary biology. Studying the costly sexual interaction of species in secondary sympatry, a phenomenon known as reproductive interference, can help to shed light on past and present isolating mechanisms, as well as the implications of sympatry for individual fitness. We investigated the roles of two sets of traits, genitalic and behavioural, in reproductive isolation and interference in two species of Australian neriid flies, *Telostylinus lineolatus* and *T. angusticollis*. Surprisingly, although these species differ markedly in male but not in female genitalia, we found evidence that genital morphology resulted in asymmetric reproductive isolation: *T. lineolatus* males could transfer sperm to *T. angusticollis* females, but *T. angusticollis* males were unable to transfer sperm to *T. lineolatus* females. However, neither type of cross produced any viable **hybrids**. Behavioural responses also contributed asymmetrically to both reproductive isolation and reproductive interference. Males pursued both conspecific and heterospecific females. Females of both species discriminated against heterospecific males, but *T. lineolatus* females exhibited stronger discrimination than *T. angusticollis* females. Curiously, *T. angusticollis* males both fought and attempted to copulate with *T. lineolatus* males, resulting in reduced mating success for *T. angusticollis* males with conspecific females. Our findings show that both genitalic and behavioural traits can play important roles in reproductive isolation, but the consequences of interspecific divergence in these traits can be surprisingly complex, resulting in asymmetric effects on many aspects of interspecific interactions. (C) 2012 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Cryptic Species of the *Anastrepha fraterculus* Complex (Diptera: Tephritidae): A Multivariate Approach for the Recognition of South American Morphotypes

Hernandez-Ortiz, V; Bartolucci, AF; Morales-Valles, P; Frias, D; Selivon, D. 2012

Although a large amount of data have been published in past years on the taxonomic status of the *Anastrepha fraterculus* (Wiedemann) species complex, there is still a need to know how many species this complex comprises, the distribution of each one, and their distinguishing features. In this study, we assessed the morphometric variability of 32 populations from the *A. fraterculus* complex, located in major biogeographical areas from the Neotropics. Multivariate techniques for analysis were applied to the measurements of 21 variables referring to the mesonotum, aculeus, and wing. For the first time, our results identified the presence of seven distinct morphotypes within this species complex. According to the biogeographical areas, populations occurring in the Mesoamerican dominion (Mexico, Guatemala, and Panama) were clustered within a single natural entity labeled as the "Mexican" morphotype; whereas in the northwestern South American dominion, samples fell into three distinct groups: the "Venezuelan" morphotype with a single population from the Caribbean lowlands of Venezuela, the "Andean" morphotype from the highlands of Venezuela and Colombia, and the third group or "Peruvian" morphotype comprised the samples from the Pacific coastal lowlands of Ecuador and Peru. Three additional groups were identified from the Chacoan and Paranaense sub-regions: the morphotype "Brazilian-1" was recognized as including the Argentinean samples with most pertaining to Brazil, and widely distributed in these biogeographical areas; the morphotype "Brazilian-2" was recognized as including two samples from the state of Sao Paulo (Ilha-Bela and Sao Sebastiao); whereas the morphotype "Brazilian-3" included a single population from Botucatu (state of Sao Paulo). Based on data published by previous authors showing genetic and karyotypic differentiation, as well as reproductive isolation, we have concluded that

such morphotypes indeed represent natural groups and distinct taxonomic entities.

INTRASPECIFIC AND INTERSPECIFIC SEX-PHEROMONE RESPONSES OF SCREWWORM AND SECONDARY SCREWWORM FLIES

HAMMACK, L. 1992

A laboratory behavioral assay examined intra- and interspecific responses to sex pheromone by screwworms, *Cochliomyia hominivorax*, and secondary screwworms, *C. macellaria*, in relation to the duration of colonization of *C. hominivorax* test males. Females of *C. macellaria*, like those of *C. hominivorax*, were found to produce a pheromone that stimulates male copulatory attempts on contact. Newly colonized (< 22 generations) *C. hominivorax* males did not respond to *C. macellaria* pheromone, indicating that pheromone contributes to reproductive isolation between these two closely related species. Although long-colonized (> 200 generations) *C. hominivorax* males did respond to *C. macellaria* females or their extract, this behavior was infrequent and significantly less common than intraspecific responses. Depriving *C. macellaria* adults of dietary protein did not affect the potency of female extracts, but did reduce male responsiveness to pheromone. These results provided little evidence that colonization reduces the ability of *C. hominivorax* males to differentiate between *C. hominivorax* and *C. macellaria* females using sex pheromones.

Cryptic species of black flies (Diptera: Simuliidae) at high elevations in the Oriental Region: The *Simulium vernum* species group in Thailand

Adler, PH; Srisuka, W; Saeung, A. 2020

High-mountain summits in tropical regions are habitat islands that provide refugia for northern taxa and opportunities for speciation. These focal hotspots of biodiversity are also threatened by increasing anthropogenic pressures and changing climatic conditions. The largest species group of black flies, the *Simulium vernum* group, reaches its southern limit in the mountains of Southeast Asia, providing an opportunity to examine macrogenetic diversification at the southern edge of the group's range. We investigated the polytene chromosomes of two morphologically similar species in the *S. vernum* group, *S. chomthongense* Takaoka, Srisuka & Choochote, and *S. khunklangense* Takaoka & Srisuka, from their type localities near the summit of Doi Inthanon, Thailand's highest mountain. Chromosomal analyses of 112 larvae demonstrated reproductive isolation between *S. chomthongense* and *S. khunklangense* and revealed a cryptic species of *S. chomthongense*. The three species have the same fixed banding sequence in five of the six chromosome arms, but they are distinguished by the successive addition of overlapping inversions in the IIIIL arm. The two cytospecies (A and B) of *S. chomthongense* s. l. are the third example of endemic or nearly endemic cryptic species pairs near the top of Doi Inthanon, suggesting the possibility of sympatric speciation and the importance of conserving high-elevation stream habitats.

High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* group species

Civetta, A; Singh, RS. 1995

The possible association between gonadal protein divergence and postzygotic reproductive isolation was investigated among species of the *Drosophila melanogaster* and *D. virilis* groups. Protein divergence was scored by high-resolution two-dimensional electrophoresis (2DE). Close to 500 protein spots from gonadal tissues (testis and ovary) and nongonadal tissues (malpighian tubules and brain) were analyzed and protein divergence was calculated based on presence vs absence. Both testis and ovary proteins showed higher divergence than nongonadal proteins, and also a highly significant positive correlation with postzygotic reproductive isolation but a weaker correlation with prezygotic reproductive isolation. Particularly, a positive and significant correlation was found between proteins expressed in the testis and postzygotic reproductive isolation among closely related species such as the within-phylad species in the *D. virilis* group. The high levels of male-reproductive-tract protein divergence between species might be associated with F-1 **hybrid** male sterility among closely related species. If so, a lower level of ovary protein divergence should be expected on the basis that F-1 female **hybrids** are fully fertile. However, this is not necessarily true if relatively few genes are responsible for the reproductive isolation observed between closely related species, as recent studies seem to suggest. We suggest that the faster rate of evolution of gonadal proteins in comparison to nongonadal proteins and the association of that rate with postzygotic reproductive isolation may be the result of episodic and/or sexual selection on male and female molecular traits.

Evidence of Adaptive Evolution and Relaxed Constraints in Sex-Biased Genes of South American and West Indies Fruit Flies (Diptera: Tephritidae)

Congrains, C; Campanini, EB; Torres, FR; Rezende, VB; Nakamura, AM; de Oliveira, JL; Lima, ALA; Chahad-Ehlers, S; Sobrinho, IS; de Brito, RA. 2018

Several studies have demonstrated that genes differentially expressed between sexes (sex-biased genes) tend to evolve faster than unbiased genes, particularly in males. The reason for this accelerated evolution is not clear, but several explanations have involved adaptive and nonadaptive mechanisms. Furthermore, the differences of sex-biased expression patterns of closely related species are also little explored out of *Drosophila*. To address the evolutionary processes involved with sex biased expression in species with incipient differentiation, we analyzed male and female transcriptomes of *Anastrepha fraterculus* and *Anastrepha obliqua*, a pair of species that have diverged recently, likely in the presence of gene flow. Using these data, we inferred differentiation indexes and evolutionary rates and tested for signals of selection in thousands of genes expressed in head and reproductive transcriptomes from both species. Our results indicate that sex-biased and reproductive-biased genes evolve faster than unbiased genes in both species, which is due to both adaptive pressure and relaxed constraints. Furthermore, among male-biased genes evolving under positive selection, we identified some related to sexual functions such as courtship behavior and fertility. These findings suggest that sex-biased genes may have played important roles in the establishment of reproductive isolation between these species, due to a combination of selection and drift, and unveil a plethora of genetic markers useful for more studies in these species and their differentiation.

Selective pollination by fungus gnats potentially functions as an alternative reproductive isolation among five *Arisaema* species

Matsumoto, TK; Hirobe, M; Sueyoshi, M; Miyazaki, Y. 2021

Background and Aims Interspecific difference in pollinators (pollinator isolation) is important for reproductive isolation in flowering plants. Species-specific pollination by fungus gnats has been discovered in several plant taxa, suggesting that they can contribute to reproductive isolation. Nevertheless, their contribution has not been studied in detail, partly because they are too small for field observations during flower visitation. To quantify their flower visitation, we used the genus *Arisaema* (Araceae) because the pitcher-like spathe of *Arisaema* can trap all floral visitors. Methods We evaluated floral visitor assemblage in an altitudinal gradient including five *Arisaema* species. We also examined interspecific differences in altitudinal distribution (geographic isolation) and flowering phenology (phenological isolation). To exclude the effect of interspecific differences in altitudinal distribution on floral visitor assemblage, we established ten experimental plots including the five *Arisaema* species in high- and low-altitude areas and collected floral visitors. We also collected floral visitors in three additional sites. Finally, we estimated the strength and contribution of these three reproductive barriers using a unified formula for reproductive isolation. Key Results Each *Arisaema* species selectively attracted different fungus gnats in the altitudinal gradient, experimental plots and additional sites. Altitudinal distribution and flowering phenology differed among the five *Arisaema* species, whereas the strength of geographic and phenological isolations were distinctly weaker than those in pollinator isolation. Nevertheless, the absolute contribution of pollinator isolation to total reproductive isolation was weaker than geographic and phenological isolations, because pollinator isolation functions after the two early-acting barriers in plant life history. Conclusions Our results suggest that selective pollination by fungus gnats potentially contributes to

reproductive isolation. Since geographic and phenological isolations can be disrupted by habitat disturbance and interannual climate change, the strong and stable pollinator isolation might compensate for the weakened early-acting barriers as an alternative reproductive isolation among the five *Arisaema* species.

The temporal pattern of mating behavior of the fruit fly, *Anastrepha zenilldae* in the laboratory

de Almeida, LM; Araujo, A; Mendesc, NHD; de Souza, JMGA; Menezese, AAL. 2011

The state of Rio Grande do Norte is an important fruit-producing and exporting area in northeastern Brazil. The success of this industry depends on fruit fly population control, especially in fly-free exporting zones. However, many fruits are not exported because of quarantine restrictions imposed by importing countries. A survey in the state has detected a considerable increase of the fruit fly, *Anastrepha zenilldae* Zucchi (Diptera: Tephritidae), probably a result of the introduction of irrigated guava orchards that make fruit available all year. Knowledge of the sexual behavior of Tephritidae has great importance to pest control programs, particularly those that employ the Sterile Insect Technique. In order to characterize the reproductive behavior of *A. zenilldae*, 32 individuals (16 males; 16 females) in each of six generations were submitted to an artificial 12:12 L:D cycle (750: < 1 lux, lights on 07:00-19:00) and observed over their lifetimes. The courtship and copulation occurred in leks and the episodes varied with the time of day, courtship being most frequent between zeitgeber time (ZT) 3 and ZT 7, peaking at ZT 5-6. Copulations occurred between ZT 2 and ZT 8, with a higher frequency between ZT 5-7 and a peak at ZT 6. Mean duration was 0.28 +/- 0.03 min/male (range: 5-163 min). Males in the leks attempted to copulate mainly between ZT 3 and ZT 7 with a peak at ZT 6, and males outside leks peaked at ZT 7. The different timing of sexual behaviors among related sympatric species, including *A. zenilldae*, may contribute to species isolation.

Reproductive isolation between *Anopheles sinensis* and *Anopheles pullus* in Korea

Kim, SJ; Choochote, W; Jitpakdi, A; Junkum, A; Ahn, DA; Baek, JA; Park, MH; Kim, MS; Chung, KW; Min, GS. 2005

Anopheles sinensis and *An. pullus*, the 2 predominant anopheline mosquito species in Korea, are major malaria vectors. Their morphological similarity makes them difficult to distinguish, and their taxonomic relationship remains unclear. Kanda and Oguma produced fertile *F*, *An. sinensis* X *An. pullus* **hybrids**, suggesting a conspecific relationship. However, our recent internal transcribed spacer 2 DNA sequence comparison of these taxa revealed 32.9% divergence, suggesting that *An. sinensis* and *An. pullus* may be distinct species. In the present study, we reassessed the relationship of *An. sinensis* and *An. pullus* in **hybridization** experiments. Laboratory-raised isofemale lines of *An. sinensis* form Band *An. pullus* form A and B were reciprocally crossed. The 3 isofemale lines were established from fully engorged females caught from the wild and morphologically identified as *An. sinensis* or *An. pullus*. The isofemale lines represented 3 distinct metaphase karyotypes: *An. sinensis* form B (X, Y-2) and *An. pullus* form A (X-1, Y-1) and form B (X-2, Y-2). The F-1 **hybrid** larvae showed low viability and completely asynaptic salivary gland polytene chromosomes. The subset of *F*, **hybrids** that developed to adults showed sex-ratio distortion; ovarian follicles developed abnormally in females, and males suffered atrophy of accessory glands and testes. These results strongly suggest that the Korean morphological species *An. sinensis* and *An. pullus* are biological species, separated by complete postzygotic reproductive isolation.

An X chromosome effect responsible for asymmetric reproductive isolation between male *Drosophila virilis* and heterospecific females

Nickel, D; Civetta, A. 2009

Reproductive isolation between closely related species is expressed through uncoordinated courtship, failed fertilization, and (or) postzygotic barriers. Behavioural components of mating often form an initial barrier to **hybridization** between species. In many animals, females are responsible for mating discrimination in both intra- and interspecific crosses; males of *Drosophila virilis* group represent an exception to this trend. Using overall productivity tests, we show that a lower proportion of *D. virilis* males sire progeny when paired with a heterospecific female (*Drosophila novamexicana* or *Drosophila americana texana*) for 2 weeks. This suggests male mate discrimination or some other kind of asymmetrical incompatibility in courtship and mating or early zygote mortality. We used males from *D. virilis* - *D. novamexicana* and from *D. virilis* - *D. a. texana* backcross populations to map chromosome effects responsible for male reproductive isolation. Results from the analysis of both backcross male populations indicate a major X chromosome effect. Further, we conduct a male behavioural analysis to show that *D. virilis* males significantly fail to continue courtship after the first step of courtship, when they tap heterospecific females. The combined results of a major X chromosome effect and the observation that *D. virilis* males walk away from females after tapping suggest that future studies should concentrate on the identification of X-linked genes affecting the ability of males to recognize conspecific females.

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

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

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

AEDES-CASPIUS (PALLAS, 1771) AND A-DORSALIS (MEIGEN, 1830) (DIPTERA, CULICIDAE) - MORPHOLOGICAL AND GENETIC ANALYSES OF 2 SYMPATRIC POPULATIONS - DEMONSTRATION OF REPRODUCTIVE ISOLATION

LAMBERT, M; PASTEUR, N; RIOUX, JA; DELALBREBELMONTE, A; BALARD, Y. 1990

Courtship song genes and speciation in sand flies

Oliveira, SG; Bottecchia, M; Bauzer, LGSR; Souza, NA; Ward, RD; Kyriacou, CP; Peixoto, AA. 2001

Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) is a vector of visceral leishmaniasis in the Americas and it might represent a complex of sibling species. Reproductive isolation between closely related species often involves differences in courtship behaviour. cacophony (cac) and period (per) are two *Drosophila* genes that control features of the "lovesong" males produce during courtship that has been implicated in the sexual isolation between closely related species. We are using gene fragments from *L. longipalpis*' homologues of these two genes to study the speciation process in this putative species complex.

Is Cote D'Ivoire a new high hybridization zone for the two major malaria vectors, *Anopheles coluzzii* and *An. gambiae* (Diptera, Culicidae)?

Anopheles gambiae and *An. coluzzii* are very closely related and recently differentiated species representing the main malaria vectors in the Afrotropical region and responsible of up to >3 infective bites/person/night in Cote D'Ivoire, where prevention and control has stagnated in recent years. The aim of the present study was to genetically and ecologically characterize *An. gambiae* and *An. coluzzii* populations from two villages of Cote D'Ivoire, lying in the coastal forest belt and 250 km inland in the Guinean savannah mosaic belt, respectively. Results reveal high frequencies of both species in both study sites and high frequencies of **hybrids** (4-33%) along the whole year of sampling. Consistently with observations for the well-known high **hybridization** zone at the far -west of the species range, **hybrid** frequencies were higher in the coastal village and highest when the two species occurred at more balanced frequencies, supporting the "frequency-dependent **hybridization**" ecological speciation theory. Pilot genotyping revealed signatures of genomic admixture in both chromosome-X and-3. Coupled with previous reports of **hybrids** in the region, the results point to the coastal region of Cote D'Ivoire as a possible regions of high **hybridization**. Preliminary characterization of parameters relevant for malaria transmission and control (e.g. possibly higher sporozoite rates and indoor biting preferences in **hybrids** than in the parental species) highlight the possible relevance of the breakdown of reproductive barriers between *An. gambiae* and *An. coluzzii* not only in the field of ecological evolution, but also in malaria epidemiology and control.

Differences in gene regulation in a tephritid model of prezygotic reproductive isolation

Raphael, KA; Sved, JA; Pearce, S; Oakeshott, JG; Gilchrist, AS; Sherwin, WB; Frommer, M. 2019

The two tephritid fruit fly pests, *Bactrocera tryoni* and *Bactrocera neohumeralis*, are unusually well suited to the study of the genetics of reproductive isolating mechanisms. Sequence difference between the species is no greater than between a pair of conspecific *Drosophila melanogaster* populations. The two species exist in close sympatry, yet do not **hybridize** in the field, apparently kept separate by a strong premating isolation mechanism involving the time of day at which mating occurs. This spurred us to search for key genes for which time of day expression is regulated differently between the species. Using replicated, quantitative transcriptomes from head tissues of males of the two species, sampled in the day and night, we identified 141 transcripts whose abundance showed a significant interaction between species and time of day, indicating a difference in gene regulation. The brain transcripts showing this interaction were enriched for genes with a neurone function and 90% of these were more abundant at night than day in *B. tryoni*. Features of the expression patterns suggest that there may be a difference in the regulation of sleep-wake cycles between the species. In particular several genes, which in *D. melanogaster* are expressed in circadian pacemaker cells, are promising candidates to further explore the genetic differentiation involved in this prezygotic reproductive isolation mechanism.

Behavioural, ecological and genetic evidence confirm the occurrence of host-associated differentiation in goldenrod gall-midges

Dorchin, N; Scott, ER; Clarkin, CE; Luongo, MP; Jordan, S; Abrahamson, WG. 2009

Host-associated differentiation (HAD) is considered a step towards ecological speciation and an important mechanism promoting diversification in phytophagous insects. Although the number of documented cases of HAD is increasing, these still represent only a small fraction of species and feeding guilds among phytophagous insects, and most reports are based on a single type of evidence. Here we employ a comprehensive approach to present behavioural, morphological, ecological and genetic evidence for the occurrence of HAD in the gall midge *Dasineura folliculi* (Diptera: Cecidomyiidae) on two sympatric species of goldenrods (*Solidago rugosa* and *S. gigantea*). Controlled experiments revealed assortative mating and strong oviposition fidelity for the natal-host species. Analysis of mitochondrial DNA showed an amount of genetic divergence between the two host-associated populations compatible with cryptic species rather than host races. Lower levels of within-host genetic divergence, gall development and natural-enemy attack in the *S. gigantea* population suggest this is the derived host.

HOST FIDELITY IS AN EFFECTIVE PREMATING BARRIER BETWEEN SYMPATRIC RACES OF THE APPLE MAGGOT FLY

FEDER, JL; OPP, SB; WLAZLO, B; REYNOLDS, K; GO, W; SPISAK, S. 1994

Models of sympatric speciation for phytophagous insects posit a central role for host plant-associated mating as a premating isolating mechanism in lieu of geographic barriers to gene flow. Here, by means of three mark-and-recapture studies, we confirm that host fidelity (i.e., the tendency of an insect to reproduce on the same host species that it used in earlier life-history stages) restricts gene flow between sympatric apple- and hawthorn-infesting races of *Rhagoletis pomonella* (Diptera: Tephritidae) to approximately 6% per generation. Genetically based differences in host preference, adult eclosion under the "correct" host species, and allochronic isolation contribute to host fidelity in various degrees in the races. The results verify that host-associated adaptation can produce reproductive isolation as a correlated character (a key premise of sympatric speciation). The study also represents one of the few or perhaps only example in animals where the intra-specific isolating effects of specific phenotypes have been quantified in nature.

Intraguild interactions promote assortative mating and affect sexual attractiveness in a phytophagous fly

Reader, T; Duce, IR. 2009

Changes in acoustic and substrate-borne sexual signals in phytophagous insects associated with host plant shifts are known to have the potential to promote assortative mating, reproductive isolation and speciation. In this article, we ask whether the switch between pure herbivory and intraguild predation (IGP), which is common amongst phytophagous insects, has similar potential. Male flies in the genus *Lipara* (Diptera: Chloropidae) search for females by vibrating reed stems and waiting for a reply. By kleptoparasitizing other phytophagous species in the genus (a form of IGP), *Lipara rufitarsis* can increase its nonsexual fitness considerably. We looked at the impact of IGP on the timing of hatching, body size and attractiveness of male calls in *L. rufitarsis*. *L. rufitarsis* males that had engaged in IGP hatched significantly earlier than purely phytophagous flies and were significantly larger, but their calls were less likely to elicit responses from females during playback experiments. We conclude that, although behavioural observations of females provided no evidence of 'like preferring like', changes in phenology associated with IGP are likely to promote assortative mating in this system. The general preference of females for the calls of smaller males is a phenomenon worthy of further study: it may have no adaptive significance, or it may indicate that mating with large males is associated with a fitness cost. (C) 2009 The Linnean Society of London, Biological Journal of the Linnean Society, 2009, 98, 171-180.

Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice

Via, S. 1999

Determining the extent and causes of barriers to gene flow between genetically divergent populations or races of single species is an important complement to post facto analyses of the causes of reproductive isolation between recognized species. Sympatric populations of pea aphids (*Acyrtosiphon pisum* Harris, Homoptera: Aphididae) on alfalfa and red clover are highly genetically divergent and locally adapted. Here, hierarchical estimates of population structure based on F_{st} suggest that gene exchange between closely adjacent aphid populations on the two hosts is highly restricted relative to that among fields of the same host plant. Although these host-associated races are presently considered to be the same subspecies, they appear to be significantly reproductively isolated, suggesting incipient speciation. Habitat (host) choice was investigated as the first in a temporal series of factors that could reduce gene exchange between these sympatric populations.

Field studies of winged colonists to newly planted fields of each host suggest pronounced habitat fidelity. This result was verified using replicated observations of the host choice behavior of different aphid genotypes for which the relative demographic performance on each host was known. These laboratory observations of behavior revealed a strong genetic correlation between habitat choice (or acceptance) and the relative performance in each habitat. Because mating occurs on the host plant, habitat choice in this system leads to assortative mating and is therefore a major cause of reproductive isolation between the sympatric pea aphid populations on alfalfa and clover. However, the extent of dispersal between hosts estimated from the field study of winged colonists (9-11%) is too great to be consistent with the genetic divergence estimated between the races. This suggests that barriers to gene flow other than host choice also exist, such as selection against migrants or **hybrids** in the parental environments, **hybrid** sterility, or **hybrid** breakdown.

