

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

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High genetic variability despite haplodiploidy in primitive sawflies of the genus *Cephalcia* (Hymenoptera, Pamphiliidae)

Boato, A; Battisti, A. 1996

Hymenoptera are haplodiploid and usually display very low genetic variation. Most data concern social or parasitic Apocrita, while the little information available for the primitive phytophagous species of the suborder Symphyta is contradictory. The present study is related to seven species of the genus *Cephalcia*, living in coniferous forests of Northern Eurasia and sharing spruce (*Picea* sp. pi.) as host plant. Individuals from 22 populations belonging to *Cephalcia abietis*, *C. alashanica*, *C. arvensis*, *C. erythrogaster*, *C. fallenii*, *C. fulva*, *C. klugii* from Europe and China were surveyed for genetic variation at 28 loci using enzyme electrophoresis. Pairs of sibling species were recognized within *C. arvensis* and *C. fallenii*, corresponding to different phenological and morphological forms. In the latter case, reproductive isolation in sympatry occurs despite low genetic distance ($D = 0.059$). Large genetic distances and fixed alternate alleles were observed between Chinese and European populations of *C. abietis* and *C. arvensis*. Expected heterozygosity of *Cephalcia* populations (0.197, SD 0.064) is significantly higher than that of other Symphyta (Tenthredinoidea) (average H_{exp} 0.059, SD 0.032) (two-tailed Mann-Whitney test, $Z = 4.39$, $p < 0.01$). These data suggest that haplodiploidy per se does not reduce the genetic variation in most *Cephalcia* populations. Most of the factors that can lower the potential for genetic diversity in a haplodiploid genetic system are not so effective in *Cephalcia* populations, which seem to be comparable to diploid insect populations in diversity. In a few isolated populations the large number of fixed loci and the large genetic distances may support the predicted faster rate of fixation, as a consequence of haplodiploidy.

The emergence of ecotypes in a parasitoid wasp: a case of incipient sympatric speciation in Hymenoptera?

Malec, P; Weber, J; Bohmer, R; Fiebig, M; Meinert, D; Rein, C; Reinisch, R; Henrich, M; Polyvas, V; Pollmann, M; von Berg, L; Konig, C; Steidle, JLM. 2021

Background To understand which reproductive barriers initiate speciation is a major question in evolutionary research. Despite their high species numbers and specific biology, there are only few studies on speciation in Hymenoptera. This study aims to identify very early reproductive barriers in a local, sympatric population of *Nasonia vitripennis* (Walker 1836), a hymenopterous parasitoid of fly pupae. We studied ecological barriers, sexual barriers, and the reduction in F1-female offspring as a postmating barrier, as well as the population structure using microsatellites. **Results** We found considerable inbreeding within female strains and a population structure with either three or five subpopulation clusters defined by microsatellites. In addition, there are two ecotypes, one parasitizing fly pupae in bird nests and the other on carrion. The nest ecotype is mainly formed from one of the microsatellite clusters, the two or four remaining microsatellite clusters form the carrion ecotype. There was slight sexual isolation and a reduction in F1-female offspring between inbreeding strains from the same microsatellite clusters and the same ecotypes. Strains from different microsatellite clusters are separated by a reduction in F1-female offspring. Ecotypes are separated only by ecological barriers. **Conclusions** This is the first demonstration of very early reproductive barriers within a sympatric population of Hymenoptera. It demonstrates that sexual and premating barriers can precede ecological separation. This indicates the complexity of ecotype formation and highlights the general need for more studies within homogenous populations for the identification of the earliest barriers in the speciation process.

Influence of postzygotic reproductive isolation on the interspecific transmission of the paternal sex ratio chromosome in *Trichogramma*

Jeong, G; Stouthamer, R. 2006

The paternal sex ratio (PSR) chromosome is a supernumerary chromosome that causes the destruction of the paternal chromosome set in the first mitosis in a fertilized egg. It is known from parasitoid wasps in the genera *Nasonia* and *Trichogramma* (Hymenoptera). In these haplodiploids, the egg fertilized by sperm carrying PSR matures as a haploid male that again carries, and is capable of transmitting, the PSR chromosome. Because of its unique transmission behavior, the PSR chromosome may be easily transmitted between species. This study tests whether the interspecific transmission of PSR between *Trichogramma kaykai* Pinto and Stouthamer and *Trichogramma deion* Pinto and Oatman (Hymenoptera: Trichogrammatidae) is affected by two types of postzygotic reproductive isolation, i.e., **hybrid** inviability and **hybrid** sterility. The results show that PSR can rescue fertilized eggs that would normally be inviable in the interspecific cross and the rescued eggs develop into male offspring that carry PSR. The results suggest that the two types of postzygotic reproductive isolation have no effect on the transmission of PSR between the two *Trichogramma* species.