Genetic identification of two sibling species of *Lutzomyia longipalpis* (Diptera : Psychodidae) that produce distinct male sex pheromones in Sobral, Ceara State, Brazil

Maingon, RDC; Ward, RD; Hamilton, JGC; Noyes, HA; Souza, N; Kemp, SJ; Watts, PC. 2003

Lutzomyia longipalpis, the main sandfly vector for New World visceral leishmaniasis is a complex of an as yet undefined number of sibling species. At present, there is no consensus on the status (single species vs. species complex) of Brazilian populations. We applied five microsatellite loci to test the hypothesis that *L. longipalpis* occurs as two sympatric cryptic species in Sobral, Ceara State, Brazil as predicted by male sex pheromone chemotypes described previously for field specimens from this site [S-9-methyl-germacrene-B (9MGB) and a cembrene compound]. Abdominal spot morphology corresponds with pheromone type at this locality (9MGB in '1 spot' males and cembrene in '2 spot' males). Genotype data from 190 wild-caught *L. longipalpis* specimens collected in October 1999 and April 2001 were used to estimate genetic differentiation between the two sex pheromone populations and sampling dates. No significant ($P > 0.05$) genetic differences were found between the 1999 and 2001 9MGB samples ($\theta = 0.018$; $R-ST = -0.005$), and genetic differentiation was low between the cembrene collections ($\theta = 0.037$, $P < 0.05$; $R-ST = -0.043$, $P > 0.05$). By contrast, highly divergent allelic frequencies (largely at two microsatellite loci) corresponded to significant ($P > 0.05$) genetic differentiation ($\theta = 0.221$; $R-ST = 0.215$) for all comparisons between samples with different pheromones. When pheromone samples were pooled across sample date, genetic differentiation was high ($\theta = 0.229$; $P < 0.001$; $N_e m = 0.84$). The allele frequency distribution at each of the five microsatellite loci was similar for males and females from the two collection years. Two of these loci showed highly divergent allele frequencies in the two sex pheromone populations. This was reflected in the highly significant genetic differentiation obtained from the male genotypes, between populations producing different pheromones ($\theta = 0.229$ - 0.268 ; $P < 0.0001$ for the 2001 and $\theta = 0.254$ - 0.558 ; $P < 0.0001$ for the 1999 collections, respectively). Similar results were obtained when the females, assigned to a pheromone type, were included in the analysis. Both a Bayesian analysis of the data set and a population assignment test provided strong evidence for two distinct populations corresponding to pheromone type. Given its genotype, the probability of assigning a 9MGB male to the original 9MGB population was 100% once the two years' collections were pooled. For cembrene-producing '2 spot' males this probability although still high, was lower than for 9MGB males, at 86%. This microsatellite data together with previously reported reproductive isolation between the two Sobral populations confirm that premating barriers are important in speciation of *L. longipalpis*.

Alternative Mating Tactics as Potential Prezygotic Barriers to Gene Flow Between Two Sister Species of Frugivorous Fruit Flies

Tadeo, E; Aluja, M; Rull, J. 2013

Rhagoletis completa Cresson and *Rhagoletis zoqui* Bush, are two sister species in the *suavis* group that were thought to occupy non-overlapping geographical ranges. Recent discovery of a contact zone in North Eastern Mexico where natural interspecific **hybrids** with intermediate morphotypes can be found has led to laboratory studies in small enclosures that failed to identify the existence of reproductive barriers capable of maintaining the genetic integrity of both species. Because the experimental approach used in such studies could have interfered with natural behavior of flies, a series of additional mating compatibility tests under much less restrictive conditions were performed. Our observations, confirmed the fact that males and females of both species engage in interspecific matings. However, *R. completa* females were more reluctant to engage in heterospecific matings than *R. zoqui* females, revealing asymmetries in sexual isolation. Also, careful scrutiny of male guarding behavior and species specific partitioning of mating location unveiled subtle differences that could result in reproductive isolation if reinforced during secondary contact.

Sequential radiation of unrelated organisms: the gall fly *Eurosta solidaginis* and the tumbling flower beetle *Mordellistena convicta*

Abrahamson, WG; Blair, CP; Eubanks, MD; Morehead, SA. 2003

Host shifts and the formation of insect-host races are likely common processes in the speciation of herbivorous insects. The interactions of goldenrods *Solidago* (Compositae), the gall fly *Eurosta solidaginis* (Diptera: Tephritidae) and the beetle *Mordellistena convicta* (Coleoptera: Mordellidae) provide behavioural, ecological and genetic evidence of host races that may represent incipient species forming via sympatric speciation. We summarize evidence for *Eurosta* host races and show that *M. convicta* has radiated from goldenrod stems to *Eurosta* galls to form host-part races and, having exploited the galler's host shift, has begun to differentiate into host races within galls. Thus, host-race formation has occurred in two interacting, but unrelated organisms representing two trophic levels, resulting in 'sequential radiation' (escalation of biodiversity up the trophic system). Distributions of host races and their behavioural isolating mechanisms suggest sympatric differentiation. Such differentiation suggests host-race formation and subsequent speciation may be an important source of biodiversity.

Male accessory gland proteins induce female monogamy in anopheline mosquitoes

Shutt, B; Stables, L; Aboagye-Antwi, F; Moran, J; Tripet, F. 2010

The role of male accessory gland (MAG) secretions in inducing refractoriness to further mating in mosquitoes (Diptera: Culicidae) was established in the late 1960s. In a set of simple experiments, MAG extract was injected intra-thoracically into the hemocoel of virgin *Aedes aegypti* (L.), *Culex pipiens pipiens* (L.) and *Anopheles quadrimaculatus* Say females. This subsequently caused most females to remain unmated when exposed to males. For anophelines these findings were later challenged by a study involving intra-abdominal injections of MAG extracts into *Anopheles gambiae* s.l. and *Anopheles albimanus* Wiedmann females, which failed to induce refractoriness to further mating. These findings led to controversy about the respective role of sperm and accessory gland peptides in inducing female monogamy in *Anopheles* and are at odds with our current understanding of the mating process in *Drosophila* spp. (Diptera: Drosophilidae) and other dipterans. Here we confirm the function of MAG secretions in anophelines experimentally by showing that intra-thoracic injections in *Anopheles stephensi* Liston and in the M and S molecular forms of *An. gambiae* s.s. result in the expected female monogamy. Cross-injections of MAG extracts between the M and S molecular forms of *An. gambiae*, two cryptic taxa within *An. gambiae* s.s. which are thought to be undergoing incipient speciation, also elicited effective refractoriness, suggesting that the two sub-taxa have not diverged with regard to sex peptides responsible for female monogamy. Importantly, this also suggests that the rare cases of re-mating following cross-mating observed in this species may not be a form of reproductive barrier between molecular forms.

Cryptic biodiversity in the cytogenome of bird-biting blackflies in North Africa

Adler, PH; Cherairia, M; Arigue, SF; Samraoui, B; Belqat, B. 2015

Bird-biting blackflies in the *Simulium* (*Eusimulium*) *aureum* group (Diptera: Simuliidae) are widespread vectors of *Leucocytozoon* and *Trypanosoma* parasites. The

polytene chromosomes of 619 larvae of the three nominal members of the *S.aureum* group in North Africa were evaluated cytogenetically for cryptic biodiversity. Seven chromosomal segregates were discovered among 29 populations in Algeria and Morocco. This diversity was based primarily on two chromosomal inversions, which have assumed unique roles in different lineages, including sex linkage, fixation, loss and autosomal polymorphism. Reproductive isolation was demonstrated for six of the seven segregates, doubling the number of species known in the area. Four species were linked with existing names: (a) *Simulium mellah* Giudicelli & Bouzidi, which is known only from North African high-salinity habitats; (b) *Simulium petricolum* (Rivosecchi), which is tentatively conspecific with continental European populations; (c) *Simulium rubzovianum* (Sherban) and its synonym *Simulium latinum* (Rubtsov), which is widely distributed from North Africa across Europe into Western Asia, and (d) *Simulium velutinum* (Santos Abreu) and its new synonym *Simulium tenerificum* Crosskey, which is restricted to North Africa and the Canary Islands. Of the remaining entities, two are new species precinctive to North Africa and one, known only from Morocco, is of undetermined taxonomic status.

Unveiling mosquito cryptic species and their reproductive isolation

Zheng, XL. 2020

Mosquitoes are major vectors of many infectious pathogens or parasites. Understanding cryptic species and the speciation of disease vectors has important implications for vector management, evolution and host-pathogen and/or host-parasite interactions. Currently, mosquito cryptic species have been reported in many studies, most of which focus on the reproductive isolation of cryptic species and mainly on *Anopheles gambiae* sensu lato complex. Emerging species within the primary malaria vector *Anopheles gambiae* show different ecological preferences and significant prezygotic reproductive isolation, while *Aedes mariae* and *Aedes zammiti* show postmating reproductive isolation. However, data reporting the reproductive isolation in *Culex* and *Aedes albopictus* mosquito cryptic species is absent. The lack of systematic studies leaves many questions open, such as whether cryptic species are more common in particular habitats, latitudes or taxonomic groups; what mosquito cryptic species evolutionary processes bring about reproductive isolation in the absence of morphological differentiation? How does *Wolbachia* infection affect in mosquitoes' reproductive isolation? In this review, we provide a summary of recent advances in the discovery and identification of sibling or cryptic species within mosquito genera.

The evolution of sex pheromones in an ecologically diverse genus of flies

Symonds, MRE; Moussalli, A; Elgar, MA. 2009

In theory, pheromones important in specific mate recognition should evolve via large shifts in composition (saltational changes) at speciation events. However, where other mechanisms exist to ensure reproductive isolation, no such selection for rapid divergence is expected. In *Bactrocera* fruit flies (Diptera: Tephritidae), males produce volatile chemicals to attract females for mating. *Bactrocera* species exhibit great ecological diversity, with a wide range of geographical locations and host plants used. They also have other mechanisms, including temporal and behavioural differences, which ensure reproductive isolation. Therefore, we predicted that their sex pheromones would not exhibit rapid divergence at speciation events. In the present study, we tested this idea by combining data on male sex pheromone composition for 19 species of *Bactrocera* with a phylogeny constructed from DNA sequence data. Analyses of the combined data revealed positive correlations between pheromone differences and nucleotide divergence between species, and between the number of pheromone changes along the phylogeny and the branch lengths associated with these changes. These results suggest a gradual rather than saltational mode of evolution. However, remarkable differences in sex pheromones composition exist, even between closely-related species. It appears therefore that the mode of evolution of sex pheromones in *Bactrocera* is best described by rapid saltational changes associated with speciation, followed by gradual divergence thereafter. Furthermore, species that do not overlap ecologically are just as different pheromonally as species that do. Thus, large changes in pheromone composition appear to be achieved, even in cases where other mechanisms to ensure reproductive isolation exist. We suggest that these differences are closely associated with rapid changes in host plant use, which is a characteristic feature of *Bactrocera* speciation. (C) 2009 The Linnean Society of London, Biological Journal of the Linnean Society, 2009, 97, 594-603.

Revisiting the particular role of host shifts in initiating insect speciation

Forbes, AA; Devine, SN; Hippee, AC; Tvedte, ES; Ward, AKG; Widmayer, HA; Wilson, CJ. 2017

The notion that shifts to new hosts can initiate insect speciation is more than 150 years old, yet widespread conflation with paradigms of sympatric speciation has led to confusion about how much support exists for this hypothesis. Here, we review 85 insect systems and evaluate the relationship between host shifting, reproductive isolation, and speciation. We sort insects into five categories: (1) systems in which a host shift has initiated speciation; (2) systems in which a host shift has made a contribution to speciation; (3) systems in which a host shift has caused the evolution of new reproductive isolating barriers; (4) systems with host-associated genetic differences; and (5) systems with no evidence of host-associated genetic differences. We find host-associated genetic structure in 65 systems, 43 of which show that host shifts have resulted in the evolution of new reproductive barriers. Twenty-six of the latter also support a role for host shifts in speciation, including eight studies that definitively support the hypothesis that a host shift has initiated speciation. While this review is agnostic as to the fraction of all insect speciation events to which host shifts have contributed, it clarifies that host shifts absolutely can and do initiate speciation.

Characterization of copulatory courtship song in the Old World sand fly species *Phlebotomus argentipes*

Araki, AS; Brazil, RP; Hamilton, JGC; Vigoder, FM. 2020

Acoustic communication in the form of courtship and mating songs are often involved in reproductive isolation between species of Diptera, such as *Drosophila*, mosquitoes and sand flies. The patterns of courtship songs in New World sand fly species evolve quickly under sexual selection; and therefore, represent an important trait that can be used as a marker to study the evolution of species complexes and may aid identification of sibling species with a complex. The ability to identify vector species within species complexes is of critical importance for effective and efficient vector control programs. Species-specific song patterns seems to contribute to reproductive isolation in New World sand fly species, suggesting that auditory communication signals may be widespread among these important vectors of leishmaniasis. The main goal of the present study was to characterize the copulatory courtship song of *Phlebotomus argentipes*, an important vector of visceral leishmaniasis in the Old World. *Ph. argentipes* males produce acoustic signals during copulation and two types of songs were observed. The one we called primary song is a 'pulse song' with similar length and amplitude to the previously observed 'P1' pattern recorded in Brazilian populations of *Lu. longipalpis* s.l. The secondary song has 'sine song' characteristics and is quite different from any song produced by New World species. The discovery of this copulation courtship songs in *Ph. argentipes* supports the possibility that acoustic communication in sandflies might be more widespread than previously thought, including Old World species. Our results highlight the importance of further research on acoustic communication in the *Ph. argentipes* species complex and other Old World vectors of leishmaniasis.

Cuticular hydrocarbons of *Glossina*. 3. Subgenera *Glossina* and *Nemorhina*

Sutton, BD; Carlson, DA. 1997

The cuticular methyl-branched alkanes of tsetse flies of the subgenera *Glossina* (sensu stricto, formerly *morsitans*) and *Nemorhina* (formerly *palpalis*) were identified and quantified by capillary gas-liquid chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Males of *Glossina* (*Nemorhina*) are differentiated

from G. (Glossina) by dominant 27-, 28-, and/or 29-carbon backbone trimethylalkanes with the methyl positions at 3,7,11-, 4,8,12-, and 3,7,11-, respectively. All females contain major quantities of long-chain internally branched di- and/or trimethylalkanes that were previously implicated as mediators of sexual behavior in males. Taxa within these two subgroups that are closely related and/or conspecific, based upon conventional morphological and ecological criteria, exhibit similar GC patterns and similar internally branched di- and trimethylalkane isomers in females. Examination of these potentially stimulatory methylalkanes may provide reasons for the reproductive isolation of closely related species from each other.

Comparative studies of stenogamous behaviour in the mosquito *Culex pipiens* complex

Kim, S; Trocke, S; Sim, C. 2018

Understanding the processes of reproductive behaviour in mosquitoes is crucial for improving mating competitiveness and mating specificity for sterile insect release programmes. The *Culex pipiens* (Linnaeus) (Diptera: Culicidae) forms *pipiens* and *molestus* (Forsk.) two biotypes of the *Cx. pipiens* complex, are vectors for West Nile virus, St Louis encephalitis virus and lymphatic filariases. **Hybridization** of these biotypes is known to occur in nature, although form *pipiens* mates above ground in large spaces (eurygamy) and form *molestus* preferentially mates in small spaces (stenogamy) such as sewage tunnels. **Hybridization** may allow gene flow of biotype-specific characteristics that are crucial in the disease transmission cycle. The present study examined and compared mating behaviours, insemination rates, fecundity and fertility in parental and F1 **hybrids** between *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus* in conditions of stenogamy. Unique mating behaviour sequences were identified in *Cx. pipiens* f. *molestus*, including tapping, mounting, co-flying and copulation. Despite the considerably high insemination rates in **hybrid** crosses, fertility and fecundity rates were varied. This observation could suggest reproductive isolation in the **hybrid** zone. The study also documents a failure of heterospecific males to produce fertile eggs in *Cx. pipiens* f. *pipiens* females, which may be attributable to gametic incompatibilities and may represent an additional barrier to gene exchange.

Histories of host shifts and cospeciation among free-living parasitoids of *Rhagoletis* flies

Hamerlinck, G; Hulbert, D; Hood, GR; Smith, JJ; Forbes, AA. 2016

Host shifts by specialist insects can lead to reproductive isolation between insect populations that use different hosts, promoting diversification. When both a phytophagous insect and its ancestrally associated parasitoid shift to the same novel host plant, they may cospeciate. However, because adult parasitoids are free living, they can also colonize novel host insects and diversify independent of their ancestral host insect. Although shifts of parasitoids to new insect hosts have been documented in ecological time, the long-term importance of such shifts to parasitoid diversity has not been evaluated. We used a genus of flies with a history of speciation via host shifting (*Rhagoletis* [Diptera: Tephritidae]) and three associated hymenopteran parasitoid genera (*Diachasma*, *Coptera* and *Utetes*) to examine cophylogenetic relationships between parasitoids and their host insects. We inferred phylogenies of *Rhagoletis*, *Diachasma*, *Coptera* and *Utetes* and used distance-based cophylogenetic methods (ParaFit and PACo) to assess congruence between fly and parasitoid trees. We used an event-based method with a free-living parasitoid cost model to reconstruct cophylogenetic histories of each parasitoid genus and *Rhagoletis*. We found that the current species diversity and host-parasitoid associations between the *Rhagoletis* flies and parasitoids are the primary result of ancient cospeciation events. Parasitoid shifts to ancestrally unrelated hosts primarily occur near the branch tips, suggesting that host shifts contribute to recent parasitoid species diversity but that these lineages may not persist over longer time periods. Our analyses also stress the importance of biologically informed cost models when investigating the coevolutionary histories of hosts and free-living parasitoids.

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

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

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

Selective maintenance of allozyme differences among sympatric host races of the apple maggot fly

Feder, JL; Roethele, JB; Wlazlo, B; Berlocher, SH. 1997

Whether phytophagous insects can speciate in sympatry when they shift and adapt to new host plants is a controversial question. One essential requirement for sympatric speciation is that disruptive selection outweighs gene flow between insect populations using different host plants. Empirical support for host-related selection (i.e., fitness trade-offs) is scant, however. Here, we test for host-dependent selection acting on apple (*Malus pumila*)- and hawthorn (*Crataegus* spp.)-infesting races of *Rhagoletis pomonella* (Diptera: Tephritidae). In particular, we examine whether the earlier fruiting phenology of apple trees favors pupae in deeper states of diapause (or with slower metabolisms/development rates) in the apple fly race. By experimentally lengthening the time period preceding winter, we exposed hawthorn race pupae to environmental conditions typically faced by apple flies. This exposure induced a significant genetic response at six allozyme loci in surviving hawthorn fly adults toward allele frequencies found in the apple race. The sensitivity of hawthorn fly pupae to extended periods of warm weather therefore selects against hawthorn flies that infest apples and helps to maintain the genetic integrity of the apple race by counteracting gene flow from sympatric hawthorn populations. Our findings confirm that postzygotic reproductive isolation can evolve as a pleiotropic consequence of host-associated adaptation, a central tenet of nonallopatric speciation. They also suggest that one reason for the paucity of reported fitness trade-offs is a failure to consider adequately costs associated with coordinating an insect's life cycle with the phenology of its host plant.

Rapid and repeatable host plant shifts drive reproductive isolation following a recent human-mediated introduction of the apple maggot fly, *Rhagoletis pomonella*

Hood, GR; Powell, THQ; Doellman, MM; Sim, SB; Glover, M; Yee, WL; Goughnour, RB; Mattsson, M; Schwarz, D; Feder, JL. 2020

Ecological speciation via host-shifting is often invoked as a mechanism for insect diversification, but the relative importance of this process is poorly understood. The shift of *Rhagoletis pomonella* in the 1850s from the native downy hawthorn, *Crataegus mollis*, to introduced apple, *Malus pumila*, is a classic example of sympatric host race formation, a hypothesized early stage of ecological speciation. The accidental human-mediated introduction of *R. pomonella* into the Pacific Northwest (PNW) in the late 1970s allows us to investigate how novel ecological opportunities may trigger divergent adaptation and host race formation on a rapid timescale. Since the introduction, the fly has spread in the PNW, where in addition to apple, it now infests native black hawthorn, *Crataegus douglasii*, and introduced ornamental

hawthorn, *Crataegus monogyna*. We use this "natural experiment" to test for genetic differentiation among apple, black, and ornamental hawthorn flies co-occurring at three sympatric sites. We report evidence that populations of all three host-associations are genetically differentiated at the local level, indicating that partial reproductive isolation has evolved in this novel habitat. Our results suggest that conditions suitable for initiating host-associated divergence may be common in nature, allowing for the rapid evolution of new host races when ecological opportunity arises.

Habitat avoidance: Overlooking an important aspect of host-specific mating and sympatric speciation?

Forbes, AA; Fisher, J; Feder, JL. 2005

Understanding speciation requires discerning how reproductive barriers to gene flow evolve between previously interbreeding populations. Models of sympatric speciation for phytophagous insects posit that reproductive isolation can evolve in the absence of geographic isolation as a consequence of an insect shifting and ecologically adapting to a new host plant. One important adaptation contributing to sympatric differentiation is host-specific mating. When organisms mate in preferred habitats, a system of positive assortative mating is established that facilitates sympatric divergence. Models of host fidelity generally assume that host choice is determined by the aggregate effect of alleles imparting positive preferences for different plant species. But negative effect genes for avoiding nonnatal plants may also influence host use. Previous studies have shown that apple and hawthorn-infesting races of *Rhagoletis pomonella* flies use volatile compounds emitted from the surface of fruit as key chemosensory cues to recognize and distinguish between their host plants. Here, we report results from field trials indicating that in addition to preferring the odor of their natal fruit, apple and hawthorn flies, and their undescribed sister species infesting flowering dogwood (*Cornus florida*), also avoid the odors of nonnatal fruit. We discuss the implications of nonnatal fruit avoidance for the evolutionary dynamics and genetics of sympatric speciation. Our findings reveal an underappreciated role for habitat avoidance as a potential postmating, as well as prezygotic, barrier to gene flow.

Molecular evolution of the cacophony IVS6 region in sandflies

Lins, RMMA; Oliveira, SG; Souza, NA; de Queiroz, RG; Justiniano, SCB; Ward, RD; Kyriacou, CP; Peixoto, AA. 2002

A number of insects produce acoustic signals during courtship. Genes involved in the control of the courtship song are particularly interesting from an evolutionary viewpoint because interspecific variation in this signal is potentially important as a reproductive isolation mechanism and, as a consequence, in the speciation process. The cacophony gene was identified by a mutation affecting the 'lovesong' in *Drosophila melanogaster*. Phlebotomine sandflies (Diptera: Psychodidae) also produce acoustic stimuli during courtship and therefore cacophony can be used as an interesting molecular marker in evolutionary studies in these important disease vectors. In this paper we have studied the molecular evolution of the IVS6 region of cacophony in sandflies. We compared the level of divergence in the exon sequences encoding this conserved domain in *Drosophila* and Phlebotomines. We also analysed the high level of variation in an intron that is present in sandflies but that was lost in *Drosophila* during evolution. The available cacophony sequences were also used for a phylogenetic analysis of some species of the Neotropical genus *Lutzomyia*.

Genetic divergence in the cacophony IVS6 intron among five Brazilian populations of *Lutzomyia longipalpis*

Bottecchia, M; Oliveira, SG; Bauzer, LGS; Souza, NA; Ward, RD; Garner, KJ; Kyriacou, CP; Peixoto, AA. 2004

Genes involved in the reproductive isolation are particularly useful as molecular markers in speciation studies. *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae), a putative species complex, is a vector of visceral leishmaniasis in Latin America. We isolated from this species a fragment homologous to cacophony, a *Drosophila* gene that encodes features of the lovesong, all acoustic signal that is important in the sexual isolation of closely related species and known to vary considerably among *L. longipalpis* putative siblings species. Using an intron of the sandfly cacophony as a marker, we analyzed the molecular variation and sequence divergence among five populations of *L. longipalpis* from Brazil, three allopatric (Jacobina, Lapinha and Natal) and two putative sympatric sibling species from the locality of Sobral. A high level of polymorphism was found and analysis of the data indicates that very little gene flow is occurring among the populations of Jacobina, Lapinha, and Natal. A high level of differentiation was also observed between the two putative sympatric species of Sobral, one of which seems to be the same sibling species found in Natal, while the other is somewhat more related to Jacobina and Lapinha. However, the amount of estimated gene flow among the Sobral siblings is about seven times higher than the previously estimated for period, another lovesong gene, perhaps indicating that introgression might be affecting cacophony, more than period. The results suggest that *L. longipalpis* is not a single species in Brazil, but it is yet not clear whether the different populations studied deserve species status rather than representing an incipient speciation process.

Evidence for X-linked introgression between molecular forms of *Anopheles gambiae* from Angola

Choi, KS; Townson, H. 2012

The M and S molecular forms of the African malaria vector *Anopheles gambiae* (Diptera: Culicidae) are morphologically identical incipient species in which reproductive isolation is incomplete, enabling low-level gene flow between forms. In an attempt to find differences between the M and S forms, sequence variation was studied at loci along the X chromosome in adult female *An. gambiae* from Angola. A high proportion of M form specimens from Angola (79% of the 456 X chromosomes sampled) were found to contain a 16-bp insertion in intron 4 of the X-linked GPRCCK1 locus, relative to the AgamP3 release of the *An. gambiae* PEST genome sequence. The insertion was in HardyWeinberg equilibrium in Angolan M form populations. The same insertion was found in all S form specimens examined, regardless of where in Africa they were sampled, but was absent from a sample of M form specimens collected in Ghana, Bioko and Mali. In M form specimens from Angola, there was an association between alleles at the GPRCCK1 locus and those at a microsatellite locus, AGXH678, close to the centromere of the X chromosome, with significant linkage disequilibrium between loci separated by 0.472 Mbp ($P < 0.033$). We show that the insertion results from introgression from the S form into the M form, rather than from the retention of an ancestral character. Gene flow from the S to M form could allow genes of adaptive value to be transferred, including those conferring insecticide resistance and others influencing ecology and behaviour, and thus malaria transmission and control. We discuss factors that may have led to this introgression event.

Sexual Selection, Epistasis and Species Boundaries in Sympatric Hawaiian Picture-winged *Drosophila*

Price, DK; Souder, SK; Russo-Tait, T. 2014

The Hawaiian picture-winged flies in the genus *Drosophila* are a spectacular example of rapid evolutionary diversification in which sexual selection is considered an important mechanism for reproductive isolation and speciation. We investigated the behavioral reproductive isolation of two closely related and sympatric Hawaiian picture-winged *Drosophila* species, *D. silvestris* and *D. heteroneura*, which are known to hybridize in nature and produce viable and fertile hybrids. We compared the mating success of parental, F1 and backcross males in pairings with *D. heteroneura* females. The F1 males were produced by mating *D. heteroneura* males with *D. silvestris* females, and the backcross males were produced by mating F1 females with *D. heteroneura* males. The mating success of backcross males paired with *D. heteroneura* females were significantly reduced relative to that of parental and F1 males. This reduced mating success occurred primarily at a late stage of courtship where female choice of mate may be important. Two- and three-gene models demonstrate that epistasis involving a few genes could account for the observed variation in male mating success. These results are consistent with negative epistasis in the backcross generation and support the importance of sexual selection

and negative epistasis in the evolution and maintenance of these species.

Don't forget the flies: dipteran diversity and its consequences for floral ecology and evolution

Raguso, RA. 2020

The attention of the global pollination community has been drawn to food safety and other ecosystem services provided by pollinators, in light of decline in social bee populations. Despite intensified research on bees, recent studies have revealed important contributions of flies to pollination success, reproductive isolation and floral diversification. Diptera is a highly diverse insect order, comprising over 125,000 described species in 110 families and representing a broad spectrum of ecological niches beyond the well-known agricultural pests and blood-feeding vectors of human and animal diseases. Flies are most appreciated as generalized pollinators in alpine habitats (anthomyiids and tachinids) and as specialized pollinators in brood-site deceptive plants that mimic fungi (drosophilids), feces (muscid) or carrion (sarcophagids and calliphorids). Syrphid and bombyliid flies visit many of the same flowers as bees and butterflies do, but with different impacts on plant fitness. Guilds of South African plants have evolved specialized relationships with long-tongued nemestrinid and tabanid flies, thanks to geographic isolation and climatic stability. Studies in Japan highlight the evolution of another plant guild, pollinated by sciarid and mycetophilid fungus gnats, whereas *Zygothrica* flies (Drosophilidae) pollinate mushroom-like *Dracula* orchids in Andean cloud forests.

Fixed Differences in the paralytic Gene Define Two Lineages within the *Lutzomyia longipalpis* Complex Producing Different Types of Courtship Songs

Lins, RMMA; Souza, NA; Brazil, RP; Maingon, RDC; Peixoto, AA. 2012

The sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae), the most important vector of American visceral leishmaniasis, is widely distributed in Latin America. There is currently a consensus that it represents a species complex, however, the number and distribution of the different siblings is still uncertain. Previous analyses have indicated that Brazilian populations of this vector can be divided into two main groups according to the type of courtship song (Burst vs. Pulse) males produce during copulation. Nevertheless, no diagnostic differences have been observed between these two groups with most molecular markers used to date. We analyzed the molecular divergence in a fragment of the paralytic (*para*) gene, a locus involved in the control of courtship songs in *Drosophila*, among a number of *Lu. longipalpis* populations from Brazil producing Burst and Pulse-type songs. Our results revealed a very high level of divergence and fixed differences between populations producing the two types of songs. We also compared *Lu. longipalpis* with a very closely related species, *Lutzomyia cruzi*, which produces Burst-type songs. The results indicated a higher number of fixed differences between *Lu. cruzi* and the Pulse-type populations of *Lu. longipalpis* than with those producing Burst-type songs. The data confirmed our previous assumptions that the presence of different sibling species of the *Lu. longipalpis* complex in Brazil can be divided into two main groups, one representing a single species and a second more heterogeneous group that probably represents a number of incipient species. We hypothesize that *para* might be one of the genes directly involved in the control of the courtship song differences between these two groups or that it is linked to other loci associated with reproductive isolation of the Brazilian species.

A novel preference for an invasive plant as a mechanism for animal hybrid speciation

Schwarz, D; Shoemaker, KD; Botteri, NL; McPherson, BA. 2007

Homoploid **hybrid** speciation-speciation via **hybridization** without a change in chromosome number is rarely documented and poorly understood in animals. In particular, the mechanisms by which animal homoploid **hybrid** species become ecologically and reproductively isolated from their parents are hypothetical and remain largely untested by experiments. For the many host-specific parasites that mate on their host, choosing the right host is the most important ecological and reproductive barrier between these species. One example of a host-specific parasite is the *Lonicera* fly, a population of tephritid fruit flies that evolved within the last 250 years likely by **hybridization** between two native *Rhagoletis* species following a host shift to invasive honeysuckle. We studied the host preference of the *Lonicera* fly and its putative parent species in laboratory experiments. The *Lonicera* fly prefers its new host, introduced honeysuckle, over the hosts of both parental species, demonstrating the rapid acquisition of preference for a new host as a means of behavioral isolation from the parent species. The parent taxa discriminate against each other's native hosts, but both accept honeysuckle fruit, leaving the potential for asymmetric gene flow from the parent species. Importantly, this pattern allows us to formulate hypotheses about the initial formation of the *Lonicera* fly. As mating partners from the two parent taxa are more likely to meet on invasive honeysuckle than on their respective native hosts, independent acceptance of honeysuckle by both parents likely preceded **hybridization**. We propose that invasive honeysuckle served as a catalyst for the local breakdown of reproductive isolation between the native parent species, a novel consequence of the introduction of an exotic weed. We describe behavioral mechanisms that explain the initial **hybridization** and subsequent reproductive isolation of the **hybrid** *Lonicera* fly. These results provide experimental support for a combination of host shift and **hybridization** as a model for **hybrid** speciation in parasitic animals.

Culex pipiens in London Underground tunnels: differentiation between surface and subterranean populations

Byrne, K; Nichols, RA. 1999

Genetic variation was quantified between surface-dwelling populations of *Culex pipiens* and the so-called molestus form found in the London Underground (the Underground) railway system. The molestus form is a commercially important biting nuisance and in the southern part of its range is also a disease vector. The surface and subterranean populations were genetically distinct, with no evidence of gene flow between closely adjacent populations of the different forms, whereas there was little differentiation between the different populations of each form. The substantially reduced heterozygosity in the Underground populations and the allelic composition suggest that colonization of the Underground has occurred once or very few times. Breeding experiments show compatibility between the Underground populations but not with those breeding above ground. There is evidence of greater gene flow and a mixing of molestus and pipiens traits in the south of the species range. This paper considers the processes that may allow establishment of reproductive isolation in the north of the species range but not in the south.

Reproductive Isolations of *Anopheles sinensis* from *Anopheles lesteri* and *Anopheles sineroides* in Korea

Park, MH; Choochote, W; Junkum, A; Joshi, D; Tuetan, B; Saeung, A; Jung, JH; Min, GS. 2008

Three species of iso-female lines of Korean anophelines (*Anopheles sinensis*, *An. lesteri*, and *An. sineroides*) were identified based on combinative diagnostic characteristics of morphology, metaphase karyotypes, crossing experiments, and comparison of DNA sequences of ribosomal DNA Internal Transcribed Spacer 2 (ITS2) and further analyzed to determine their genetic relationships. Upon comparison of the F-1 **hybrids** with those of their parent species as the control, differences in viabilities were observed. The viability of F-1 **hybrids** with high larval mortalities, the failure of all pupae to emerge as adults, and the F-1 **hybrid**'s salivary gland polytene chromosomes showing complete asynapsis of all arms from the crosses between *An. sinensis* and *An. lesteri*, as well as *An. sinensis* and *An. sineroides*, suggested that these three cryptic species exhibited signs of an entirely reproductive isolation.

REPRODUCTIVE ISOLATION BETWEEN FLORIDA STRAINS OF AEDES-AEGYPTI AND AEDES-ALBOPICTUS

HARPER, JP; PAULSON, SL. 1994

The dynamics of heterospecific and conspecific mating between Florida strains of *Aedes aegypti* and *Aedes albopictus* was examined. In nonchoice experiments where conspecific males were not available, dissection of the spermathecae showed that heterospecific insemination was an infrequent event for both species combinations (10.6% for *Ae. albopictus* with *Ae. aegypti* males, 3.6% for the reciprocal cross). Few eggs were produced from heterospecific crosses and all were nonviable. Frequency of heterospecific mating was not increased when the hindtarsi of females were removed, eliminating a significant mechanism for fending off unwanted courtship. When held with males of both species, females mated with conspecifics and oviposited without regard to the presence of heterospecifics. In low density experiments, a single female of either species caged with an excess of heterospecific males, the conspecific male always located and inseminated the female. These results indicate that significant prezygotic and postzygotic reproductive isolation exists between *Ae. aegypti* and *Ae. albopictus*.

Evolution in the *Drosophila ananassae* species subgroup

Matsuda, M; Ng, CS; Doi, M; Kopp, A; Tobari, YN. 2009

Drosophila ananassae and its relatives have many advantages as a model of genetic differentiation and speciation. In this report, we examine evolutionary relationships in the *ananassae* species subgroup using a multi-locus molecular data set, karyotypes, meiotic chromosome configuration, chromosomal inversions, morphological traits, and patterns of reproductive isolation. We describe several new taxa that are the closest known relatives of *D. ananassae*. Analysis of Y-chromosomal and mitochondrial haplotypes, shared chromosome arrangements, pre-mating isolation and **hybrid** male sterility suggests that these taxa represent a recent evolutionary radiation and may experience substantial gene flow. We discuss possible evolutionary histories of these species and give a formal description of one of them as *D. parapallidosa* Tobari sp. n. The comparative framework established by this study, combined with the recent sequencing of the *D. ananassae* genome, will facilitate future studies of reproductive isolation, phenotypic variation and genome evolution in this lineage.

Reproductive Isolation and Ecological Niche Partition among Larvae of the Morphologically Cryptic Sister Species *Chironomus riparius* and *C. piger*

Pfenninger, M; Nowak, C. 2008

Background: One of the central issues in ecology is the question what allows sympatric occurrence of closely related species in the same general area? The non-biting midges *Chironomus riparius* and *C. piger*, interbreeding in the laboratory, have been shown to coexist frequently despite of their close relatedness, similar ecology and high morphological similarity. Methodology/Principal Findings: In order to investigate factors shaping niche partitioning of these cryptic sister species, we explored the actual degree of reproductive isolation in the field. Congruent results from nuclear microsatellite and mitochondrial haplotype analyses indicated complete absence of interspecific gene-flow. Autocorrelation analysis showed a non-random spatial distribution of the two species. Though not dispersal limited at the scale of the study area, the sister species occurred less often than expected at the same site, indicating past or present competition. Correlation and multiple regression analyses suggested the repartition of the available habitat along water chemistry gradients (nitrite, conductivity, CaCO₃), ultimately governed by differences in summer precipitation regime. Conclusions: We show that these morphologically cryptic sister species partition their niches due to a certain degree of ecological distinctness and total reproductive isolation in the field. The coexistence of these species provides a suitable model system for the investigation of factors shaping the distribution of closely related, cryptic species.

Exploring Heterogeneity in the Tachinid Uzi^{fly}, *Exorista sorbillans*

Prakash, BM; Murthy, BCK; Puttaraju, HP. 2009

The uzi^{fly}, *Exorista sorbillans* (= *E. bombycis*) a tachinid endoparasitoid of silkworm, *Bombyx mori* L., known to cause considerable damage to the silk industry. Earlier studies on various aspects of the uzi^{fly} indicated for possible presence of a number of cryptic species. Though the fly entered into South India 25 years ago, the basis for the existence of cryptic species was not known. To ascertain whether the genome of this parasitoid is differentiating into discrete gene pools in contrasting eco and geo-climatic conditions, an attempt has been made in the present study the molecular profiling of 8 populations (Chittoor, Kuppam, Erode, Bangarapete, Sira, Mandya, Chitradurga, and Kanakapura) from south India with 16 RAPD primers and also to explore the possibility of its endosymbiont *Wolbachia* as a causative agent as it known to cause cytoplasmic incompatibility in its host uzi^{fly}. The hierarchical clustering done on the basis of RAPD data and the Euclidean distance matrix put Chitradurga populations at the maximum distance from other south Indian populations studied and reveals the occurrence of spectacular region-wise pairing with separate clusters. Further, when we have crossed within and between selected populations for the *Wolbachia* induced reproductive isolation, the results showed the lower level (28-32%), of reproductive isolation within and between populations. The RAPD and crossing data clearly indicates the micro-level divergence in the uzi^{fly}, *E. sorbillans*. The significance of this study with a tachinid fly pest is discussed in the context of understanding the diversification of uzi^{fly} and also establishing this pest as a relevant biological material for studying microevolution in nature.

Sexual and natural selection on pollen morphology in *Taraxacum*

Lynn, A; Piotter, E; Harrison, E; Galen, C. 2020

Premise Spiny pollen has evolved independently in multiple entomophilous lineages. Sexual selection may act on exine traits that facilitate male mating success by influencing the transfer of pollen from the anther to the body of the pollinator, while natural selection acts to increase pollen survival. We postulated that relative to sexual congeners, apomictic dandelions undergo relaxed selection on traits associated with male mating success. Methods We explored sexual selection on exine traits by measuring the propensity for *Taraxacum* spp. pollen to attach to hairs of flower-visiting bumblebees (*Bombus* spp.) or flies (Diptera: Syrphidae and Muscoidea) and assessed natural selection by testing whether pollen traits defend against consumption. Results Pollen picked up by bumblebees exhibited a narrower subset of spine-spacing phenotypes, consistent with stabilizing selection. Flies picked up larger pollen from flowers than expected at random. Surveys of corbiculae (pollen basket) contents from foraging bumblebees and feces of flies showed that pollen grains consumed by both kinds of visitors are similar in spine characteristics and size to those produced by the donor. When bees visit inflorescences of apomictic *T. officinale*, they pick up pollen with spine-spacing phenotypes above the mean and shifted toward those of sexual *T. ceratophorum*. Conclusions We demonstrate that traits under sexual selection during pollen pickup vary among pollinators, while natural selection for pollen defense is nil in *T. ceratophorum*. In **hybrid** zones between apomictic and sexual dandelions, pollen traits place apomictic donors at a dispersal disadvantage, potentially reinforcing reproductive isolation.

Host plant-related genomic differentiation in the European cherry fruit fly, *Rhagoletis cerasi*

Bakovic, V; Schuler, H; Schebeck, M; Feder, JL; Stauffer, C; Ragland, GJ. 2019

Elucidating the mechanisms and conditions facilitating the formation of biodiversity are central topics in evolutionary biology. A growing number of studies imply that divergent ecological selection may often play a critical role in speciation by counteracting the homogenising effects of gene flow. Several examples involve

phytophagous insects, where divergent selection pressures associated with host plant shifts may generate reproductive isolation, promoting speciation. Here, we use ddRADseq to assess the population structure and to test for host-related genomic differentiation in the European cherry fruit fly, *Rhagoletis cerasi* (L., 1758) (Diptera: Tephritidae). This tephritid is distributed throughout Europe and western Asia, and has adapted to two different genera of host plants, *Prunus* spp. (cherries) and *Lonicera* spp. (honeysuckle). Our data imply that geographic distance and geomorphic barriers serve as the primary factors shaping genetic population structure across the species range. Locally, however, flies genetically cluster according to host plant, with consistent allele frequency differences displayed by a subset of loci between *Prunus* and *Lonicera* flies across four sites surveyed in Germany and Norway. These 17 loci display significantly higher F_{ST} values between host plants than others. They also showed high levels of linkage disequilibrium within and between *Prunus* and *Lonicera* flies, supporting host-related selection and reduced gene flow. Our findings support the existence of sympatric host races in *R. cerasi* embedded within broader patterns of geographic variation in the fly, similar to the related apple maggot, *Rhagoletis pomonella*, in North America.

Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character

Rice, WR. 1987

A diverse group of theoretical and empirical studies are integrated into a composite model of sympatric speciation via habitat specialization. It is shown that disruptive selection on a continuous distribution of habitat preference can lead to the evolution of prezygotic reproductive isolation as a correlated character. The form of selection eliminates the major theoretical objections to the process of sympatric speciation. The principal difference between this model and the allopatric model of speciation is that the initial barrier to gene flow between subpopulations is produced by the evolution of gaps in the phenotypic distribution of spatial/temporal habitat use, rather than an extrinsic geographical barrier.

Latitudinal Variation in Parasitoid Guild Composition and Parasitism Rates of North American Hawthorn Infesting *Rhagoletis*

Rull, J; Wharton, R; Feder, JL; Guillen, L; Sivinski, J; Forbes, A; Aluja, M. 2009

Rhagoletis pomonella (Diptera: Tephritidae) populations in North America have diverged by exploiting host plants with varying fruiting phenologies in environments that differ markedly in temperature and humidity. As a result, four genetically and ecologically distinct *R. pomonella* populations that display partial reproductive isolation have evolved. Host shifting by *Rhagoletis* and similar evolutionary histories could have had cascading effects across trophic levels, influencing the diversity and distribution of associated parasitoid guilds. To establish the basis for a future understanding of the possible effect of divergence in *R. pomonella* populations on the parasitoids attacking these flies, we surveyed parasitoids from five different species of hawthorns distributed over 15 states in Mexico and 2 states in the midwestern United States. Emerging parasitoids were identified, parasitism rates were calculated, and regional fly and parasitoid emergence schedules were determined. Parasitism rate, emergence schedules, Shannon-Weiner diversity indexes, and species accumulation curves were compared across three main geographical regions. Parasitism levels varied greatly among regions from an overall high of 27.2% in the United States to 5.5% in the Sierra Madre Oriental (SMO) mountains of Mexico, to as low as 0.19% in the Eje Volcánico Trans Mexicano (EVTM). Shannon-Weiner diversity indexes showed that parasitoid species diversity was similar across the distribution range of *R. pomonella* in Mexico and the United States because of the fact that total parasitism was dominated by only two species, one of them recovered across the whole North American range of hawthorn infesting *Rhagoletis*. Nevertheless, eight parasitoids were found attacking *R. pomonella* in Mexico compared with only four collected in the United States. Only two diapausing parasitoid species were shared between the U.S. and Mexican *R. pomonella* populations: *Utetes canaliculatus* and *Diachasmimorpha mellea*. Interestingly, many subtropical parasitoid species, usually associated to flies in the subtropical genus *Anastrepha*, were recovered in the SMO in low numbers. The wide distribution of *U. canaliculatus* and *D. mellea* offers an ideal opportunity to test for a shared biogeography and co-evolution between fly and parasitoids. In this regard, one factor contributing to the success of *U. canaliculatus* seems to be the wasp's ability to modulate its eclosion time to track regional variation in hawthorn fruiting phenology and host (i.e., fly larvae) availability. Both *R. pomonella* and *U. canaliculatus* from southern sites emerged later than insects from northern populations, mirroring seasonal differences in hawthorn fruiting times across Mexico and the United States. These results suggest that molecular studies and crossing experiments could show, as they have for *Rhagoletis*, recent speciation events for parasitoid species of Nearctic origin that were found to be ecologically tracking environmentally driven divergence of their tephritid hosts.

Phylogeography and recent emergence of the Old World screwworm fly, *Chrysomya bezziana*, based on mitochondrial and nuclear gene sequences

Ready, PD; Testa, JM; Wardhana, AH; Al-Izzi, M; Khalaj, M; Hall, MJR. 2009

A previous study had identified an African and an Asian race of the Old World screwworm fly, *Chrysomya bezziana* Villeneuve (Diptera: Calliphoridae), based on the 3' terminal 279 basepairs (bp) of the mitochondrial cytochrome b gene. The Current study improved the phylogeographic resolution of cytochrome b for this species by characterizing more of the gene (the 3' terminal 715 bp) and by sampling more geographical populations, including Oman, Iran, Hong Kong and the Indonesian islands of Sulawesi and East Sumba. Strong support was found for recognizing all African race, but not for a monophyletic Asian race. The cladistic and genealogical relationships among the Asian populations were complex. There was sufficient genetic homogeneity throughout separate regions (mainland Asia and each Indonesian island) to suggest that there are no reproductive barriers within each region that might necessitate the production of more than one strain for control by the sterile insect technique (SIT). Primers were developed for the amplification by polymerase chain reaction of two nuclear loci, the highly conserved elongation factor-1 alpha gene and the less conserved white gene, and the preliminary results indicated that these genes showed the same pattern of small-scale regional variation as cytochrome b. The cytochrome b haplotypes are useful markers for identifying the geographical origins of any emerging infestations of the species: the absence of Indonesian and African haplotypes in the Middle East demonstrates that the large-scale transport of livestock is not spreading Old World screwworm.

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

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

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

females is that the same gene influences both female and male host preference, but it is a sex-influenced gene. Thus, males carrying the gene for *S. gigantea* preference have an intermediate host preference, whereas females have a strong host preference to *S. gigantea*. In summary, we found that the host preference that produces host-associated mating is inherited nonadditively at a relatively small number of loci on autosomal genes. This mode of inheritance meets the assumptions of models of sympatric speciation, indicating that the host races could have evolved in sympatry.

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.