Immigrant inviability promotes reproductive isolation among host-associated populations of the gall wasp *Belonocnema treatae*

Zhang, LY; Driscoll, A; Izen, R; Toussaint, C; Ott, JR; Egan, SP. 2017

Ecological speciation describes the evolutionary process whereby divergent natural selection between environments generates reproductive isolation. Studying the magnitude of sequential reproductive barriers between ecologically divergent populations improves our understanding of the way these barriers evolve and how each contributes to the speciation process. Immigrant inviability describes the lower fitness of immigrants in non-native environments and is an important, but long underexplored, reproductive barrier. In this study, we test the role of immigrant inviability among host-associated populations of the gall wasp *Belonocnema treatae* Mayr (Hymenoptera: Cynipidae) by measuring the ability of gall wasps to initiate and complete gall formation, while avoiding host immune responses, on closely related native and non-native live oaks, *Quercus virginiana* Mill., *Quercus fusiformis* Small, and *Quercus geminata* Small (Fagaceae). In general, we found evidence for immigrant inviability when *B. treatae* populations colonized non-native host species. However, patterns were variable among years, suggesting that episodic events may play an important role in connecting ecologically divergent populations.

Diversity, Host Ranges, and Potential Drivers of Speciation Among the Inquiline Enemies of Oak Gall Wasps (Hymenoptera: Cynipidae)

Ward, AKG; Sheikh, SI; Forbes, AA. 2020

Animals that exploit living spaces of other animals (inquilines) may have specialized traits that adapt them to extended phenotypes of their 'hosts'. These adaptations to host traits may incur fitness trade-offs that restrict the host range of an inquiline such that shifts to new hosts might trigger inquiline diversification. Speciation via host shifting has been studied in many animal parasites, but we know less about the role of host shifts in inquiline speciation. *Synergus* Hartig (Hymenoptera: Cynipidae: Synergini) is a speciose but taxonomically challenging genus of inquilines that feed inside galls induced by oak gall wasps (Hymenoptera: Cynipidae: Cynipini). Here, we report on a large collection of *Synergus* reared from galls of 33 oak gall wasp species in the upper Midwestern United States. We integrated DNA barcodes, morphology, ecology, and phenology to delimit putative species of *Synergus* and describe their host ranges. We find evidence of at least 23 *Synergus* species associated with the 33 gall wasp hosts. At least five previously described *Synergus* species are each complexes of two to five species, while three species fit no prior description. We find evidence that oak tree phylogeny and host gall morphology define axes of specialization for *Synergus*. The North American *Synergus* have experienced several transitions among gall hosts and tree habitats and their host use is correlated with reproductive isolation. It remains too early to tell whether shifts to new hosts initiate speciation events in *Synergus* inquilines of oak gall wasps, or if host shifts occur after reproductive isolation has already evolved.

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: Eucolilinae) 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.

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.

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

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

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

Effects of intraspecific hybridization on the fitness of the egg parasitoid *Trichogramma galloi*

Bertin, A; Pavinato, VAC; Parra, JRP. 2018

Successive rearing in laboratory conditions can result in the loss of genetic diversity, inbreeding depression and adaptation to the captive environment, affecting the quality of the insects reared and compromising their field performance. Introduction of genetic variation by admixing different populations may increase the fitness of populations, minimizing the negative effects of rearing many generations in artificial conditions. We experimentally investigated the role of intraspecific **hybridization** in enhancing the fitness of the egg parasitoid *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae), by reciprocally crossing three populations. Our results showed that the mating type did not affect the number of crosses that produced viable daughters. Homotypic crosses produced 94% viable daughters, while heterotypic crosses produced 92%. There were neither mating incompatibilities nor reproductive barriers between these populations. However, we observed a low fitness value for females from one of the populations studied. The fitness of **hybrids** was either unchanged or improved (in one case) when compared to the parental populations. We discuss the implications of our results and suggest future research directions.

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

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

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

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

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

Courtship behaviors of insect populations can vary across the range of a species. Populations exhibiting divergent courtship behavior may indicate genetic

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

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.