Timing strains of the marine insect *Clunio marinus* diverged and persist with gene flow

Kaiser, TS; von Haeseler, A; Tessmar-Raible, K; Heckel, DG. 2021

Genetic divergence of populations in the presence of gene flow is a central theme in speciation research. Theory predicts that divergence can happen with full range overlap - in sympatry - driven by ecological factors, but there are few empirical examples of how ecologically divergent selection can overcome gene flow and lead to reproductive isolation. In the marine midge *Clunio marinus* (Diptera: Chironomidae) reproduction is ecologically restricted to the time of the lowest tides, which is ensured through accurate control of development and adult emergence by circalunar and circadian clocks. As tidal regimes differ along the coastline, locally adapted timing strains of *C. marinus* are found in different sites across Europe. At the same time, ecologically suitable low tides occur at both full and new moon and twice a day, providing *C. marinus* with four nonoverlapping temporal niches at every geographic location. Along the coast of Brittany, which is characterized by a steep gradient in timing of the tides, we found an unusually large number of differentially adapted timing strains, and the first known instances of sympatric *C. marinus* strains occupying divergent temporal niches. Analysis of mitochondrial genotypes suggests that these timing strains originated from a single recent colonization event. Nuclear genotypes show strong gene flow, sympatric timing strains being the least differentiated. Even when sympatric strains exist in nonoverlapping temporal niches, timing adaptations do not result in genome-wide genetic divergence, suggesting timing adaptations are maintained by permanent ecological selection. This constitutes a model case for incipient ecological divergence with gene flow.

Reproductive Isolation between Host Races of *Phytomyza glabricola* on *Ilex coriacea* and *I. glabra*

Hebert, JB; Scheffer, SJ; Hawthorne, DJ. 2013

Recently diverged taxa often show discordance in genetic divergence among genomic loci, where some loci show strong divergence and others show none at all. Genetic studies alone cannot distinguish among the possible mechanisms but experimental studies on other aspects of divergence may provide guidance in the inference of causes of observed discordances. In this study, we used no-choice mating trials to test for the presence of reproductive isolation between host races of the leaf-mining fly, *Phytomyza glabricola* on its two holly host species, *Ilex coriacea* and *I. glabra*. These trials inform our effort to determine the cause of significant differences in the degree of divergence of nuclear and mitochondrial loci of flies collected from the two host plants. We present evidence of reproductive isolation between host races in a controlled greenhouse setting: significantly more mate pairs consisting of flies from the same host plant species produced offspring than inter-host mate pairs, which produced no offspring. We also tested whether the presence of the natal or non-natal host plant affects reproductive success. Flies collected from *I. coriacea* were more likely to produce offspring when in the presence of the natal host, whereas the presence or absence of either the natal or non-natal host had no effect on flies collected from *I. glabra*. The results indicate discordant patterns of nuclear and mitochondrial divergence among host races of *P. glabricola* are likely due to incomplete lineage sorting, and the host races may be well on their way to becoming biological species.

Cryptic speciation and host-race formation in a purportedly generalist tumbling flower beetle

Blair, CP; Abrahamson, WG; Jackman, JA; Tyrrell, L. 2005

Host-race formation remains controversial as a source of herbivorous insect diversity, and examples of host races are still fairly scarce. In this study, analysis of five enzyme loci in the ostensibly generalist tumbling flower beetle *Mordellistena convicta* (Coleoptera: Mordellidae) revealed hidden host-plant and plant-organ related genetic differentiation. *Mordellistena convicta* turned out to be a complex of cryptomorphic species, each with fewer hosts than the nominal species. These cryptic species, in turn, were divided into taxa that showed host-race characteristics: samples from different host plants and organs exhibited (1) genetic indications of partial reproductive isolation, (2) differences in size and emergence timing that suggested divergent host-related selection, and (3) among-host selective differences in mortality from parasitoids. Host-race formation in *M. convicta*, which has a somewhat different life history from the well-studied host races, enlarges the group of insects considered likely to undergo this process. The widespread sympatry of the *M. convicta* species complex, along with its spectrum of host-correlated genetic differentiation, suggests that these host specialist taxa developed in sympatry.

Predicting the occurrence of host-associated differentiation in parasitic arthropods: a quantitative literature review

Harrison, K; Tarone, AM; DeWitt, T; Medina, RF. 2022

Parasite populations associated with different host species can encounter a variety of isolating reproductive barriers, leading to each population independently accumulating genome-wide genetic differences due to their host associations. This phenomenon is called host-associated differentiation (HAD) and has been proposed as an indicator of early diversification among parasitic arthropods. Although many parasite-host case study systems have been tested for the genetic signature of HAD (e.g., $F_{ST} \geq 0.15$ between sympatric, host-associated populations in the absence of allopatry), it is unknown which isolating reproductive barriers best predict the general occurrence of HAD. HAD development has been attributed to biological and ecological factors that either directly generate reproductive isolation between parasites living on different hosts, such as 'immigrant inviability' (i.e., lower fitness of immigrants in non-native environments), or that promote the

accumulation of host-specific genetic adaptations, such as the gallmaking feeding mode. In fact, some of these factors are shared across multiple case studies, suggesting that the occurrence of HAD is generalizable and can be predicted based on the incidence of significant biological and ecological factors. By means of a discriminant function analysis (DFA), this research assessed 108 arthropod parasite-host case studies for ecological and biological factors significantly correlated with the occurrence of HAD and whether these factors could be used to distinguish the presence of HAD from its absence. The DFA demonstrated that case studies that developed HAD could be distinguished from case studies that did not develop HAD. The results of the DFA were corroborated by a 'non-iterative partial least squares' (NIPALS) discriminant model and a nominal logistic regression. Case studies with HAD could be robustly separated from case studies without HAD based on the incidence of these predictive factors: immigrant inviability, gallmaking, endophagy, recent range invasions of either hosts or parasites, differential host phenology, and differential parasite morphology. These results were used in an infinite random forest analysis to generate a hierarchy of conditional probabilities that separated HAD presence from absence. The results provide researchers with a tool for reliably predicting which untested parasite-host system would likely develop HAD. Immigrant inviability, gallmaking, and their combination were strongly correlated with the presence of HAD, which indicated parasite-host systems with these traits were highly likely to develop HAD. Contrary to expectation, endophagous feeding was negatively correlated with HAD presence, which indicated phytophagous endophagous feeders (excepting gallmakers) were highly unlikely to develop HAD. Furthermore, parasitoids were shown to be just as likely to develop HAD as not. Unfortunately, potentially significant predictive factors (e.g., allochrony) were excluded from analysis because too few case studies have been specifically tested for these factors. Furthermore, this analysis was biased by the lack of 'negative' publication results and the overrepresentation of research laboratories that primarily study HAD. Future research should accumulate novel HAD case studies that specifically test for allochrony, differential microbial associations, and morphological differentiation.

Differences in timing of mating swarms in sympatric populations of *Anopheles coluzzii* and *Anopheles gambiae* s.s. (formerly *An. gambiae* M and S molecular forms) in Burkina Faso, West Africa

Sawadogo, SP; Costantini, C; Pennetier, C; Diabate, A; Gibson, G; Dabire, RK. 2013

Background: The M and S molecular forms of *Anopheles gambiae* s.s. Giles appear to have speciated in West Africa and the M form is now formally named *An. coluzzii* Coetzee & Wilkerson sp.n. and the S form retains the nominotypical name (abbreviated here to *An. gambiae*). Reproductive isolation is thought to be the main barrier to **hybridisation**; even though both species are found in the same mating swarms, **hybrid** fertilisations in copulae have not been found in the study area. The aim of the study, therefore, was to determine whether differences in circadian and/or environmental control over the timing of swarming in the two species contribute to reproductive isolation. Methods: The timing of male swarming in these species was recorded four nights per month over four years at five swarming sites in each of two villages. The timing of the start and end of swarming, and the concurrent environmental parameters, temperature, humidity and light intensity, were recorded for n = 20 swarms/month/species. The timing of 'spontaneous' activity at dusk of individual *An. coluzzii* and *An. gambiae* males was video-recorded in an actograph outdoors for 21 nights. Results: Of the environmental parameters considered, swarming was most strongly correlated with sunset ($r(2) > 0.946$). *Anopheles gambiae* started and stopped swarming earlier than *An. coluzzii* (3:35 +/- 0:68 min:sec and 4:51 +/- 1:21, respectively), and the mean duration of swarming was 23:37 +/- 0:33 for *An. gambiae* and 21:39 +/- 0:33 for *An. coluzzii*. Accordingly, in principle, whenever both species swarm over the same marker, a mean of 15.3 +/- 3.1% of *An. gambiae* swarming would occur before *An. coluzzii* males arrived, and 19.5 +/- 4.55% of *An. coluzzii* swarming would occur after *An. gambiae* males had stopped swarming. These results are consistent with the finding that *An. gambiae* males became active in the actograph 09:35 +/- 00:22 min:sec earlier than *An. coluzzii* males. Conclusions: The timing of swarming and spontaneous activity at dusk are primarily under circadian control, with the phase linked closely to sunset throughout the year. The mating activity of these two species is temporally segregated for 15-20% of the swarming period, which may contribute to the observed reproductive isolation of these species in local sympatric populations.

IN VITRO HYBRIDIZATION OF HAEMOPROTEUS SPP.: AN EXPERIMENTAL APPROACH FOR DIRECT INVESTIGATION OF REPRODUCTIVE ISOLATION OF PARASITES

Valkiunas, G; Iezhova, TA; Krizanauskiene, A; Palinauskas, V; Bensch, S. 2008

In spite of their potential as model organisms and their medical importance, parasite speciation processes have been insufficiently discussed in the literature. Scarcity of experimental data regarding reproductive isolation of parasites is a serious obstacle. Toward this end we developed a method for the investigation of **hybridization** of hemosporidian parasites *in vitro*. Five species of *Haemoproteus* (Haemosporida, Haemoproteidae) were isolated from naturally infected passerine birds. They were identified to species based on morphology of their gametocytes and PCR amplification of a segment of the parasite's mitochondrial cytochrome b gene. **Hybridization** of *Haemoproteus* spp. was initiated *in vitro* by mixing blood containing mature gametocytes of different species with a 3.7% solution of sodium citrate and exposure of the mixture to air. The following **hybridization** experiments were performed: (1) *Haemoproteus minutus* x *Haemoproteus pallidus*, (2) *Haemoproteus balmoralis* fringillae x *H. tartakovskyi*. The development of ookinetes of both species x *Haemoproteus tartakovskyi*, and (3) *Haemoproteus* was blocked in the first experiment. Ookinetes of all species developed in other experiments, but presumed **hybrids** were distinguished only in the third experiment. Illustrations of ookinete species are given. The present communication indicates that controlled experiments for direct **hybridization** of hemosporidians, can be carried out *in vitro*. Such experimental research can be used to reconcile molecular and morphological data and to define biological species for this group of parasites.

The molecular forms of *Anopheles gambiae*: A phenotypic perspective

Lehmann, T; Diabate, A. 2008

The African malaria mosquito *Anopheles gambiae* is undergoing speciation, being split into the M and S molecular forms. Speciation is the main process promoting biological diversity, thus, new vector species might complicate disease transmission. Genetic differentiation between the molecular forms has been extensively studied, but phenotypic differences between them, the evolutionary forces that generated divergence, and the mechanisms that maintain their genetic isolation have only recently been addressed. Here, we review recent studies suggesting that selection mediated by larval predation and competition promoted divergence between temporary and permanent freshwater habitats. These differences explain the sharp discontinuity in distribution of the molecular forms between rice fields and surrounding savanna, but they can also explain the concurrent cline between humid and arid environments due to the dependence on permanent habitats in the latter. Although less pronounced, differences in adult body size, reproductive output, and longevity also suggest that the molecular forms have adapted to distinct niches. Reproductive isolation between the molecular forms is achieved by spatial swarm segregation, although within-swarm mate recognition appears to play a role in certain locations. The implications of these results to disease transmission and control are discussed and many of the gaps in our understanding are highlighted. Published by Elsevier B.V.

Assortative mating and maintenance of intrapopulation polymorphism in wild populations and laboratory cultures of insects

Benkovskaya, GV; Nikonov, YM. 2015

Speciation as a micro-evolutionary process begins with emerging of intraspecific differentiation, which is associated with establishment of reproductive isolation. One of intrinsic isolation factors ensuring physiological isolation is assortative mating. In course of long-term field observations in the Southern Urals (Republic of Bashkortostan) and prolonged laboratory experiments, we have detected assortative mating in populations of potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Three morphotypes were singled out by the extent of integument melanization, namely achromists (A), melanists (M), and intermediate type (I), and frequency of occurrence of these morphotypes imago pairs significantly deviated from random distribution, thus manifesting the assortative mating. Under in copulo conditions, mating between achromist males (A) and melanist females (M) where active choice belonged to males was not registered. In laboratory experiments, in the sample of 40 artificially formed pairs, there were detected significant differences in longevity and fecundity of different morphotype imagoes.

Achromists and melanists had significantly (almost two times) higher longevity as compared with intermediate type. Females of intermediate type were significantly more fecund in homonomous crossings than achromists and melanists. It is shown that pairs offspring also differs significantly in viability, with highest viability being characteristic for offspring from females of A- and I-type. The differences revealed are indicative of different reproductive strategies that exist in populations. In laboratory line S of house fly *Musca domestica* L. (Diptera: Muscidae) the presence of individuals with different reproductive strategies related to longevity is detected. Maximum longevity in inbred lines Sh28 (short living) and L2 (long living), isolated from the line S, significantly differed almost twofold. In the line of short living flies, mass reproduction occurs on the first second week of imago stage, while in long living flies it is put off to the third fourth week. In reciprocal crossings between the lines, drastic reduction in females fecundity is observed as well as reduction in viability of females from **hybrid** offspring. The results obtained are indicative of substantial role of assortative mating and frequencydependent selection in maintenance of intrapopulation diversity.

It's about time: the evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella*, and its implications for fitness trade-offs in phytophagous insects

Feder, JL; Filchak, KE. 1999

The apple maggot fly, *Rhagoletis pomonella*, Walsh (Diptera: Tephritidae), provides a unique opportunity to address the issue of host-related fitness trade-offs for phytophagous insects. *Rhagoletis pomonella* has been controversial since the 1860's when Benjamin Walsh cited the fly's shift from hawthorn (*Crataegus* spp.) to apple (*Malus pumila*) as an example of an incipient sympatric speciation event. Allozyme and mark-release-recapture studies have subsequently confirmed the status of apple and hawthorn flies as partially reproductively isolated and genetically differentiated 'host races', the hypothesized initial stage in sympatric divergence. Here, we review the ecological and genetic evidence for host-plant mediated selection in *R. pomonella*. We reach the following three major conclusions: First, although developmental timing is not everything, it is a good deal of the story. Differences in the fruiting phenologies of apple and hawthorn trees exert different selection pressures on the diapause and eclosion time characteristics of the host races. In particular, the similar to 3-week earlier mean fruiting phenology of apples in eastern North America appears to select for a slower rate of metabolism or deeper pupal diapause in apple than hawthorn flies. Second, host-related fitness trade-offs for *R. pomonella* may not be due to disruptive selection affecting any one specific life-history stage. Rather, it is the sum total of directional selection pressures acting across different life-stages that generates divergent selection on apple and hawthorn flies. For example, selection favors the alleles Me 100, Acon-2 95 and Mpi 37 (or linked genes) in the larval stage in both host races. However, these same alleles are disfavored in the pupal stage to follow, where they correlate with early adult eclosion, and by inference premature diapause termination. Because apple trees fruit an average of similar to 3 weeks earlier than hawthorn trees, this counter-balancing selection is stronger on apple-fly pupae. The net result is that the balance of selective forces is different between apple and hawthorn flies, helping to maintain the genetic integrity of the host races in sympatry in the face of gene flow. Finally, natural *R. pomonella* populations harbor a good deal of genetic variation for development-related traits. This variation allows fly populations to rapidly respond to temporal vagaries in local environmental conditions across years, as well as to broad-scale geographic differences that exist across the range of the species. Perhaps most importantly, this variation gives *R. pomonella* the flexibility to explore and adapt to novel plants. Taken together, our results underscore how difficult it can be to document host plant-related fitness trade-offs for phytophagous insects due to the need to consider details of the entire life-cycle of a phytophagous insect. Our findings also show how reproductive isolation can arise as a by-product of host-associated adaptation in insects, a central theme for models of sympatric speciation via host shifts.

Ecological adaptation and reproductive isolation in sympatry: genetic and phenotypic evidence for native host races of *Rhagoletis pomonella*

Powell, THQ; Forbes, AA; Hood, GR; Feder, JL. 2014

Ecological speciation with gene flow may be an important mode of diversification for phytophagous insects. The recent shift of *Rhagoletis pomonella* from its native host downy hawthorn (*Crataegus mollis*) to introduced apple (*Malus domestica*) in the northeastern United States is a classic example of sympatric host race formation. Here, we test whether *R. pomonella* has similarly formed host races on four native *Crataegus* species in the southern United States: western mayhaw (*C. opaca*), blueberry hawthorn (*C. brachyacantha*), southern red hawthorn (*C. mollis* var. *texana*) and green hawthorn (*C. viridis*). These four southern hosts differ from each other in their fruiting phenology and in the volatile compounds emitted from the surface of their fruits. These two traits form the basis of ecological reproductive isolation between downy hawthorn and apple flies in the north. We report evidence from microsatellite population surveys and eclosion studies supporting the existence of genetically differentiated and partially reproductively isolated host races of southern hawthorn flies. The results provide an example of host shifting and ecological divergence involving native plants and imply that speciation with gene flow may be commonly initiated in *Rhagoletis* when ecological opportunity presents itself.

Identification of Host Fruit Volatiles from Snowberry (*Symphoricarpos albus*), Attractive to *Rhagoletis zephyria* Flies from the Western United States

Cha, DH; Olsson, SB; Yee, WL; Goughnour, RB; Hood, GR; Mattsson, M; Schwarz, D; Feder, JL; Linn, CE. 2017

A mixture of behaviorally active volatiles was identified from the fruit of snowberry, *Symphoricarpos albus laevigatus*, for *Rhagoletis zephyria* flies reared from snowberry fruit. A nine-component blend containing 3-methylbutan-1-ol (3%), dimethyl trisulfide (1%), 1-octen-3-ol (40%), myrcene (8%), nonanal (9%), linalool (13%), (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT, 6%), decanal (15%), and beta-caryophyllene (5%) was identified that gave consistent electroantennogram activity and was behaviorally active in flight tunnel tests. In other flight tunnel assays, snowberry flies from two sites in Washington state, USA, displayed significantly greater levels of upwind oriented flight to sources with the snowberry volatile blend compared with previously identified volatile blends from domestic apple (*Malus domestica*) and downy hawthorn (*Crataegus mollis*) fruit from the eastern USA, and domestic apple, black hawthorn (*C. douglasii*) and ornamental hawthorn (*C. monogyna*) from Washington state. Selected subtraction assays showed that whereas removal of DMNT or 1-octen-3-ol significantly reduced the level of upwind flight, removal of myrcene and beta-caryophyllene, or dimethyl trisulfide alone did not significantly affect the proportion of upwind flights. Our findings add to previous studies showing that populations of *Rhagoletis* flies infesting different host fruit are attracted to unique mixtures of volatile compounds specific to their respective host plants. Taken together, the results support the hypothesis that differences among flies in their behavioral responses to host fruit odors represent key adaptations involved in sympatric host plant shifts, contributing to host specific mating and generating prezygotic reproductive isolation among members of the *R. pomonella* sibling species complex.

Testing the potential contribution of *Wolbachia* to speciation when cytoplasmic incompatibility becomes associated with host-related reproductive isolation

Bruzzese, DJ; Schuler, H; Wolfe, TM; Glover, MM; Mastroni, JV; Doellman, MM; Tait, C; Yee, WL; Rull, J; Aluja, M; Hood, GR; Goughnour, RB; Stauffer, C; Nosil, P; Feder, JL. NA

Endosymbiont-induced cytoplasmic incompatibility (CI) may play an important role in arthropod speciation. However, whether CI consistently becomes associated or coupled with other host-related forms of reproductive isolation (RI) to impede the transfer of endosymbionts between **hybridizing** populations and further the divergence process remains an open question. Here, we show that varying degrees of pre- and post-mating RI exist among allopatric populations of two interbreeding cherry-infesting tephritid fruit flies (*Rhagoletis cingulata* and *R. indifferens*) across North America. These flies display allochronic and sexual isolation among populations, as well as unidirectional reductions in egg hatch in **hybrid** crosses involving southwestern USA males. All populations are infected by a *Wolbachia* strain, wCin2, whereas a second strain, wCin3, only co-infests flies from the southwest USA and Mexico. Strain wCin3 is associated with a unique mitochondrial DNA haplotype and unidirectional postmating RI, implicating the strain as the cause of CI. When coupled with nonendosymbiont RI barriers, we estimate the strength of CI associated with wCin3 would not prevent the strain from introgressing from infected southwestern to uninfected populations elsewhere in the USA if populations were to come into secondary contact and **hybridize**. In contrast, cytoplasmic-nuclear coupling may impede the transfer of wCin3 if Mexican and USA populations

were to come into contact. We discuss our results in the context of the general paucity of examples demonstrating stable *Wolbachia* **hybrid** zones and whether the spread of *Wolbachia* among taxa can be constrained in natural **hybrid** zones long enough for the endosymbiont to participate in speciation.

Interspecific Competition and Speciation in Endoparasitoids

Hood, GR; Egan, SP; Feder, JL. 2012

Ecological speciation occurs when inherent reproductive barriers to gene flow evolve between populations as a result of divergent natural selection. Frequency dependent effects associated with intraspecific resource competition are thought to be one important source of divergent selection facilitating ecological speciation. Interspecific competition may also play an important role in promoting population divergence. Although evidence for interspecific competition in nature is ubiquitous, there is currently little empirical data supporting its role in the speciation process. Here, we discuss two general models in which interspecific competition among species can promote ecological speciation among populations within a species. In both models, interspecific competition is the source of divergent selection driving adaption to different portions of the resource distribution, generating ecological reproductive isolation from other conspecific populations. We propose that the biology of endoparasitoids that attack phytophagous insects make model systems for studying the role of interspecific competition in ecological speciation. We describe details for one such system, the community of endoparasitic braconid wasps attacking *Rhagoletis* fruit flies, as a potential model for investigating competitive speciation. We conclude by hypothesizing that a model in which interspecific competition forces an inferior competitor to alternative fly hosts may be a common theme contributing to parasitoid diversification in the *Rhagoletis*-parasitoid system.

Incomplete concerted evolution and reproductive isolation at the rDNA locus uncovers nine cryptic species within *Anopheles longirostris* from Papua New Guinea

Alquezar, DE; Hemmerter, S; Cooper, RD; Beebe, NW. 2010

Background: Nuclear ribosomal DNA (rDNA) genes and transcribed spacers are highly utilized as taxonomic markers in metazoans despite the lack of a cohesive understanding of their evolution. Here we follow the evolution of the rDNA second internal transcribed spacer (ITS2) and the mitochondrial DNA cytochrome oxidase I subunit in the malaria mosquito *Anopheles longirostris* from Papua New Guinea (PNG). This morphospecies inhabits a variety of ecological environments indicating that it may comprise a complex of morphologically indistinguishable species. Using collections from over 70 sites in PNG, the mtDNA was assessed via direct DNA sequencing while the ITS2 was assessed at three levels - crude sequence variation through restriction digest, intragenomic copy variant organisation (homogenisation) through heteroduplex analysis and DNA sequencing via cloning. Results: Genetic evaluation of over 300 individuals revealed that *A. longirostris* comprises eight ITS2 PCR-RFLP genotypes and nine ITS2 heteroduplex genotypes showing distinct copy variant organization profiles after PCR amplification. Seven of these nine genotypes were found to be sympatric with other genotypes. Phylogenetic analysis of cloned ITS2 PCR products and mtDNA COI confirmed all nine clades with evidence of reproductive isolation at the rDNA locus. Compensatory base changes in the ITS2 secondary structure or in pseudoknots were absent when closely related species were assessed. Individuals from each ITS2 genotype showed the same copy variant heteroduplex profile suggesting that the rDNA array is fixed within each genotype. Conclusion: The centromere-proximal position of the rDNA array in *Anopheles* mosquitoes has probably reduced interchromosomal recombination leaving intrachromosomal events responsible for the observed pattern of concerted evolution we see in these mosquitoes. The stability of these intragenomic ITS2 copy variants within individuals and interbreeding populations suggests that rDNA is moving as a single evolutionary unit through natural populations to fixation and has provided a complementary diagnostic tool to the restriction digest for studying genetic discontinuities and species boundaries. In this, the utility of the ITS2 as a universal taxonomic marker is probably contingent on several factors pertaining to spacer dimensions and the genomic location of the rDNA array with respect to recombination and proximity to regions potentially under selection.

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.

Divergent diapause life history timing drives both allochronic speciation and reticulate hybridization in an adaptive radiation of *Rhagoletis* flies

Inskeep, KA; Doellman, MM; Powell, THQ; Berlocher, SH; Seifert, NR; Hood, GR; Ragland, GJ; Meyers, PJ; Feder, JL. NA

Divergent adaptation to new ecological opportunities can be an important factor initiating speciation. However, as niches are filled during adaptive radiations, trait divergence driving reproductive isolation between sister taxa may also result in trait convergence with more distantly related taxa, increasing the potential for reticulated gene flow across the radiation. Here, we demonstrate such a scenario in a recent adaptive radiation of *Rhagoletis* fruit flies, specialized on different host plants. Throughout this radiation, shifts to novel hosts are associated with changes in diapause life history timing, which act as "magic traits" generating allochronic reproductive isolation and facilitating speciation-with-gene-flow. Evidence from laboratory rearing experiments measuring adult emergence timing and genome-wide DNA-sequencing surveys supported allochronic speciation between summer-fruiting *Vaccinium* spp.-infesting *Rhagoletis mendax* and its hypothesized and undescribed sister taxon infesting autumn-fruiting sparkleberries. The sparkleberry fly and *R. mendax* were shown to be genetically discrete sister taxa, exhibiting no detectable gene flow and allochronically isolated by a 2-month average difference in emergence time corresponding to host availability. At sympatric sites across the southern USA, the later fruiting phenology of sparkleberries overlaps with that of flowering dogwood, the host of another more distantly related and undescribed *Rhagoletis* taxon. Laboratory emergence data confirmed broadly overlapping life history timing and genomic evidence supported on-going gene flow between sparkleberry and flowering dogwood flies. Thus, divergent phenological adaptation can drive the initiation of reproductive isolation, while also enhancing genetic exchange across broader adaptive radiations, potentially serving as a source of novel genotypic variation and accentuating further diversification.

COURTSHIP BEHAVIOR ANALYSIS IN THREE SIBLING SPECIES OF THE *DROSOPHILA VIRILIS* GROUP

Belkina, EG; Vedenina, VY; Sorokina, SY; Lazebny, OE. 2018

Courtship behavior was studied in three sibling species of the *Drosophila virilis* group, namely, *D. virilis*, *D. lummei*, and *D. littoralis*. The latter species was represented

by two strains, the founders of which had been caught in the habitats of the southern and northern races of *D. littoralis*, whose status is equivalent to subspecies. Con- and heterospecific tests were analyzed by video-typing. Analyses of conspecific tests of *D. virilis* and *D. lummei* revealed no differences in the duration of courtship elements and their latencies. Conversely, a comparison of heterospecific tests female *D. virilis* + male *D. lummei* and female *D. lummei* + male *D. virilis* showed a strong decrease in the duration of all main courtship elements (touching, licking, and singing), as well as a significant decrease in the percentage of copulation. Comparisons of the con- and heterospecific tests of the northern and southern races of *D. littoralis* revealed some differences in courtship structure. However, we did not find any obstacles for a successful mating in the heterospecific tests of the northern and southern races of *D. littoralis*. By contrast, large differences in the structure of the courtship ritual in heterospecific reciprocal tests of *D. littoralis* + *D. virilis* and *D. littoralis* + *D. lummei* were found. The males of the *D. virilis* phylad lost their interest to *D. littoralis* females immediately after the beginning of touching. On the contrary, *D. littoralis* males demonstrated a complete courtship towards both *D. lummei* and *D. virilis* females. At the same time, all heterospecific tests of *D. lummei* + *D. littoralis* and *D. virilis* + *D. littoralis* were characterized by the lowest percent of copulation.

Structure and Dynamics of Male Swarms of *Anopheles gambiae*

Manoukis, NC; Diabate, A; Abdoulaye, A; Diallo, M; Dao, A; Yaro, AS; Ribeiro, JMC; Lehmann, T. 2009

Mosquito swarms are poorly Understood mating aggregations. In the malaria vector *Anopheles gambiae* Giles, they are known to depend on environmental conditions, such as the presence of a marker on the ground, and they may be highly relevant to reproductive isolation. We present quantitative measurements of individual *An. gambiae* positions within swarms from Donegoubougou, Mali, estimated by stereoscopic video image analysis. Results indicate that swarms in this species are approximately spherical, with all unexpectedly high density of individuals close to the swarm centroid. This high density may be the result of individual males maximizing their probability of encountering a female or a product of mosquito orientation through cues within the swarm. Our analysis also suggests a difference in swarm organization between putative incipient species of *An. gambiae* with increasing numbers of males. This may be related to a difference in marker use between these groups, supporting the hypothesis that swarming behavior is a mechanism of mate recognition and ultimately reproductive isolation,

The *Diachasmimorpha longicaudata* complex: Reproductive isolation and geometric patterns of the wing

Kitthawee, S; Dujardin, JP. 2009

Diachasmimorpha longicaudata is an endoparasitoid of Tephritid fruit fly larvae and is regarded as an important biocontrol agent. However, it is likely that under this specific name several biological species may be contained, the correct identification of which is essential for effective use in control programs. In this paper, three populations (DLA, DLB and DLBB) of *D. longicaudata* designated according to geography and/or natural hosts were reared in the same laboratory. They were tested for reproductive compatibility and characterized by morphometric analyses. Forced-contact mating technique showed either complete lack of inter-population reproductive compatibility or the production of rare, sterile female offspring. The three populations, indistinguishable on the basis of morphological characters alone, were readily identified by the geometry of the wing. Results strongly suggest that the DLA, DLB and DLBB are distinct biological species, and highlight the usefulness of wing geometry to distinguish them. (C) 2009 Elsevier Inc. All rights reserved.

EVIDENCE THROUGH CROSSMATING EXPERIMENTS OF A SPECIES COMPLEX IN *ANOPHELES-PSEUDOPUNCTIPENNIS SENSU-LATO* - A PRIMARY MALARIA VECTOR OF THE AMERICAN CONTINENT

ESTRADA FRANCO, JG; MA, MC; GWADZ, RW; SAKAI, R; LANZARO, GC; LAUGHINGHOUSE, A; GALVANSANCHEZ, C; CESPEDES, JL; VARGASSAGARNAGA, R. 1993

Crossmating experiments were conducted to determine if postmating reproductive barriers are involved in the maintenance of genetic divergence among populations of *Anopheles pseudopunctipennis sensu lato*, a primary malaria vector of the American continent. Reciprocal crosses were conducted between colony and wild strains from Mexico, Bolivia, and Peru. **Hybridization** experiments revealed unidirectional male/female **hybrid** sterility in crosses between Mexican females and South American males. The data presented provide the first evidence that genetic differences exist among geographic strains of *An. pseudopunctipennis* in neotropical America. There is a consistent pattern suggesting the presence of at least two allopatric sibling species. One species occurs in central Mexico, the other in the South American Andean Cordillera.

"Patterns of speciation in *Drosophila*" revisited

Coyne, JA; Orr, HA. 1997

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 1.2-1.6 million 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.

Interspecific hybridization between *Liriomyza trifolii* and *Liriomyza sativae*

Sakamaki, Y; Miura, K; Chi, YC. 2005

Experimental interspecific **hybridization** between the notorious, worldwide horticultural crop pests *briomyza trifolii* (Burgess) and *Liriomyza sativae* Blanchard is reported. Premating reproductive isolation was easily disrupted under no-choice crossing conditions; **hybrid** flies were obtained only in one mating combination (female *L. sativae* X male *L. trifolii*). Crosses between them yielded only female progeny, which had some superficial characters identified as *L. sativae*, but the shape of the genitalia was similar to that of *L. trifolii*. All of the F1 **hybrids** were sterile and could not be backcrossed to either parental species. An electrophoretic analysis

of internal transcribed spacer region sequences on ribosomal DNA genes and a nucleotide sequence analysis of mitochondrial DNA cytochrome oxidase subunit I DNA sequences confirmed that the progeny were not the result of parthenogenetic reproduction.

Population genetics of *Anopheles koliensis* through Papua New Guinea: New cryptic species and landscape topography effects on genetic connectivity

Ambrose, L; Hanson, JO; Riginos, C; Xu, WX; Fordyce, S; Cooper, RD; Beebe, NW. 2019

New Guinea is a topographically and biogeographically complex region that supports unique endemic fauna. Studies describing the population connectivity of species through this region are scarce. We present a population and landscape genetic study on the endemic malaria-transmitting mosquito, *Anopheles koliensis* (Owen). Using mitochondrial and nuclear sequence data, as well as microsatellites, we show the evidence of geographically discrete population structure within Papua New Guinea (PNG). We also confirm the existence of three rDNA ITS2 genotypes within this mosquito and assess reproductive isolation between individuals carrying different genotypes. Microsatellites reveal the clearest population structure and show four clear population units. Microsatellite markers also reveal probable reproductive isolation between sympatric populations in northern PNG with different ITS2 genotypes, suggesting that these populations may represent distinct cryptic species. Excluding individuals belonging to the newly identified putative cryptic species (ITS2 genotype 3), we modeled the genetic differences between *A. koliensis* populations through PNG as a function of terrain and find that dispersal is most likely along routes with low topographic relief. Overall, these results show that *A. koliensis* is made up of geographically and genetically discrete populations in Papua New Guinea with landscape topography being important in restricting dispersal.

"Singing on the Wing" as a Mechanism for Species Recognition in the Malarial Mosquito *Anopheles gambiae*

Pennetier, C; Warren, B; Dabire, KR; Russell, IJ; Gibson, G. 2010

Anopheles gambiae, responsible for the majority of malaria deaths annually, is a complex of seven species and several chromosomal/molecular forms. The complexity of malaria epidemiology and control is due in part to *An. gambiae*'s remarkable genetic plasticity, enabling its adaptation to a range of human-influenced habitats. This leads to rapid ecological speciation when reproductive isolation mechanisms develop [1-6]. Although reproductive isolation is essential for speciation, little is known about how it occurs in sympatric populations of incipient species [2]. We show that in such a population of "M" and "S" molecular forms, a novel mechanism of sexual recognition (male-female flight-tone matching [7-9]) also confers the capability of mate recognition, an essential precursor to assortative mating; frequency matching occurs more consistently in same-form pairs than in mixed-form pairs ($p > 0.001$). Furthermore, the key to frequency matching is "difference tones" produced in the nonlinear vibrations of the antenna by the combined flight tones of a pair of mosquitoes and detected by the Johnston's organ. By altering their wing-beat frequencies to minimize these difference tones, mosquitoes can match flight-tone harmonic frequencies above their auditory range. This is the first description of close-range mating interactions in incipient *An. gambiae* species.

Genetic differentiation of *Ganaspis brasiliensis* (Hymenoptera: Figitidae) from East and Southeast Asia

Nomano, FY; Kasuya, N; Matsuura, A; Suwito, A; Mitsui, H; Buffington, ML; Kimura, MT. 2017

Ganaspis brasiliensis (Ihering) (Hymenoptera: Figitidae: Eucilinae) is a *Drosophila* parasitoid that has often been misidentified as *G. xanthopoda* (Ashmead) in recent studies. This study aims to clarify genetic differentiation of *G. brasiliensis* based on the nucleotide sequences of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene and three nuclear DNA regions, the inter-transcribed spacers 1 and 2 (ITS1 and ITS2) and putative 60S ribosomal protein L37 (RpL37), as well as crossing experiments. Four lineages are recognized in individuals assigned as *G. brasiliensis* by morphology, (1) individuals occurring in Japan and probably South Korea, (2) individuals from a small subtropical island of Japan, Iriomote-jima, (3) individuals from temperate lowlands of Japan and high altitude areas of Southeast Asia, and (4) individuals occurring widely in Asia, America, Hawaii and Africa. The first lineage is a specialist of *Drosophila suzukii* (Matsumura), a pest of fresh fruit, and also the fourth lineage has a capacity to parasitize this pest species. The first, third and fourth lineages occur sympatrically at least in Tokyo. The third and fourth lineages differed in mate choice and host use to some extent, but post-mating isolation between them was almost absent.

Chemically mediated sexual signals restrict hybrid speciation in a flea beetle

Xue, HJ; Segreaves, KA; Wei, J; Zhang, B; Nie, RE; Li, WZ; Yang, XK. 2018

The evolution of reproductive isolation following **hybridization** is a major obstacle that may limit the prevalence of **hybrid** speciation among specific groups of organisms. Here, we use a flea beetle system to offer a behavioral hypothesis for why there are so few examples of homoploid **hybrid** speciation among insects. Specifically, we examined cuticular hydrocarbon (CHC) mating signals and mate-choice decisions of *Altica fragariae* and *A. viridicyanea* to test whether the signals produced by **hybrids** cause prezygotic reproductive isolation. Although **hybrids** of *A. fragariae* and *A. viridicyanea* had unique CHC profiles as compared to the parental species, mate-choice trials indicated that these differences were insufficient to prevent gene flow between **hybrids** and parental species. We found that mate-choice decisions and CHC signals were not correlated. Considering the ubiquity of CHC signaling molecules in insects, we propose that decoupling of CHC signals and mate choice may be a general mechanism limiting **hybrid** speciation in insects.

Hybridization and sequential components of reproductive isolation between parapatric walnut-infesting sister species *Rhagoletis completa* and *Rhagoletis?zoqui*

Rull, J; Tadeo, E; Aluja, M; Guillen, L; Egan, SP; Feder, JL. 2012

Hybridization can provide a window into how populations diverge to form new species. Here, we confirm **hybridization** between *Rhagoletis completa* Cresson, 1929 and *Rhagoletis zoqui* Bush, 1966, two species of walnut husk-infesting flies that geographically overlap in a narrow area of parapatry in Northeastern Mexico. *Rhagoletis completa* and *R. zoqui* are members of a species group (*Rhagoletis suavis*) that has been hypothesized to speciate in allopatry. Sexual selection has been argued to be a potentially important factor for generating pre-mating isolation among walnut husk flies, because of the differences in wing morphology and body coloration, and the existence of sexual dimorphism within species. However, there was no evidence for pre-mating isolation between *R. completa* and *R. zoqui*, based on choice and no-choice mating experiments conducted on adults of fly populations outside the contact zone. There was also no support for reduced fertility of **hybrid** matings or for F1 inviability; however, F1 **hybrids** appeared to have lower fertility and F2 offspring have reduced survivorship. Postzygotic isolation in later generation **hybrids** of mixed ancestry therefore appears to be the first intrinsic barrier to gene flow evolving between *R. completa* and *R. zoqui*. We discuss the implications of our results for allopatric speciation in walnut flies and the potential evolutionary fate of *R. zoqui* and *R. completa* if they were to come into broad geographic contact in the future. (C) 2012 The Linnean Society of London, Biological Journal of the Linnean Society, 2012, , .

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.

Exon-Enriched Libraries Reveal Large Genic Differences Between *Aedes aegypti* from Senegal, West Africa, and Populations Outside Africa

Dickson, LB; Campbell, CL; Juneja, P; Jiggins, FM; Sylla, M; Black, WC. 2017

Aedes aegypti is one of the most studied mosquito species, and the principal vector of several arboviruses pathogenic to humans. Recently failure to oviposit, low fecundity, and poor egg-to-adult survival were observed when *Ae. aegypti* from Senegal (SenAae) West Africa were crossed with *Ae. aegypti* (Aaa) from outside of Africa, and in SenAae intercrosses. Fluorescent in situ **hybridization** analyses indicated rearrangements on chromosome 1, and pericentric inversions on chromosomes 2 and 3. Herein, high throughput sequencing (HTS) of exon-enriched libraries was used to compare chromosome-wide genetic diversity among Aaa collections from rural Thailand and Mexico, a sylvatic collection from southeastern Senegal (PK10), and an urban collection from western Senegal (Kaolack). Sex-specific polymorphisms were analyzed in Thailand and PK10 to assess genetic differences between sexes. Expected heterozygosity was greatest in SenAae. F-ST distributions of 15,735 genes among all six pairwise comparisons of the four collections indicated that Mexican and Thailand collections are genetically similar, while F-ST distributions between PK10 and Kaolack were distinct. All four comparisons of SenAae with Aaa indicated extreme differentiation. F-ST was uniform between sexes across all chromosomes in Thailand, but were different, especially on the sex autosome 1, in PK10. These patterns correlate with the reproductive isolation noted earlier. We hypothesize that cryptic *Ae. aegypti* taxa may exist in West Africa, and the large genic differences between Aaa and SenAae detected in the present study have accumulated over a long period following the evolution of chromosome rearrangements in allopatric populations that subsequently cause reproductive isolation when these populations became sympatric.

Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish

Selz, OM; Thommen, R; Maan, ME; Seehausen, O. 2014

Hybrid speciation is constrained by the homogenizing effects of gene flow from the parental species. In the absence of post-mating isolation due to structural changes in the genome, or temporal or spatial premating isolation, another form of reproductive isolation would be needed for homoploid **hybrid** speciation to occur. Here, we investigate the potential of behavioural mate choice to generate assortative mating among **hybrids** and parental species. We made three-first-generation **hybrid** crosses between different species of African cichlid fish. In three-way mate-choice experiments, we allowed **hybrid** and non**hybrid** females to mate with either **hybrid** or non**hybrid** males. We found that **hybrids** generally mated nonrandomly and that **hybridization** can lead to the expression of new combinations of traits and preferences that behaviourally isolate **hybrids** from both parental species. Specifically, we find that the phenotypic distinctiveness of **hybrids** predicts the symmetry and extent of their reproductive isolation. Our data suggest that behavioural mate choice among **hybrids** may facilitate the establishment of isolated **hybrid** populations, even in proximity to one or both parental species.

Intraspecific morphological variation of the scorpionfly *Dicerapanorpa magna* (Chou) (Mecoptera: Panorpidae) based on geometric morphometric analysis of wings

Liu, M; Ma, N; Hua, BZ. 2016

Wing shape variation was investigated between the sexes and among four populations of the scorpionfly *Dicerapanorpa magna* (Chou, 1981) endemic to the Qin-Ba Mountains area, China through the landmark-based geometric morphometric approach. The results show that sexual dimorphism exists both in wing size and shape in *D. magna*. Significant differences exist in female wing size and shape among *D. magna* populations. The possible reasons of the wing variation are discussed based on the divergence time of *D. magna* in combination with the tectonic and climatic events in the Qin-Ba Mountains during the late Miocene-Pleistocene period. Whether reproductive isolation exists between different populations needs further research.

Cytotypes of the *Simulium arcticum* species complex in the American Pacific Northwest

Shields, GF. NA

The larval polytene chromosomes of 17 of the 22 known cytotypes of the *Simulium arcticum* Malloch complex were analyzed and compared at 59 collection sites in the Pacific Northwest of the United States. Cytotypes range from those that were broadly distributed, such as IIL-9, which was found at 25 different sites, to those that were found at only one or two sites, such as IIL-73-74. Cytotypes are "clustered" in a geographic sense, suggesting that once a new cytotype is described, if it is found again, it will be found nearby. The cytotype *S. arcticum* IIL-9 was found at 11 of the 25 sites where *S. arcticum* IIL-19 exists. Finally, based on its sibling species status and widespread distribution throughout the Pacific Northwest, *Simulium brevicercum* may be an early ancestor of the complex given that it occurs at 44 of the 59 sites sampled. The observations and conclusions of this research are scientifically important because previous research suggests that reproductive isolation may have begun among these cytotypes at these sites, and unless their locations are reported, they cannot be studied further. Future work should incorporate a multi-locus approach to study the putative initial divergences within this complex.

Species delimitation and mitonuclear discordance within a species complex of biting midges

Shults, P; Hopken, M; Eyer, PA; Blumenfeld, A; Mateos, M; Cohnstaedt, LW; Vargo, EL. 2022

The inability to distinguish between species can be a serious problem in groups responsible for pathogen transmission. Culicoides biting midges transmit many pathogenic agents infecting wildlife and livestock. In North America, the *C. variipennis* species complex contains three currently recognized species, only one of which is a known vector, but limited species-specific characters have hindered vector surveillance. Here, genomic data were used to investigate population structure and genetic differentiation within this species complex. Single nucleotide polymorphism data were generated for 206 individuals originating from 17 locations throughout the United States and Canada. Clustering analyses suggest the occurrence of two additional cryptic species within this complex. All five species were significantly differentiated in both sympatry and allopatry. Evidence of **hybridization** was detected in three different species pairings indicating incomplete reproductive isolation.

Additionally, COI sequences were used to identify the **hybrid** parentage of these individuals, which illuminated discordance between the divergence of the mitochondrial and nuclear datasets.

Bridges and barriers to host shifts resulting from host plant genotypic variation

Craig, TP; Itami, JK; Ohgushi, T; Ando, Y; Utsumi, S. 2011

Host-associated differentiation is hypothesized to be one of the primary means by which new species of herbivorous insects evolve. Divergent selection for host plant use following a host shift is a critical assumption of host-associated differentiation. Host shifts are more likely to occur between closely related host species, and the probability of a host shift and of the evolution of reproductive isolation depends on the genetic variation within both the host plant population and the herbivorous insect. We have been studying the evolution of reproductive isolation in populations of *Eurosta solidaginis* that form galls on *Solidago altissima altissima* and *Solidago altissima gilvocanescens*. Each of the *Eurosta* populations is a host race that is partially but incompletely isolated from the other host races by its adaptation to its host plants. The *Solidago* sp. populations show high intraspecific variation within and among populations in their resistance to attack by the host races which are adapted to them. There is also evidence that they vary in their susceptibility to host races adapted to other *Solidago* species. We examine the hypothesis that the intraspecific *Solidago* variation determines the degree of gene flow among *Eurosta* populations and that this can create barriers or bridges to gene flow and therefore determine the course of speciation in these insects. The intraspecific host plant variation can influence the interactions with herbivores when it is an invasive species. The restricted range of genetic variation introduced into a new region may be a good or poor match with populations of herbivores that are also introduced into the new area.

Genetic diversity within and among populations of *Diprion pini* (Hym., Diprionidae) determined by random amplified polymorphic DNA-polymerase chain reaction of haploid males

Baumann, R; Schubert, R; Heitland, W; Auger-Rozenberg, MA; Faivre-Rimpant, P; Muller-Starck, G. 2003

Based on haploid males, random amplified polymorphic DNA (RAPD) markers were used to study genetic variation within and among four French populations and one Finnish outgroup population of the common pine sawfly, *Diprion pini* (L.), representing a severe European forest pest associated with mass outbreaks. Taking into account that all multilocus haplotypes were detected, a total of 140 individuals were completely discriminated by means of 17 polymorphic markers (present or absent), which were amplified from nine selected random-decamer primers. All populations shared the same genetic types, but pronounced population-specific frequency distributions were found, indicating that on average 84% of the present genetic variation exists within populations and the remaining smaller part counts for interpopulational variation. The haplotype differentiation registered was able to distinguish between the class of lowland populations and the class of highland populations. Reproductive isolation and therefore limited altitudinal gene flow, indicated in case of a French highland population by an enlarged number of fixed markers, and hitchhiking effects with respect to selective processes at certain loci following local adaptation and speciation are discussed to explain the population structures found. A possible selection is indicated by five markers, showing significantly different frequency distributions between the class of highland populations and the class of lowland populations.

Delineating taxonomic boundaries in the largest species complex of black flies (Simuliidae) in the Oriental Region

Low, VL; Takaoka, H; Pramual, P; Adler, PH; Ya'cob, Z; Huang, YT; Pham, XD; Ramli, R; Chen, CD; Wannaket, A; Sofian-Azirun, M. 2016

Perspicuous assessments of taxonomic boundaries and discovery of cryptic taxa are of paramount importance in interpreting ecological and evolutionary phenomena among black flies (Simuliidae) and combating associated vector-borne diseases. *Simulium tani* Takaoka & Davies is the largest and perhaps the most taxonomically challenging species complex of black flies in the Oriental Region. We use a DNA sequence-based method to delineate currently recognized chromosomal and morphological taxa in the *S. tani* complex on the Southeast Asian mainland and Taiwan, while elucidating their phylogenetic relationships. A molecular approach using multiple genes, coupled with morphological and chromosomal data, supported recognition of cytoform K and morphoform 'b' as valid species; indicated that *S. xuandei*, cytoform L, and morphoform 'a' contain possible cryptic species; and suggested that cytoform B is in the early stages of reproductive isolation whereas lineage sorting is incomplete in cytoforms A, C, and G.

Phylogenetic relationships among holarctic populations of *Chironomus entis* and *Chironomus plumosus* in view of possible horizontal transfer of mitochondrial genes

Guryev, VP; Blinov, AG. 2002

In eight Holarctic populations of two typical chironomid sibling species of the plumosus group, *Chironomus entis* and *Chironomus plumosus*, nucleotide sequences of mitochondrial (cytb) and nuclear (gb2b) gene regions were examined. The phylogenetic trees reflecting the evolutionary histories of the nuclear and mitochondrial markers exhibited significant differences. On the tree based on the nuclear gene sequences the populations clustered according to their species affiliation, whereas on the tree based on the mitochondrial gene sequences the populations were grouped according to their geographic position. This discrepancy is probably explained by mitochondrial gene flow between sympatric species with incomplete reproductive isolation (sibling species). Based on our results together with the earlier data on nuclear and mitochondrial gene sequences of some other species from the phylogenetic group plumosus, a scheme of phylogenetic relationships within this group is proposed. This scheme is in many ways different from the traditional view on the evolutionary relationships among species of the plumosus group.

Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation

Favia, G; dellaTorre, A; Bagayoko, M; Lanfrancotti, A; Sagnon, N; Toure, YT; Coluzzi, M. 1997

Three chromosomal forms of *Anopheles gambiae* s.s., designated as Bamako, Mopti and Savanna, were studied for diagnostic PCR assays based on the analysis of the X-linked ribosomal DNA (rDNA). The study was performed on a 1.3 kb fragment containing part of the 28S coding region and part of the intergenic spacer region. The amplified material was cut with fourteen restriction enzymes to detect Restriction Fragment Length Polymorphisms (RFLPs). The enzymes Tru9I and HhaI produced patterns of DNA bands which differentiated Mopti from Savanna and Bamako; moreover, a distinct '**hybrid**' pattern was recognized in the F₁ female progeny from the cross of Mopti with either one of the other two chromosomal forms. The diagnostic significance of the PCR-RFLP assay was verified on 203 karyotyped females from field samples collected in two villages in Mali and one village in Burkina Faso. Agreement was observed between the chromosomal and the molecular identifications. No '**hybrid**' molecular patterns were detected even among carriers of rare heterokaryotypes hypothetically produced by crosses between Mopti and Savanna. The results confirm previous observations indicating barriers to gene flow within *An. gambiae* s.s. and supporting the specific status of the taxonomic units proposed on cytogenetic ground.

Inter-population mating success in Australian dengue vector mosquitoes: effects of laboratory colonization and implications for the spread of transgenics

Richardson, AJ; Williams, CR. 2013

Variability between *Aedes aegypti* populations in north Queensland, Australia, has the potential to impact the successful implementation of new population replacement mosquito releases for dengue control. Four *Ae. aegypti* colonies originating from different locations (Cairns, Mareeba, Innisfail, and Charters Towers), along with one F1 field-derived population from Cairns, were inter-crossed to determine any incompatibilities in copulation, insemination, and production of viable offspring. Greater copulation and insemination rates were observed when males recently introduced from the wild (Cairns-Wild' population) were mated with long-term laboratory females. Egg viability rates for all crosses ranged from 90.298.2%, with no significant differences observed between crosses. Greater egg production was seen in some populations, and when corrected for wing-length, egg production was greatest in a Mareeba x Innisfail cross (19.55 eggs/mm wing length) and lowest for the Charters Towers intra-population cross (14.35 eggs/mm). Additionally, behavioral differences were observed between laboratory and wild mosquitoes from the Cairns location, suggesting possible laboratory conditioning. Finally, despite controlled larval rearing conditions, size differences between populations existed with Charters Towers mosquitoes consistently smaller than the other populations. The spread of genes or bacterial symbionts between these populations is unlikely to be hindered by pre-existing reproductive barriers.

Fruit odor discrimination and sympatric host race formation in *Rhagoletis*

Linn, C; Feder, JL; Nojima, S; Dambroski, HR; Berlocher, SH; Roelofs, W. 2003

Rhagoletis pomonella is a model for incipient sympatric speciation (divergence without geographic isolation) by host-plant shifts. Here, we show that historically derived apple- and ancestral hawthorn-infesting host races of the fly use fruit odor as a key olfactory cue to help distinguish between their respective plants. In flight-tunnel assays and field tests, apple and hawthorn flies preferentially oriented to, and were captured with, chemical blends of their natal fruit volatiles. Because *R. pomonella* rendezvous on or near the unabsorbed fruit of their hosts to mate, the behavioral preference for apple vs. hawthorn fruit odor translates directly into premating reproductive isolation between the fly races. We have therefore identified a key and recently evolved (<150 years) mechanism responsible for host choice in *R. pomonella* bearing directly on sympatric host race formation and speciation.

The saga of mosquito evolution

Rai, KS. 2011

In view of their importance as vectors, many mosquito genera and species in Dipteran family Culicidae have been the subjects of extensive cytological, genetic and evolutionary studies over the last more than 50 years. As a result, a great deal of information has accumulated on chromosome morphology and evolution, heterochromatin distribution and differentiation, and molecular structure, organization and evolution of genomes in this family. Numerically the haploid chromosome number ($n = 3$) has remained virtually unchanged. However extensive variation exists at different levels of genomic structure and organization. Anophelinae have heteromorphic sex chromosomes, a small genome size and repetitive elements are distributed in a long-period interspersed pattern. In contrast, Culicinae have homomorphic sex chromosomes and repetitive DNA is organized in a short-period interspersed pattern. There has been a general increase in genome size during the evolution of Culicine taxa. Heteromorphic sex chromosomes evolved early in the evolution of Anophelinae and a long-period interspersed pattern was retained. Intensive multi-point linkage map studies suggest that recombination frequencies per genome have remained relatively constant over the course of culicid evolution. As Anophelinae possess relatively small genome size hence taxa in Anophelinae have a higher amount of recombination per haploid genome size than Culicinae. Undoubtedly, family Culicidae represents one of the best studied systems of genome evolution in animals. It is helpful to ascertain the mechanics of sympatric v/s allopatric speciation in closely related group of species.

Frequency of multiple inseminations in field-collected *Anopheles gambiae* females revealed by DNA analysis of transferred sperm

Tripet, F; Toure, YT; Dolo, G; Lanzaro, GC. 2003

We investigated the frequencies of single and multiple matings in field-collected female *Anopheles gambiae* by conducting microsatellite DNA analyses on the sperm contained within their spermatheca. Amplification by a polymerase chain reaction (PCR) at four loci allowed the detection of sperm extracts exhibiting more than two alleles per locus, thereby revealing the occurrence of multiple inseminations. Polyandry was found in six of 239 females examined, or 2.5% of the samples. Previous analyses of the molecular form of the sperm and female extracts using a PCR-based diagnostic procedure showed that two of these multiple inseminations involved cross-mating between two chromosomal/molecular forms of *An. gambiae* s.s. Thus polyandry occurred within-form in 1.7% of examined females while other multiple inseminations may be linked to processes of reproductive isolation between forms of *An. gambiae*.

Age-dependent insemination success of sterile Mediterranean fruit flies

Taylor, PW; Kaspi, R; Mossinson, S; Yuval, B. 2001

The Sterile Insect Technique (SIT) is used in many regions worldwide to manage wild populations of the Mediterranean fruit fly ('medfly'). an important pest species. This technique relies on released sterile males outcompeting their wild counterparts in fertilizing ova of wild females. Numerous studies have investigated the ability of sterile males to secure copulations, an essential step toward overall success. Here we progress further along the mating sequence by studying reproductive barriers that may remain ahead of sterile males that manage to secure copulations in field cage experiments and whether ability to pass these barriers is influenced by a males age, diet and size, or the size of his mate. Amongst those virgin males that succeeded in copulating, both the number of sperm stored by mates and the chances of having any sperm stored at all decreased with age. Sperm tended to be stored asymmetrically between the females two spermathecae, and this tendency was more apparent when few sperm were stored. In accord with effects of male age on number of sperm stored, sperm of older males were stored more asymmetrically than that of young males. We found no evidence that male size, male diet or female size influenced copula duration, number of sperm stored or allocation of sperm between the female's two spermathecae. The decline in number of sperm stored as males aged was not accompanied by age-dependent changes in copula duration, indicating that copula duration and insemination success are not deterministically linked. We discuss these results in light of their relevance to SIT and the medfly mating system.

Divergence and correlated evolution of male wing spot and courtship display between *Drosophila nepalensis* and *D. trilineata*

Mo, WZ; Li, ZM; Deng, XM; Chen, AL; Ritchie, MG; Yang, DJ; He, ZB; Toda, MJ; Wen, SY. NA

Male-specific wing spots are usually associated with wing displays in the courtship behavior of *Drosophila* and may play important roles in sexual selection. Two closely related species, *D. nepalensis* and *D. trilineata*, differ in wing spots and scissoring behavior. Here, we compare male morphological characters, pigmentation intensity of male wing spots, wing-scissoring behavior, courtship songs, and reproductive isolation between 2 species. F1 fertile females and sterile males result from the cross between females of *D. nepalensis* and males of *D. trilineata*. The pigmentation of wing spots is significantly weaker in *D. trilineata* than in *D. nepalensis* and the

F1 hybrid. Males scissor both wings in front of the female during courtship, with a posture spreading wings more widely, and at a faster frequency in *D. nepalensis* than in *D. trilineata* and the F1s. Males of *D. trilineata* vibrate wings to produce 2 types (A and B) of pulse songs, whereas *D. nepalensis* and the F1s sing only type B songs. The incidence of wing vibration and scissoring during courtship suggests that wing vibration is essential but scissoring is a facultative courtship element for successful mating in both species. The association between the darker wing spots with more elaborate scissoring might be the consequence of correlated evolution of these traits in *D. nepalensis*; however, *D. trilineata* retains wing scissoring during courtship despite having weaker pigmentation of wing spots. The genetic architecture of 2 traits differs in the F1s, consistent with maternal or sex-linked effects for spots but nonadditive effects for scissoring.

***Drosophila yakuba mayottensis*, a new model for the study of incipient ecological speciation**

Yassin, A. 2017

A full understanding of how ecological factors drive the fixation of genetic changes during speciation is obscured by the lack of appropriate models with clear natural history and powerful genetic toolkits. In a recent study, we described an early stage of ecological speciation in a population of the generalist species *Drosophila yakuba* (melanogaster subgroup) on the island of Mayotte (Indian Ocean). On this island, flies are strongly associated with the toxic fruits of noni (*Morinda citrifolia*) and show a partial degree of pre-zygotic reproductive isolation. Here, I mine the nuclear and mitochondrial genomes and provide a full morphological description of this population. Only 29 nuclear sites ($< 4 \times 10^{-7}$) of the genome are fixed in this population and absent from 3 mainland populations and the closest relative *D. santomea*, but no mitochondrial or morphological character distinguish Mayotte flies from the mainland. This result indicates that physiological and behavioral traits may evolve faster than morphology at the early stages of speciation. Based on these differences, the Mayotte population is designated as a new subspecies, *Drosophila yakuba mayottensis* subsp. nov., and its strong potential in understanding the genetics of speciation and plant-insect interactions is discussed.

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.

Tracking factors modulating cytoplasmic incompatibilities in the mosquito *Culex pipiens*

Duron, O; Bernard, C; Unal, S; Berthomieu, A; Berticat, C; Weill, M. 2006

Wolbachia are maternally inherited endosymbiotic bacteria that infect many arthropod species and may induce cytoplasmic incompatibility (CI), resulting in abortive embryonic development. One *Wolbachia* host, *Culex pipiens* complex mosquitoes, displays high levels of variability in both CI crossing types (cytotypes) and DNA markers. We report here an analysis of 14 mosquito strains, containing 13 *Wolbachia* variants, and with 13 different cytotypes. Cytotypes were *Wolbachia*-dependent, as antibiotic treatment rendered all strains tested compatible. Cytotype distributions were independent of geographical distance between sampling sites and host subspecies, suggesting that *Wolbachia* does not promote a reproductive isolation depending on these parameters. Backcross analysis demonstrated a mild restoring effect of the nuclear genome, indicating that CI is mostly cytoplasmically determined for some crosses. No correlation was found between the phenotypic and genotypic variability of 16 WO prophage and transposon markers, except for the WO prophage Gp15 gene, which encodes a protein similar to a bacterial virulence factor. However, Gp15 is partially correlated with CI expression, suggesting that it could be just linked to a CI gene.

Forced-contact mating: A technique for crossing experiments with the fruit fly parasitoid, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera : Braconidae)

Kitthawee, S. 2008

In this paper, a new technique is described for successfully manipulating the mating of a braconid parasitoid (Hymenoptera: Braconidae) of tephritid fruit flies. This forced-contact-mating technique was first developed for cross-mating experiments to determine the inheritance of winglessness in *Diachasmimorpha longicaudata* (Ashmead). Since female *D. longicaudata* only result from fertilized eggs (unfertilized eggs become males), this mating technique has advantages in the mass production of females for biocontrol releases as well as in investigations on the inheritance of the wingless trait and studies of reproductive isolation among different populations of these parasitoids. Free-mated colonies were generally all winged and predominantly male. Wingless males occurred occasionally but wingless females were rare. Virgin, winged females were immobilized by chilling and placed in close contact with wingless males. Active wingless males readily mounted and mated with immobilized female. Progeny of these mated females were all winged (similar to 83% male and 17% female). When F1 females remained unmated they produced both winged and wingless males (similar to 1: 1 ratio) but when immobilized F1 females were back-crossed with wingless males, both winged and wingless females (similar to 5:1 ratio) were produced in addition to winged and wingless males. The wingless character was thus determined to be controlled by a recessive gene. Crossing experiments between two different Thai populations of *D. longicaudata* provided evidence that these populations were reproductively isolated. Among free-mated pairs, some sperm transfer occurred but almost no female progeny were produced. Similarly, among forced-mated pairs, more than double the numbers of females had sperm transferred to their spermatheca, but few female progeny were still produced. This suggests that these two populations are reproductively isolated and are part of a closely related species complex. (c) 2007 Elsevier Inc. All rights reserved.

Differences in morphological traits between two sibling species, *Drosophila ananassae* and *D. pallidosa*

Vishalakshi, C; Singh, BN. 2008

Capturing the mechanisms of speciation that appear in the early stages of reproductive isolation has been of recent interest to evolutionary biologists. In the present study, we investigated interspecific variations in several morphological traits, the degree of crossability, productivity, and the sex ratio in 2 sibling species, *Drosophila ananassae* and *D. pallidosa*, and their **hybrids**. The present species pair is unique due to the presence of strong sexual isolation and the absence of postmating barriers such as **hybrid** inviability or sterility. The 2 sibling species significantly differed in the following morphological traits: thorax length, sternopleural bristle number, wing length, wing-to-thorax ratio, sex comb tooth number, and ovariole number in males and females. Interspecific **hybrids** also significantly differed from their parental species in all morphological traits. Further, the degree of crossability and productivity was greater with conspecific matings than with heterospecific matings. Moreover, we found no sex ratio distortion in interspecific **hybrids** suggesting that there are fewer genetic incompatibilities between these 2 sibling species.

These results are interpreted in terms of the evolutionary divergence between *D. ananassae* and its sibling species, *D. pallidosa*.

Breeding structure of the sand fly *Lutzomyia longipalpis* (Lutz & Neiva) in Brazil

Mutebi, JP; Alexander, B; Sherlock, I; Wellington, J; Souza, AA; Shaw, J; Rangel, EF; Lanzaro, GC. 1999

Eleven populations of *Lutzomyia longipalpis* (Lutz & Neiva), the sand fly vector of *Leishmania chagasi*, from different areas of Brazil were analyzed for genetic variation at 16 enzyme loci. In this region, the prevalence of visceral leishmaniasis (VL) caused by *L. chagasi* is spotty and reproductive isolation among populations of *L. longipalpis* has been reported. It is thought that morphologically similar cryptic species with varying vectorial capacity may be responsible for the discontinuous distribution of VL. The aim was to study the genetic structure of populations within this region and to identify demes that may represent sibling species. Genotypic frequencies within populations were in close compliance to Hardy-Weinberg expectations, suggesting there are no sympatric species among these 11 populations. Levels of genetic distance between pairs of populations were very low (< 0.03), consistent with local populations within a single sand fly species. When genotypic frequency data for all populations were pooled, 9 of the 13 polymorphic loci deviated from Hardy-Weinberg expectations, indicating some degree of genetic substructuring. Estimates of effective migration rates ($N(e)m$) among all populations were low, 2.73, suggesting that gene flow is restricted among populations, which is probably the reason for the observed genetic substructuring.

Symbionts do not affect the mating incompatibility between the Brazilian-1 and Peruvian morphotypes of the *Anastrepha fraterculus* cryptic species complex

Devescovi, F; Conte, CA; Augustinos, A; Martinez, EIC; Segura, DF; Caceres, C; Lanzavecchia, SB; Bourtzis, K. 2019

The South American fruit fly, *Anastrepha fraterculus*, is clearly undergoing a speciation process. Among others, two of their morphotypes, the Brazilian-1 and Peruvian, have accumulated differences in preand post-zygotic mechanisms resulting in a degree of reproductive isolation. Both harbor a different strain of *Wolbachia*, which is a widespread endosymbiotic bacterium among many invertebrates producing a range of reproductive effects. In this paper, we studied the role of this bacterium as one of the factors involved in such isolation process. Infected and cured laboratory colonies were used to test pre- and post-zygotic effects, with special emphasis in uni- and bi-directional cytoplasmic incompatibility (CI). We showed that *Wolbachia* is the only known reproductive symbiont present in these morphotypes. *Wolbachia* reduced the ability for embryonic development in crosses involving cured females and infected males within each morphotype (uni-directional CI). This inhibition showed to be more effective in the Peruvian morphotype. Bi-directional CI was not evidenced, suggesting the presence of compatible *Wolbachia* strains. We conclude that *Wolbachia* is not directly involved in the speciation process of these morphotypes. Other mechanisms rather than CI should be explored in order to explain the reduced mating compatibility between the Brazilian-1 and Peruvian morphotypes.

Genetic differentiation and cryptic speciation in natural populations of *Drosophila lacertosa*

He, LP; Watabe, H; Xiangyu, JG; Gao, JJ; Liang, XC; Aotsuka, T; Zhang, YP. 2007

Drosophila lacertosa, an Oriental member of the robusta species group in the virilis-repleta radiation, has a wide distribution from northern India throughout China to the Far East. Phylogenetic analyses of mitochondrial ND2 gene sequences revealed two genetically significantly diverged lineages with 2.1% DNA sequence divergence. These two lineages are largely allopatric: one is mainly found in southwest China (lineage A), whereas the other ranges central and east of China to northeast Asia (lineage B). The geographic distributions of these two clades narrowly overlap near the provincial boundaries of Yunnan and Guizhou or Guangxi, probably as a consequence of population expansion and secondary contact. These two lineages were estimated to have diverged about 0.56 Mya, and the estimated time of expansion was approximately 70,000 years ago for lineage A population, and 110,000 years ago for lineage B population. The present molecular analysis, together with nearly complete reproductive isolation between those two lineages, strongly suggests that the two lineages might be incipient species. (c) 2006 Elsevier Inc. All rights reserved.

Pre-copula acoustic behaviour of males in the malarial mosquitoes *Anopheles coluzzii* and *Anopheles gambiae* s.s. does not contribute to reproductive isolation

Simoes, PMV; Gibson, G; Russell, IJ. 2017

We reveal that males of two members of the *Anopheles gambiae* s. l. species complex, *Anopheles coluzzii* and *Anopheles gambiae* s. s. (hereafter *A. gambiae*), which are both malaria vectors, perform a stereotypical acoustic behaviour in response to pure tones at frequencies that encompass the frequency range of the female's flight-tones. This behaviour resembles that described for *Culex quinquefasciatus* and consists of phonotactic flight initiated by a steep increase in wing-beat frequency (WBF) followed by rapid frequency modulation (RFM) of WBF when in close proximity to the sound source. RFM was elicited without acoustic feedback or the presence of a live female, but it appears to be a stereotypic behaviour in the immediate lead up to copula formation. RFM is an independent and different behavioural process from harmonic convergence interactions used by male-female pairs for mate recognition at earlier stages of mating. Acoustic threshold for RFM was used to plot behavioural audiograms from free-flying *A. coluzzii* and *A. gambiae* males. These audiograms were almost identical (minima similar to 400 Hz) and encompassed the WBF ranges of *A. coluzzii* (378-601 Hz) and *A. gambiae* (373-590 Hz) females, indicating that males of the two species share similar frequency tuning and range. Furthermore, no differences were found between the two species in their WBFs, RFM behaviour or harmonic convergence ratios. These results indicate that assortativemating between *A. coluzzii* and *A. gambiae* is unlikely to be based on male-specific acoustic behaviours during RFM. The significance of these findings in relation to possible mechanisms for assortative mating is discussed.

The evolution of insect mating structures through sexual selection

Sirof, LK. 2003

Mating structures are of interest to a wide range of biologists because, in many taxa, mating structures are incredibly diverse and range widely in elaboration even between closely related species. As a result of this diversity, mating structures have been useful in species identification. Historically, the evolution of diverse mating structures has been attributed to post-zygotic selection for pre-zygotic isolation to avoid production of **hybrid** offspring. More recently, sexual selection has been proposed as an alternative explanation for the rapid diversification of mating structures. Mating structures could diversify between populations through sexual selection if sexual selection acted differently on mating structures in different populations. Eberhard (1985) wrote a comprehensive book explaining how sexual selection could result in the diversification of mating structures and providing examples to support the hypothesis, but none of the examples were experimental tests of the hypothesis. Since 1985, a few studies have experimentally tested this hypothesis. However, there have been no empirical studies that connect intraspecific selection with interspecific diversification. In this paper, I review the reproductive isolation and sexual selection hypotheses and two recent experimental tests of the sexual selection hypothesis. Then, I provide a description of a system that may allow one to establish a connection between sexual selection on mating structures within a species and diversification of mating structures between species.

Discrimination of Eubazus (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation

Villemant, C; Simbolotti, G; Kenis, M. 2007

Complexes of sibling and cryptic species are encountered frequently in parasitic Hymenoptera. Geometric morphometrics is a useful tool to detect minimal morphological variations, which often are undetectable by traditional morphological studies and even by classical morphometric approaches. We applied geometric morphometrics to wing venation to assess a complex case of sibling species in the genus *Eubazus* (Hymenoptera, Braconidae), parasitoids of conifer bark weevils of the genus *Pissodes* (Coleoptera, Curculionidae). The results and methods were compared with previous taxonomic studies on the same species, involving classical multivariate morphometrics, isoenzyme analyses, cross-mating experiments and biological observations. Geometric morphometrics confirmed the previous division into four distinct species. However, this approach enabled the four species to be separated simultaneously, with a reliability of 98.6% for well-classified females and 93.1% for males. A similar result in previous studies was obtained only by combining isoenzyme analyses and several canonical variate analyses, including many morphometric characters. Furthermore, measurements of wing venation were less time-consuming, more reliable and required less prior knowledge of braconid taxonomy than the measurements needed for the classical morphometrics methods. Geometric morphometrics was used also to test the effect of host species on wing shape. Several female populations of *Eubazus semirugosus* originating from three different *Pissodes* spp. were compared. Significant differences were found in wing shape between conspecific *Eubazus* from different host species. The results are discussed in relation to reproductive isolation and genetic flow between the four species.

A REVIEW ON THE POLLINATION OF AROIDS WITH BISEXUAL FLOWERS

Jimenez, PD; Hentrich, H; Aguilar-Rodriguez, PA; Kromer, T; Chartier, M; MacSwiney, MC; Gibernau, M. 2019

This paper presents an exhaustive review of the current knowledge on pollination of Araceae genera with bisexual flowers. All available studies on floral morphology, flowering sequence, floral scent, floral thermogenesis, floral visitors, and pollinators were carefully examined, with emphasis on the species-rich genera *Anthurium* Schott, *Monstera* Adans., and *Spathiphyllum* Schott. Genera with bisexual flowers are among the early-diverging lineages in Araceae, but present adaptations in their floral ecology to a great variety of pollination vectors, such as bees, beetles, flies, and, unusually, wind. These clades have developed highly derived pollination systems, involving the use of floral scent as a reward. We conclude that floral scent chemistry plays a key role in the pollination biology of the plants and that, in some genera, reproductive isolation through variation in the emitted floral volatile compounds may have been the decisive factor in the speciation processes of sympatric species.

Chromosome and Genome Divergence between the Cryptic Eurasian Malaria Vector-Species *Anopheles messeae* and *Anopheles daciae*

Naumenko, AN; Karagodin, DA; Yurchenko, AA; Moskaev, AV; Martin, OI; Baricheva, EM; Sharakhov, IV; Gordeev, MI; Sharakhova, MV. 2020

Chromosomal inversions are important drivers of genome evolution. The Eurasian malaria vector *Anopheles messeae* has five polymorphic inversions. A cryptic species, *An. daciae*, has been discriminated from *An. messeae* based on five fixed nucleotide substitutions in the internal transcribed spacer 2 (ITS2) of ribosomal DNA. However, the inversion polymorphism in *An. daciae* and the genome divergence between these species remain unexplored. In this study, we sequenced the ITS2 region and analyzed the inversion frequencies of 289 *Anopheles* larvae specimens collected from three locations in the Moscow region. Five individual genomes for each of the two species were sequenced. We determined that *An. messeae* and *An. daciae* differ from each other by the frequency of polymorphic inversions. Inversion X1 was fixed in *An. messeae* but polymorphic in *An. daciae* populations. The genome sequence comparison demonstrated genome-wide divergence between the species, especially pronounced on the inversion-rich X chromosome (mean $F_{st} = 0.331$). The frequency of polymorphic autosomal inversions was higher in *An. messeae* than in *An. daciae*. We conclude that the X chromosome inversions play an important role in the genomic differentiation between the species. Our study determined that *An. messeae* and *An. daciae* are closely related species with incomplete reproductive isolation.

New arrangements on several species subcomplexes of *Triatoma* genus based on the chromosomal position of ribosomal genes (Hemiptera - Triatominae)

Pita, S; Lorite, P; Nattero, J; Galvao, C; Alevi, KCC; Teves, SC; Azeredo-Oliveira, MTV; Panzera, F. 2016

The hemipteran subfamily Triatominae includes 150 blood-sucking species, vectors of Chagas disease. By far the most speciose genus is *Triatoma*, assembled in groups, complexes and subcomplexes based on morphological similarities, geographic distribution and genetic data. However, many molecular studies questioned the species integration of several subcomplexes as monophyletic units. In triatomines, chromosomal position of major ribosomal DNA (rDNA) loci is extremely variable but seems to be species-specific and an evolutionary conserved genetic trait, so that closely related species tend to have ribosomal clusters in the same chromosomal location. Considering that the autosomal position as the ancestral character for all heteropteran species, including triatomines, we suggest that the movement of rDNA loci from autosomes to sex chromosomes rapidly established reproductive barriers between divergent lineages. We proposed that the rDNA translocation from the autosomes to the sex chromosomes restrict reproductive compatibility and eventually promote speciation processes. We analyzed the chromosomal position of 45S rDNA clusters in almost all species of the *matogrossensis*, *rubrovaria*, *maculata* and *sordida* subcomplexes. The fluorescent *in situ* hybridization results are discussed considering the available genetic data and we proposed new arrangements in the species that constitute each one of these subcomplexes. (C) 2016 Elsevier B.V. All rights reserved.

Postzygotic isolating factor in sympatric speciation in *Rhagoletis* flies: Reduced response of hybrids to parental host-fruit odors

Linn, CE; Darnbroski, HR; Feder, JL; Berlocher, SH; Nojima, S; Roelofs, WL. 2004

Rhagoletis pomonella is a model for sympatric speciation (divergence without geographic isolation) by means of host-plant shifts. Many *Rhagoletis* species are known to use fruit odor as a key olfactory cue to distinguish among their respective host plants. Because *Rhagoletis* rendezvous on or near the unabsorbed fruit of their hosts to mate, behavioral preferences for fruit odor translate directly into premating reproductive isolation among flies. Here, we report that reciprocal F₁ hybrids between the apple and hawthorn host races of *R. pomonella*, as well as between the host races and an undescribed sibling species infesting *Cornus florida* (flowering dogwood) do not respond to host fruit volatiles in wind-tunnel assays at doses that elicit maximal directed flight in parental flies. The reduced ability of hybrids to orient to fruit volatiles could result from a conflict between neural pathways for preference and avoidance behaviors, and it suggests that hybrids might suffer a fitness disadvantage for finding fruit in nature. Therefore, host-specific mating may play a dual role as an important postzygotic as well as a premating reproductive barrier to isolate sympatric *Rhagoletis* flies.

Differences in the reliance on cuticular hydrocarbons as sexual signaling and species discrimination cues in parasitoid wasps

Buellesbach, J; Vetter, SG; Schmitt, T. 2018

Background: Cuticular hydrocarbons (CHC) have been documented to play crucial roles as species- and sex-specific cues in the chemical communication systems of a wide variety of insects. However, whether they are sufficient by themselves as the sole cue triggering sexual behavior as well as preference of con-over

heterospecific mating partners is rarely assessed. We conducted behavioral assays in three representative species of parasitoid wasps (Hymenoptera: Pteromalidae) to determine their reliance on CHC as species-specific sexual signaling cues. Results: We found a surprising degree of either unspecific or insufficient sexual signaling when CHC are singled out as recognition cues. Most strikingly, the cosmopolitan species *Nasonia vitripennis*, expected to experience enhanced selection pressure to discriminate against other co-occurring parasitoids, did not discriminate against CHC of a partially sympatric species from another genus, *Trichomalopsis sarcophagae*. Focusing on the latter species, in turn, it became apparent that CHC are even insufficient as the sole cue triggering conspecific sexual behavior, hinting at the requirement of additional, synergistic sexual cues particularly important in this species. Finally, in the phylogenetically and chemically most divergent species *Muscidifurax uniraptor*, we intriguingly found both CHC-based sexual signaling as well as species discrimination behavior intact although this species is naturally parthenogenetic with sexual reproduction only occurring under laboratory conditions. Conclusions: Our findings implicate a discrepancy in the reliance on and specificity of CHC as sexual cues in our tested parasitoid wasps. CHC profiles were not sufficient for unambiguous discrimination and preference behavior, as demonstrated by clear cross-attraction between some of our tested wasp genera. Moreover, we could show that only in *T. sarcophagae*, additional behavioral cues need to be present for triggering natural mating behavior, hinting at an interesting shift in signaling hierarchy in this particular species. This demonstrates the importance of integrating multiple, potentially complementary signaling modalities in future studies for a better understanding of their individual contributions to natural sexual communication behavior.

Strain- and sex-specific differences in daily flight activity and the circadian clock of *Anopheles gambiae* mosquitoes

Rund, SSC; Lee, SJ; Bush, BR; Duffield, GE. 2012

Anopheles gambiae, the primary African malaria vector, is currently speciating into two incipient species, the so-called "molecular forms" M and S. While some geographic areas may contain only one form, in many areas both forms are found coexisting, but reproductively isolated. It appears that spatial segregation of mating swarms may contribute significantly to reproductive isolation as in many locales single-form swarms exist almost exclusively even though they are in close geographic proximity. The mechanism causing this spatial segregation is not well understood. Here, we compare the locomotor flight activity of M and S form male and female *An. gambiae* mosquitoes with the goal of identifying potential strain-specific temporal differences that could potentially serve as a mating barrier. We use an infrared beam break method to monitor flight activity of individual mosquitoes with a minute-to-minute time resolution under both LD cycle and constant dark conditions. We compare daily total flight activity, activity onset, peak in early nocturnal activity, the build up of dusk-related activity, and the free-running circadian period length. Our investigations revealed strain- and sex-specific differences in total daily activity. In both forms, males commenced nightly flight activity earlier than females, and this corresponded with a significantly shorter circadian period length in males compared to females. We note strain-specific differences in this response to dusk as males of the M form have a pronounced build up in flight activity relative to the S form males prior to complete darkness. This is likely driven by a differential response to the decreasing light intensity at dusk. We hypothesize that this behavioral difference could be a temporal factor contributing to the assembly of single-form swarms. (C) 2012 Elsevier Ltd. All rights reserved.

Male diet affects female fitness and sperm competition in human- and bat-associated lineages of the common bedbug, *Cimex lectularius*

Kremenova, J; Bartonicka, T; Balvin, O; Massino, C; Reinhardt, K; Sasinkova, M; Weig, AR; Otti, O. 2021

Sperm performance can vary in ecologically divergent populations, but it is often not clear whether the environment per se or genomic differences arising from divergent selection cause the difference. One powerful and easily manipulated environmental effect is diet. Populations of bedbugs (*Cimex lectularius*) naturally feed either on bat or human blood. These are diverging genetically into a bat-associated and a human-associated lineage. To measure how male diet affects sperm performance, we kept males of two HL and BL populations each on either their own or the foreign diet. Then we investigated male reproductive success in a single mating and sperm competition context. We found that male diet affected female fecundity and changed the outcome of sperm competition, at least in the human lineage. However, this influence of diet on sperm performance was moulded by an interaction. Bat blood generally had a beneficial effect on sperm competitiveness and seemed to be a better food source in both lineages. Few studies have examined the effects of male diet on sperm performance generally, and sperm competition specifically. Our results reinforce the importance to consider the environment in which sperm are produced. In the absence of gene flow, such differences may increase reproductive isolation. In the presence of gene flow, however, the generally better sperm performance after consuming bat blood suggests that the diet is likely to homogenise rather than isolate populations.

Integration of molecular, ecological, morphological and endosymbiont data for species delimitation within the *Phnigalio 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 *Phnigalio 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.

Insect Speciation Rules: Unifying Concepts in Speciation Research

Mullen, SP; Shaw, KL. 2014

The study of speciation is concerned with understanding the connection between causes of divergent evolution and the origin and maintenance of barriers to gene exchange between incipient species. Although the field has historically focused either on examples of recent divergence and its causes or on the genetic basis of reproductive isolation between already divergent species, current efforts seek to unify these two approaches. Here we integrate these perspectives through a discussion of recent progress in several insect speciation model systems. We focus on the evolution of speciation phenotypes in each system (i.e., those phenotypes causally involved in reducing gene flow between incipient species), drawing an explicit connection between cause and effect (process and pattern). We emphasize emerging insights into the genomic architecture of speciation as well as timely areas for future research.

Population structure of *Wolbachia* and cytoplasmic introgression in a complex of mosquito species

Dumas, E; Atyame, CM; Milesi, P; Fonseca, DM; Shaikovich, EV; Unal, S; Makoundou, P; Weill, M; Duron, O. 2013

Background: The maternally inherited bacterium *Wolbachia* often acts as a subtle parasite that manipulates insect reproduction, resulting potentially in reproductive

isolation between host populations. Whilst distinct *Wolbachia* strains are documented in a group of evolutionarily closely related mosquitoes known as the *Culex pipiens* complex, their impact on mosquito population genetics remains unclear. To this aim, we developed a PCR-RFLP test that discriminates the five known *Wolbachia* groups found in this host complex. We further examined the *Wolbachia* genetic diversity, the variability in the coinherited host mitochondria and their partitioning among members of the *Cx. pipiens* complex, in order to assess the impact of *Wolbachia* on host population structure. Results: There was a strong association between *Wolbachia* and mitochondrial haplotypes indicating a stable co-transmission in mosquito populations. Despite evidence that members of the *Cx. pipiens* complex are genetically distinct on the basis of nuclear DNA, the association of *Wolbachia* and mtDNA with members of the *Cx. pipiens* complex were limited. The *Wolbachia* wPip-I group, by far the most common, was associated with divergent *Cx. pipiens* members, including *Cx. quinquefasciatus*, *Cx. pipiens pipiens* form *pipiens* and *Cx. pipiens pipiens* form *molestus*. Four other wPip groups were also found in mosquito populations and all were shared between diverse *Cx. pipiens* members. Conclusion: This data overall supports the hypothesis that wPip infections, and their allied mitochondria, are associated with regular transfers between *Cx. pipiens* members rather than specific host associations. Overall, this is suggestive of a recent and likely ongoing cytoplasmic introgression through **hybridization** events across the *Cx. pipiens* complex.

Evolutionary history of a mosquito endosymbiont revealed through mitochondrial hitchhiking

Rasgon, JL; Cornel, AJ; Scott, TW. 2006

Due to cytoplasmic inheritance, spread of maternally inherited *Wolbachia* symbionts can result in reduction of mitochondrial variation in populations. We examined sequence diversity of the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene in *Wolbachia*-infected (South Africa (SA), California and Thailand) and uninfected (SA) *Culex pipiens* complex populations. In total, we identified 12 haplotypes (A-L). In infected populations, 99% of individuals had haplotype K. In the uninfected SA population, 11 haplotypes were present, including K. Nuclear allozyme diversity was similar between infected and uninfected SA populations. Analysis of nuclear DNA sequences suggested that haplotype K presence in uninfected SA *Cx. pipiens* was probably due to a shared ancestral polymorphism rather than **hybrid** introgression. These data indicate that *Wolbachia* spread has resulted in drastic reduction of mitochondrial variability in widely separated *Cx. pipiens* complex populations. In contrast, the uninfected SA population is probably a cryptic species where *Wolbachia* introgression has been prevented by reproductive isolation, maintaining ancestral levels of mitochondrial diversity. Molecular clock analyses suggest that the *Wolbachia* sweep occurred within the last 47 000 years. The effect of *Wolbachia* on mitochondrial dynamics can provide insight on the potential for *Wolbachia* to spread transgenes into mosquito populations to control vector-borne diseases.

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.

Asymmetric Phenotypes of Sterile Hybrid Males From Reciprocal Crosses Between Species of the *Anopheles gambiae* Complex

Liang, JT; Hodge, JM; Sharakhov, IV. 2021

Haldane's rule of speciation states that sterility or inviability affects the heterogametic sex of inter-species **hybrids**. Darwin's corollary to Haldane's rule implies that there are asymmetric phenotypes in inter-species **hybrids** from reciprocal crosses. Studying the phenotypes of F1 **hybrids** among closely related species of malaria mosquitoes can assist researchers in identifying the genetic factors and molecular mechanisms of speciation. To characterize phenotypes of sterile **hybrid** males in the *Anopheles gambiae* complex, we performed crosses between laboratory strains of *An. merus* and either *An. gambiae* or *An. coluzzii*. The reproductive tracts had normal external morphology in **hybrid** males from crosses between female *An. merus* and male *An. gambiae* or *An. coluzzii*. Despite being sterile, these males could copulate with females for a normal period of time and could transfer a mating plug to induce female oviposition and monogamy. In contrast, the entire reproductive tracts in **hybrid** males from crosses between female *An. gambiae* or *An. coluzzii* and male *An. merus* were severely underdeveloped. These males had atrophic testes and reduced somatic organs of the reproductive system including male accessory glands and ejaculatory duct. In addition, **hybrid** males with underdeveloped reproductive tracts displayed a shorter copulation time with females and failed to induce female oviposition and monogamy due to their inability to form and transfer a plug to females during mating. The asymmetry of the phenotypes associated with **hybrid** male sterility suggests that different genetic factors and molecular mechanisms are responsible for reproductive isolation in reciprocal crosses among species of the *An. gambiae* complex.

Differential gene expression in incipient species of *Anopheles gambiae*

Cassone, BJ; Mouline, K; Hahn, MW; White, BJ; Pombi, M; Simard, F; Costantini, C; Besansky, NJ. 2008

A speciation process is ongoing in the primary vector of malaria in Africa, *Anopheles gambiae*. Assortatively mating incipient species known as the M and S forms differentially exploit larval breeding sites associated with different ecological settings. However, some ongoing gene flow between M and S limits significant genomic differentiation mainly to small centromere-proximal regions on chromosomes X and 2L, termed 'speciation islands' with the expectation that they contain the genes responsible for reproductive isolation. As the speciation islands exhibit reduced recombination and low polymorphism, more detailed genetic analysis using fine-scale mapping is impractical. We measured global gene expression differences between M and S using oligonucleotide microarrays, with the goal of identifying candidate genes that could be involved in this ongoing speciation process. Gene expression profiles were examined in two independent colonies of both forms at each of three developmental periods of interest: fourth instar larvae, virgin females, and gravid females. Patterns were validated on a subset of genes using quantitative real-time reverse transcription polymerase chain reaction of RNA samples from laboratory colonies and wild mosquitoes collected from Cameroon and Burkina Faso. Considered across all three developmental periods, differentially expressed genes represented similar to 1-2% of all expressed genes. Although disproportionately represented in the X speciation island, the vast majority of genes were located outside any speciation island. Compared to samples from the other developmental periods, virgin females were characterized by more than twice as many differentially expressed genes, most notably those implicated in olfaction and potentially, mate recognition.

The role of fetid olfactory signals in the shift to saprophilous fly pollination in *Jaborosa* (Solanaceae)

More, M; Mulieri, P; Battan-Horenstein, M; Cocucci, AA; Raguso, RA. 2019

Floral scents can act as important contributing factors to plant reproductive isolation mediated by pollinators. Plants may utilize fetid floral odors that specifically lure saprophilous flies seeking high protein content substrates, such as dung or carrion, to reach sexual maturity or as food sources for their larvae. In this work, we used baits with fetid volatile organic compounds (VOCs) that are produced during the decay of animal protein substrates (oligosulfides and a fermented bone-meal blend) to evaluate the role that olfactory signals may have played in the shift to saprophilous fly pollination in species of the nightshade genus *Jaborosa* Juss. Traps with the fetid baits attracted the same assemblage of saprophilous fly species that were recorded pollinating the flowers in different populations of the Andean-distributed species *J. laciniata*, whereas no flies were attracted to the control traps using mineral oil. Furthermore, the addition of oligosulfides to flowers of *J. integrifolia*, a lowland distributed species pollinated by nocturnal hawkmoths, resulted in the nearly immediate attraction of saprophilous flies (mainly Calliphoridae) to the flowers. These results provide evidence that the emission of fetid floral VOCs is sufficient to attract flies to flowers irrespective of other flower features and geographic region. This suggests that the evolutionary shift to saprophilous fly pollination in the genus *Jaborosa* could have been initiated with novel floral visitors attracted by the emission of fetid VOCs and then followed by major changes in other flower traits such as corolla color and morphology to optimize pollen export and placement.

The role of aedeagus size and shape in failed mating interactions among recently diverged taxa in the *Drosophila mojavensis* species cluster

Richmond, MP. 2014

Background: Investigating the evolution of species-specific insect genitalia is central to understanding how morphological diversification contributes to reproductive isolation and lineage divergence. While many studies evoke some form of sexual selection to explain genitalia diversity, the basis of selection and the mechanism of failed heterospecific mate exclusion remains vague. I conducted reciprocal mate pair trials in the *Drosophila mojavensis* species cluster to quantify the frequency of failed insemination attempts, historically referred to as pseudocopulation, between lineages with discrete size and shape differences of the male aedeagus. Results: In cross-taxon matings aedeagus size had a significant effect on pseudocopulation frequencies, while aedeagus shape and genetic distance did not. The direction of the size difference was an important factor for successful mating. When females were mated to a cross-taxon male with a larger aedeagus than males from her own species, the pair could not establish a successful mating interaction. Females mated to cross-taxon males with a smaller aedeagus than conspecific males were able to establish the mating interaction but had issues disengaging at the end of the interaction. Conclusions: The results of this study support a role for aedeagus size in the male-female mating interaction, with a secondary role for aedeagus shape. In natural populations, mating failure based on aedeagus size could serve as an important reproductive isolating mechanism resulting in failed insemination attempts after both the male and female show a willingness to mate.

Characterization and Host-Feeding Patterns of *Culex pipiens* s.l. Taxa in a West Nile Virus-Endemic Area in Southeastern Romania

Tiron, GV; Stancu, IG; Dinu, S; Prioteasa, FL; Falcuta, E; Ceianu, CS; Cotar, AI. 2021

Culex pipiens sensu lato has been documented as West Nile virus (WNV) vector in southeastern Romania. Bucharest, the densely populated capital city of Romania, and the surrounding Ilfov county are WNV hotspots. In this area, the morphologically indistinguishable biotypes of *Cx. pipiens*, namely *pipiens* and *molestus*, are usually differentiated by their behavioral and physiological traits. Their involvement in WNV transmission, as suggested by entomological investigations, was not previously documented for each biotype. We used a Real-Time PCR assay based on CQ11 microsatellite to identify the *Cx. pipiens* biotypes and their **hybrids** collected in various habitats in the Bucharest metropolitan area. A sympatric distribution of both biotypes was observed, with a preference of green areas for *pipiens*, and human settings and animal farmlands for *molestus*. In the latter habitats, *pipiens* and *molestus* were found in mixed aboveground populations. A low number of **hybrids** was found suggesting existence of reproductive isolation. In subway tunnels *molestus* was dominant with a higher number of **hybrids** recorded than aboveground. Blood-engorged mosquitoes were identified to biotype and the blood meal source identified by DNA barcoding. Overall, *Cx. pipiens* s.l. fed mainly on birds, commonly on house sparrows, collared doves, and blackbirds, which are potential WNV-amplifying hosts. The preference for avian hosts was expressed strongest by *pipiens* biotype, while *molestus* was substantially less specific, feeding on avian and mammal hosts with similar frequency, with humans representing 20% of the hosts. **Hybrids** had a host choice closer to that of *molestus*. These findings highlight the role of *pipiens* biotype as enzootic/epizootic vector, and specifically show *molestus* as the bridge vector for WNV. The *pipiens* and *molestus* biotypes show important differences in habitat preferences, including oviposition; these findings demonstrate that targeted mosquito control to limit WNV transmission may be possible.

THE EFFECTS OF CLIMATE, HOST-PLANT PHENOLOGY AND HOST FIDELITY ON THE GENETICS OF APPLE AND HAWTHORN INFESTING RACES OF RHAGOLETIS-POMONELLA

FEDER, JL; HUNT, TA; BUSH, GL. 1993

True fruit flies belonging to the *Rhagoletis pomonella* (Walsh) sibling species complex have been proposed to speciate sympatrically by shifting and adapting to new host plants. Here, we report the results from a series of ecological and genetic experiments conducted at a study site near Grant, Michigan, U.S.A., aimed at clarifying the relationship between host specialization and reproductive isolation for these flies. Our findings indicate that apple (*Malus pumila*) and hawthorn (*Crataegus mollis*) infesting populations of *R. pomonella* are partially allochronically isolated. Differences in the timing of adult eclosion account for part of the allochronic divergence, as apple adults emerge approximately ten days earlier than hawthorn flies in the field. Genetic analyses across different life history stages of the fly show that adults do not randomly move between apple and hawthorn trees, but tend to attack the same species of plant that they infested as larvae. Estimates of interhost migration from the allozyme data suggest that from 2.8 to 10% of the apple population is of hawthorn origin and that over 20% of the hawthorn population is of apple origin. The length and quality of the growing season appear to affect the genetic composition of the host races, as allele frequencies in the hawthorn population are correlated with ambient temperature and rainfall during the spring of the preceding year. Finally, allele frequencies for six allozyme loci displaying host associated differentiation also show significant linear regressions with the timing of adult eclosion within both races. These regressions establish a link between allozyme loci displaying inter-host differentiation and a developmental trait (adult eclosion) responsible for partially isolating the races. The slopes of the regressions are paradoxical, however, as they suggest that apple adults should eclose later, not earlier, than hawthorn flies. We conclude by discussing potential resolutions to the eclosion time paradox.

Host races in plant-feeding insects and their importance in sympatric speciation

Dres, M; Mallet, J. 2002

The existence of a continuous array of sympatric biotypes-from polymorphisms, through ecological or host races with increasing reproductive isolation, to good species-can provide strong evidence for a continuous route to sympatric speciation via natural selection. Host races in plant-feeding insects, in particular, have often been used as evidence for the probability of sympatric speciation. Here, we provide verifiable criteria to distinguish host races from other biotypes: in brief, host races are genetically differentiated, sympatric populations of parasites that use different hosts and between which there is appreciable gene flow. We recognize host races as kinds of species that regularly exchange genes with other species at a rate of more than ca. 1% per generation, rather than as fundamentally distinct taxa. Host races provide a convenient, although admittedly somewhat arbitrary intermediate stage along the speciation continuum. They are a heuristic device to aid in evaluating the probability of speciation by natural selection, particularly in sympatry. Speciation is thereby envisaged as having two phases: (i) the evolution of host races from within polymorphic, panmictic populations; and (ii) further reduction of gene flow between host races until the diverging populations can become generally accepted as species. We apply this criterion to 21 putative host race systems. Of these, only three are unambiguously classified as host races, but a further eight are

strong candidates that merely lack accurate information on rates of **hybridization** or gene flow. Thus, over one-half of the cases that we review are probably or certainly host races, under our definition. Our review of the data favours the idea of sympatric speciation via host shift for three major reasons: (i) the evolution of assortative mating as a pleiotropic by-product of adaptation to a new host seems likely, even in cases where mating occurs away from the host; (ii) stable genetic differences in half of the cases attest to the power of natural selection to maintain multilocus polymorphisms with substantial linkage disequilibrium, in spite of probable gene flow; and (iii) this linkage disequilibrium should permit additional host adaptation, leading to further reproductive isolation via pleiotropy, and also provides conditions suitable for adaptive evolution of mate choice (reinforcement) to cause still further reductions in gene flow. Current data are too sparse to rule out a cryptic discontinuity in the apparently stable sympatric route from host-associated polymorphism to host-associated species, but such a hiatus seems unlikely on present evidence. Finally, we discuss applications of an understanding of host races in conservation and in managing adaptation by pests to control strategies, including those involving biological control or transgenic parasite-resistant plants.

Host Plant Adaptation in Cactophilic Species of the *Drosophila buzzatii* Cluster: Fitness and Transcriptomics

Hasson, E; De Panis, D; Hurtado, J; Mensch, J. 2019

Host plant shifts in herbivorous insects often involve facing new environments that may speed up the evolution of oviposition behavior, performance-related traits, morphology, and, incidentally, reproductive isolation. In the genus *Drosophila*, cactophilic species of the repleta group include emblematic species in the study of the evolution of host plant utilization. The South American *D. buzzatii* and its sibling *D. koepferae* are a model system for the study of differential host plant use. Although these species exhibit a certain degree of niche overlap, the former breeds primarily on decaying cladodes of *Opuntia* cacti while *D. koepferae* main hosts are columnar cacti of the genus *Trichocereus*. *Opuntia sulphurea* and *Trichocereus terscheckii* are among the main hosts in nature. These cacti differ in ecological (spatial and temporal predictability) and chemical characteristics. Particularly relevant is the presence of toxic alkaloids in *T. terscheckii*. Studies of the effects of these cacti and alkaloids revealed the remarkable impact on oviposition behavior, viability, developmental time, wing morphology, mating success, and developmental stability in both species. Recent whole-genome expression studies showed that expression profiles are massively affected by the rearing cactus, and that the presence of alkaloids is the main factor modulating gene expression in *D. buzzatii*. Functional enrichment analysis indicated that differentially expressed genes are related to detoxification processes and stress response-though genes involved in development are an important part of the transcriptomic response. The implications of our studies in the evolution of host plant use in the repleta group are discussed.

Assessment of the ecologically dependent post-zygotic isolation between *Anopheles coluzzii* and *Anopheles gambiae*

Niang, A; Sawadogo, SP; Dabire, RK; Tripet, F; Diabate, A. 2020

Within the *Anopheles gambiae* complex, the sibling species *An. coluzzii* and *An. gambiae* are undergoing sympatric speciation. These species are characterized by rare **hybrids** in most of their geographical distribution. A strong assortative mating mediated by spatial swarm segregation has been shown whereas no intrinsic post-zygotic barriers have been found in laboratory conditions. To test the role of the **hybridisation** in reproductive isolation in natural populations transplant experiment are therefore needed to establish the significance of post-zygotic barriers. Previous studies indicated that predation is one of the major forces driving ecological divergence between *An. gambiae* and *An. coluzzii*. Here we extended these studies to their **hybrids**. Parental species and their F1 **hybrids** from reciprocal crosses were generated by the forced-mating technique as follows: female *An. coluzzii* x male *An. coluzzii*; female *An. coluzzii* x male *An. gambiae*; female *An. gambiae* x male *An. coluzzii* and female *An. gambiae* x male *An. gambiae*. First instar larvae of each group from the crossing (here after *An. coluzzii*, **Hybrid** COL/GAM, **Hybrid** GAM/COL and *An. gambiae*, respectively) were transplanted in a field experiment with predation effect. Emergence success, development time of larvae and body size of the newly emerging adults were estimated as fitness components and then compared between parental species and F1 **hybrids** in absence and in presence of predators. Our findings confirm that *An. coluzzii* had higher fitness than *An. gambiae* in presence of predators versus in absence of predators. Moreover, the fitness of the F1 **hybrid** COL/GAM whose female parent was *An. coluzzii* matched that of *An. coluzzii* while that of the F1 reciprocal **hybrid** GAM/COL was similar to *An. gambiae*.

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.

Cryptic Species of Parasitoids Attacking the Soybean Aphid (Hemiptera: Aphididae) in Asia: *Binodoxys communis* and *Binodoxys koreanus* (Hymenoptera: Braconidae: Aphidinae)

Desneux, N; Stary, P; Delebecque, CJ; Garipey, TD; Barta, RJ; Hoelmer, KA; Heimpel, GE. 2009

Collections of parasitoids attacking the soybean aphid, *Aphis glycine* Matsumura (Hemiptera: Aphididae), in South Korea yielded specimens that were originally identified as *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae). We report here on laboratory studies indicating that this population is actually a previously unknown species of *Binodoxys*. Four classes of comparisons were made between the Korean population and a Chinese population that also had been identified as *B. communis*. The comparisons included 1) mating trials coupled with behavioral observations and spermathecal examinations, 2) assessment of nucleotide divergence at two mitochondrial and two nuclear gene loci, 3) patterns of host use, and 4) reassessment of morphological characters. These studies revealed premating reproductive isolation of the two populations and minor nucleotide differences in mitochondrial cytochrome oxidase I sequences and nuclear internal transcriber spacer 1 sequences, providing strong indications that they are different species. Subtle morphological differences also were discovered that confirmed that the Chinese population corresponds to *B. communis*, whereas the Korean population does not. We propose the name *Binodoxys koreanus* Stary, sp. n. for the Korean Population. The two species exhibited similar host ranges in the laboratory, the most notable exception being that *B. koreanus*, sp. n. is better able to develop in a Population of *Aphis craccivora* Koch that harbors the bacterial endosymbiont *Hamiltonella defensa* Moran, which seems to strongly interfere with the development of *B. communis*. We discuss the implications of our results for biological control introductions against the soybean aphid in North America.

Male mating preference of two cryptic species of the herbivorous insect *Ecritotarsus catarinensis*

Ismail, M; Brooks, M. 2018

When using biological control against pest populations, more than one biocontrol agent might be introduced simultaneously. This could be counterproductive in the event of negative interactions between the biocontrol agents. Within and between species interactions have a strong impact on mating behaviour and reproduction, and can have an impact on the effectiveness of biological control. We studied the reproductive compatibility between two geographically isolated strains (Brazil and Peru) of *Ecritotarsus catarinensis* (Heteroptera: Miridae), a biocontrol agent against the invasive aquatic weed, *Eichhornia crassipes*. By performing inter- and intra-species mating experiments, we investigated whether or not males from each of the cryptic species would be able to distinguish between partners from either species, and if they would mate with partners from the opposite species. Our results showed the decrease in lifetime fecundity, and most importantly, the lack of production of offspring from eggs resulting from forced **hybridisation**. We showed that Peruvian males mated only with females from their own species, and did not mate with females from the Brazilian species. In contrast, Brazilian males mated equally with females from both species, but needed significantly more time in order to commence a mating, and no offspring were produced from eggs resulting from **hybridisation**. Although future studies demand more rigorous controls, our results indicate asymmetrical sexual isolation between the two species. We speculate on mechanisms involved in reproductive isolation in the two cryptic species, and the possible implications for effective biocontrol, and include some morphological measurements that might support our assumptions.

Mitochondrial genome sequences reveal deep divergences among *Anopheles punctulatus* sibling species in Papua New Guinea

Logue, K; Chan, ER; Phipps, T; Small, ST; Reimer, L; Henry-Halldin, C; Sattabongkot, J; Siba, PM; Zimmerman, PA; Serre, D. 2013

Background: Members of the *Anopheles punctulatus* group (AP group) are the primary vectors of human malaria in Papua New Guinea. The AP group includes 13 sibling species, most of them morphologically indistinguishable. Understanding why only certain species are able to transmit malaria requires a better comprehension of their evolutionary history. In particular, understanding relationships and divergence times among *Anopheles* species may enable assessing how malaria-related traits (e.g. blood feeding behaviours, vector competence) have evolved. Methods: DNA sequences of 14 mitochondrial (mt) genomes from five AP sibling species and two species of the *Anopheles dirus* complex of Southeast Asia were sequenced. DNA sequences from all concatenated protein coding genes (10,770 bp) were then analysed using a Bayesian approach to reconstruct phylogenetic relationships and date the divergence of the AP sibling species. Results: Phylogenetic reconstruction using the concatenated DNA sequence of all mitochondrial protein coding genes indicates that the ancestors of the AP group arrived in Papua New Guinea 25 to 54 million years ago and rapidly diverged to form the current sibling species. Conclusion: Through evaluation of newly described mt genome sequences, this study has revealed a divergence among members of the AP group in Papua New Guinea that would significantly predate the arrival of humans in this region, 50 thousand years ago. The divergence observed among the mtDNA sequences studied here may have resulted from reproductive isolation during historical changes in sea-level through glacial minima and maxima. This leads to a hypothesis that the AP sibling species have evolved independently for potentially thousands of generations. This suggests that the evolution of many phenotypes, such as insecticide resistance will arise independently in each of the AP sibling species studied here.

A test of genomic modularity among life-history adaptations promoting speciation with gene flow

Ragland, GJ; Doellman, MM; Meyers, PJ; Hood, GR; Egan, SP; Powell, THQ; Hahn, DA; Nosil, P; Feder, JL. 2017

Speciation with gene flow may require adaptive divergence of multiple traits to generate strong ecologically based reproductive isolation. Extensive negative pleiotropy or physical linkage of genes in the wrong phase affecting these diverging traits may therefore hinder speciation, while genetic independence or "modularity" among phenotypic traits may reduce constraints and facilitate divergence. Here, we test whether the genetics underlying two components of diapause life history, initial diapause intensity and diapause termination timing, constrain differentiation between sympatric hawthorn and apple-infesting host races of the fly *Rhagoletis pomonella* through analysis of 10,256 SNPs measured via genotyping-by-sequencing (GBS). Loci genetically associated with diapause termination timing were mainly observed for SNPs mapping to chromosomes 1-3 in the genome, most notably for SNPs displaying higher levels of linkage disequilibrium (LD), likely due to inversions. In contrast, selection on initial diapause intensity affected loci on all five major chromosomes of the genome, specifically those showing low levels of LD. This lack of overlap in genetically associated loci suggests that the two diapause phenotypes are largely modular. On chromosome 2, however, intermediate level LD loci and a subgroup of high LD loci displayed significant negative relationships between initial diapause intensity and diapause termination time. These gene regions on chromosome 2 therefore affected both traits, while most regions were largely independent. Moreover, loci associated with both measured traits also tended to exhibit highly divergent allele frequencies between the host races. Thus, the presence of nonoverlapping genetic modules likely facilitates simultaneous, adaptive divergence for the measured life-history components.

Cryptic Diversity within the Major Trypanosomiasis Vector *Glossina fuscipes* Revealed by Molecular Markers

Dyer, NA; Ravel, S; Choi, KS; Darby, AC; Causse, S; Kapitano, B; Hall, MJR; Steen, K; Lutumba, P; Madinga, J; Torr, SJ; Okedi, LM; Lehane, MJ; Donnelly, MJ. 2011

Background: The tsetse fly *Glossina fuscipes* s.l. is responsible for the transmission of approximately 90% of cases of human African trypanosomiasis (HAT) or sleeping sickness. Three *G. fuscipes* subspecies have been described, primarily based upon subtle differences in the morphology of their genitalia. Here we describe a study conducted across the range of this important vector to determine whether molecular evidence generated from nuclear DNA (microsatellites and gene sequence information), mitochondrial DNA and symbiont DNA support the existence of these taxa as discrete taxonomic units. Principal Findings: The nuclear ribosomal Internal transcribed spacer 1 (ITS1) provided support for the three subspecies. However nuclear and mitochondrial sequence data did not support the monophyly of the morphological subspecies *G. f. fuscipes* or *G. f. quanzensis*. Instead, the most strongly supported monophyletic group was comprised of flies sampled from Ethiopia. Maternally inherited loci (mtDNA and symbiont) also suggested monophyly of a group from Lake Victoria basin and Tanzania, but this group was not supported by nuclear loci, suggesting different histories of these markers. Microsatellite data confirmed strong structuring across the range of *G. fuscipes* s.l., and was useful for deriving the interrelationship of closely related populations. Conclusion/Significance: We propose that the morphological classification alone is not used to classify populations of *G. fuscipes* for control purposes. The Ethiopian population, which is scheduled to be the target of a sterile insect release (SIT) programme, was notably discrete. From a programmatic perspective this may be both positive, given that it may reflect limited migration into the area or negative if the high levels of differentiation are also reflected in reproductive isolation between this population and the flies to be used in the release programme.

DNA barcoding: species delimitation in tree peonies

Zhang, JM; Wang, JX; Xia, T; Zhou, SL. 2009

Delimitations of species are crucial for correct and precise identification of taxa. Unfortunately "species" is more a subjective than an objective concept in taxonomic practice due to difficulties in revealing patterns of intra- or inter-specific variations. Molecular phylogenetic studies at the population level solve this problem and lay a sound foundation for DNA barcoding. In this paper we exemplify the necessity of adopting a phylogenetic concept of species in DNA barcoding for tree peonies (*Paonia* sect. *Moutan*). We used 40 samples representing all known populations of rare and endangered species and several populations of widely distributed tree peonies. All currently recognized species and major variants have been included in this study. Four chloroplast gene fragments, i.e. *ndhF*, *rps16-trnQ*, *trnL-F* and *trnS-G* (a total of 5040 characters, 96 variable and 69 parsimony-informative characters) and one variable and single-copy nuclear *GPAT* gene fragment (2093-2197 bp, 279 variable and 148 parsimony-informative characters) were used to construct phylogenetic relationships among the taxa. The evolutionary lineages revealed by the nuclear gene and the chloroplast genes are inconsistent with the current circumscriptions of *P. decomposita*, *P. jishanensis*, *P. quii*, and *P. rockii* based on

morphology. The inconsistencies come from (1) significant chloroplast gene divergence but little nuclear GPAT gene divergence among population systems of *P. decomposita* + *P. rockii*, and (2) well-diverged nuclear GPAT gene but little chloroplast gene divergence between *P. jishanensis* and *P. qiui*. The incongruence of the phylogenies based on the chloroplast genes and the nuclear GPAT gene is probably due to the chloroplast capture event in evolutionary history, as no reproductive barriers exist to prevent inter-specific **hybridization**. We also evaluated the suitability of these genes for use as DNA barcodes for tree peonies. The variability of chloroplast genes among well-defined species or population systems of a species complex is 4.82 times the figure within the groups, and the GPAT gene is twice as variable between the groups as within the groups. The number of completely divergent sites is sufficient to mark the two subsections, the two species in subsection *Delavayanae*, and the well-divergent species in subsection *Vaginatae*. But the genes currently used either from the chloroplast genome or from the nuclear genome alone cannot correctly assign samples of *P. decomposita*, *P. jishanensis*, *P. qiui*, or *P. rockii* to the species as currently defined. We conclude that (1) DNA barcoding should be based on prior phylogenetic studies to understand the evolutionary lineages and how well the taxonomic species correspond to the lineages; (2) it is unlikely to find a single short fragment as a barcode for every plant and such a fragment could result in misidentification when a chloroplast capture event happened in the evolutionary history of plants like tree peonies; and (3) we suggest striving for a universal marker at the familial level and locally universal barcodes within a family instead of looking for a universal barcode for all plants.

FURTHER OBSERVATIONS ON IN VITRO HYBRIDIZATION OF HEMOSPORIDIAN PARASITES: PATTERNS OF OOKINETE DEVELOPMENT IN HAEMOPROTEUS SPP.

Valkiunas, G; Palinauskas, V; Krizanauskienė, A; Bernotienė, R; Kazlauskienė, R; Iezhova, TA. 2013

Increasingly frequent outbreaks of zoonotic infections call for studies of wildlife parasites to reach a better understanding of the mechanisms of host switch, leading to the evolution of new diseases. However, speciation processes have been insufficiently addressed in experimental parasitology studies, primarily due to difficulties in determining and measuring mate-recognition signals in parasites. We investigated patterns of sexual process and ookinete development in avian *Haemoproteus* (*Parahaemoproteus*) spp. (*Haemosporida*, *Haemoproteidae*) using in vitro experiments on between-lineage **hybridization**. Eleven mitochondrial cytochrome b (cyt b) lineages belonging to 9 species of hemoproteid were isolated from naturally infected passerine birds. The parasites were identified to species on the basis of morphology of their gametocytes and polymerase chain reaction amplification of segments of the cyt b gene. Sexual process and ookinete development were initiated in vitro by mixing blood containing mature gametocytes with a 3.7% solution of sodium citrate and exposing the mixture to air. Ookinetes of all lineages except *Haemoproteus payevskii* (lineage hRW1) and *Haemoproteus nucleococondensus* (hGRW1) developed; the 2 latter species did not exflagellate. Between-lineage **hybridization** was initiated by mixing blood containing mature gametocytes of 2 different parasites; the following experiments were performed: (1) *Haemoproteus pallidus* (lineage hPFC1) x *Haemoproteus minutus* (lineage hTURDUS2); (2) *H. pallidus* (hPFC1) x *Haemoproteus tartakovskyi* (hSISKIN1); (3) *Haemoproteus belopolskyi* (hHICT3) x *Haemoproteus lanii* (hRB1); (4) *Haemoproteus balmorali* (hSFC1) x *H. pallidus* (hPFC1); (5) *H. belopolskyi* (hHICT1) x *Haemoproteus parabelopolskyi* (hSYBOR1); (6) *H. tartakovskyi* (hHAWF1) x *H. tartakovskyi* (hSISKIN1); (7) *H. pallidus* (hPFC1) x *H. lanii* (hRB1); (8) *H. tartakovskyi* (hHAWF1) x *H. parabelopolskyi* (hSYBOR1). We report 4 patterns of between-lineage interactions that seem to be common and might prevent mixing lineages during simultaneous sexual process in wildlife: (1) the blockage of ookinete development of both parasites; (2) the development of ookinetes of 1 parasite and blockage of ookinete development of the other; (3) selective within-lineage mating resulting in ookinete development of both parent species and absence of **hybrid** organisms; (4) absence of selective within-lineage mating resulting in presence of ookinetes of both parents and also development of **hybrid** organisms with unclear potential for further sporogony. The present study indicates directions for collection of source material in the investigation of mechanisms of reproductive isolation leading to speciation in these parasites. The next steps in these studies should be the development of nuclear markers for distinguishing hemosporidian **hybrid** organisms and the experimental observation of further development of **hybrid** ookinetes in vectors.

Sex aggregation and species segregation cues in swarming mosquitoes: role of ground visual markers

Poda, SB; Nignan, C; Gnankine, O; Dabire, RK; Diabate, A; Roux, O. NA

Background: Mating swarm segregation in closely related insect species may contribute to reproductive isolation. Visual markers are used for swarm formation; however, it is unknown whether they play a key role in swarm location, species segregation and sex aggregation. Methods: Using two sympatric closely related species of the *Anopheles gambiae* complex, *An. coluzzii* and *An. gambiae* (s.s.), we investigated in both laboratory and semi-field conditions (i) whether males of the two species use visual markers (black cloths) to locate their swarm; and (ii) whether the presence/absence and size of the marker may differentially affect swarm characteristics. We also investigated whether conspecific virgin females use these markers to join male swarm sites. Results: We showed that males of the two species used visual markers but in different ways: *An. coluzzii* swarm right above the marker whereas *An. gambiae* (s.s.) locate their swarm at a constant distance of 76.4 +/- 0.6 cm from a 20 x 20 cm marker in the laboratory setup and at 206 +/- 6 cm from a 60 x 60 cm marker in the semi-field setup. Although increased marker size recruited more mosquitoes and consequently increased the swarm size in the two species, *An. coluzzii* swarms flew higher and were stretched both vertically and horizontally, while *An. gambiae* (s.s.) swarms were only stretched horizontally. Virgin females displayed a swarm-like behavior with similar characteristics to their conspecific males. Conclusions: Our results provided experimental evidence that both *An. coluzzii* and *An. gambiae* (s.s.) males use ground visual markers to form and locate their swarm at species-specific locations. Moreover, the marker size differentially affected swarm characteristics in the two species. Our results also showed that virgin females displayed a swarm-like behavior. However, these "swarms" could be due to the absence of males in our experimental conditions. Nevertheless, the fact that females displayed these "swarms" with the same characteristics as their respective males provided evidence that visual markers are used by the two sexes to join mating spots. Altogether, this suggests that visual markers and the way species and sexes use them could be key cues in species segregation, swarm location and recognition.

Diversity and distribution of *Wolbachia* in relation to geography, host plant affiliation and life cycle of a heterogonic gall wasp

Schuler, H; Egan, SP; Hood, GR; Busbee, RW; Driscoll, AL; Ott, JR. 2018

Background: The maternally inherited endosymbiont *Wolbachia* is widespread in arthropods and nematodes and can play an important role in the ecology and evolution of its host through reproductive manipulation. Here, we survey *Wolbachia* in *Belonocnema treatae*, a widely distributed North American cynipid gall forming wasp that exhibits regional host specialization on three species of oaks and alternation of sexually and asexually reproducing generations. We investigated whether patterns of *Wolbachia* infection and diversity in *B. treatae* are associated with the insect's geographic distribution, host plant association, life cycle, and mitochondrial evolutionary history. Results: Screening of 463 individuals from 23 populations including sexual and asexual generations from all three host plants across the southern U.S. showed an average infection rate of 56% with three common *Wolbachia* strains: wTre1-3 and an additional rare variant wTre4. Phylogenetic analysis based on wsp showed that these strains are unrelated and likely independently inherited. We found no difference in *Wolbachia* infection frequency among host plant associated populations or between the asexual and sexual generations, or between males and females of the sexual generation. Partially incomplete *Wolbachia* transmission rates might explain the occurrence of uninfected individuals. A parallel analysis of the mitochondrial cytochrome oxidase I gene in *B. treatae* showed high mtDNA haplotype diversity in both infected and uninfected populations suggesting an ancestral infection by *Wolbachia* as well as a clear split between eastern and western *B. treatae* mtDNA clades with a sequence divergence of > 6%. The strain wTre1 was present almost exclusively in the western clade while wTre2 and wTre3 occur almost exclusively in eastern populations. In contrast, the same strains co-occur as double-infections in Georgia and triple-infections in two populations in central Florida. Conclusions: The diversity of *Wolbachia* across geographically and genetically distinct populations of *B. treatae* and the co-occurrence of the same strains within three populations highlights the complex infection dynamics in this system. Moreover, the association of distinct *Wolbachia* strains with mitochondrial haplotypes of its host in populations infected by different *Wolbachia* strains suggests a potential role of the endosymbiont in reproductive isolation in *B. treatae*.