Irreversible thelytokous reproduction in *Muscidifurax uniraptor*

Gottlieb, Y; Zchori-Fein, E. 2001

Vertically transmitted bacteria of the genus *Wolbachia* are obligatory endosymbionts known to cause thelytokous (asexual) reproduction in many species of parasitic Hymenoptera. In these species production of males can be induced, but attempts to establish sexual lines have failed in all but one genus. We have found three reproductive barriers between antibiotic-induced males and conspecific females of *Muscidifurax uniraptor* Kogan and Legner (Hymenoptera: Pteromalidae): males do not produce mature sperm. females are reluctant to mate, and a major muscle is absent from the spermatheca. These findings suggest that *Wolbachia*-induced thelytokous reproduction in *M. uniraptor* is irreversible, and are consistent with the idea that since sexual reproduction has ceased, selection on sexual traits has been removed leading to the disappearance or reduction in these traits. Because under these circumstances asexual reproduction is irreversible, the host has become totally dependent on the symbiont for reproduction.

Rapid range expansion in *Lasius neglectus* (Hymenoptera, Formicidae) - an Asian invader swamps Europe

Seifert, B. 2000

After its first observation in Europe in 1974, *Lasius neglectus* Van Loon, Boomsma & Andrasfalvy 1990 has performed a rapid range expansion throughout the entire Mediterranean area and has reached Central Europe. The present range is delimited by 1 degrees E and 75 degrees E and by 36 degrees N and 49 degrees N with 38 sites known. Regarding colony structure, mating, mode of distribution, local dominance, pest status, and impact on local ant faunas, *neglectus* is comparable to *Linepithema humile* (Mayr 1868). *L. neglectus*, however, can establish permanent colonies in regions with mean January temperatures of -5 degreesC. Climatic barriers ending the northern expansion of *Linepithema* and preventing its long-term outdoor persistence in Central Europe and S Scandinavia will not stop *neglectus* that has the potency to develop into a most important pest species. Morphology, genetics, and zoogeography suggest a very recent separation of *neglectus* from *Lasius turcicus* Santschi 1921 with a most probable radiation centre in Asia Minor. The behavioural change from normal aerial mating to exclusively intranidal mating is considered as deciding step leading to complete reproductive isolation from *turcicus*. The obvious absence of *turcicus* from huge parts of *neglectus*' range is a further argument against considering the two as expression of intraspecific polymorphism. The loss of flight behaviour in queens is still not accompanied by significant morphological changes: wing load and development of flight muscles or fat tissue are comparable to well-flying and clausally founding *Lasius* species.

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.

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 F I females remained unmated they produced both winged and wingless males (similar to 1: 1 ratio) but when immobilized F I 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.

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

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

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

Altered host plant volatiles are proxies for sex pheromones in the gall wasp *Antistrophus rufus*

Tooker, JF; Koenig, WA; Hanks, LM. 2002

We describe a previously uncharacterized function for changes in plant chemistry induced by phytophagous insects: to provide cues for mate location. Larvae of the gall wasp *Antistrophus rufus* Gillette (Hymenoptera: Cynipidae) feed within inconspicuous galls inside the flowering stems of the prairie perennials *Silphium laciniatum* L. and *Silphium terebinthinaceum* Jacquin (Asteraceae). Adult male *A. rufus* emerge before females and are challenged with locating mates that are sequestered within dead plant stems that occur in a matrix of dead vegetation. Allozyme studies revealed complete reproductive isolation between wasp subpopulations in the two plant species. In laboratory bioassays, males responded only to their natal plant species, antennating the stem surface. Males from *S. laciniatum* also responded to hexane extracts of *S. laciniatum* stems, and extracts contained much higher concentrations of monoterpenes (alpha-pinene, beta-pinene, and camphene) than did *S. terebinthinaceum*. Ratios of "+" and "-" enantiomers of alpha- and beta-pinene approximated 50:50 for nongalled *S. laciniatum* stems but strongly differed from 50:50 in galled stems, with "+" and "-" enantiomers strongly dominant in different plants. In bioassays, male wasps from *S. laciniatum* responded to a synthetic blend of the monoterpenes in enantiomeric ratios characteristic of galled stems. Male *A. rufus* rely entirely on olfaction to locate females within stems in a complex prairie habitat, and gall wasps themselves apparently influence the plant to modify ratios of monoterpene enantiomers. These plant volatiles serve as a signal for males, acting as a sex pheromone proxy for females concealed within plant tissues.

Comparative analysis of volatile components from labial glands of male Japanese bumblebees (*Bombus* spp.)

Kubo, R; Ono, M. NA

The volatile components from the labial glands of males of six Japanese bumblebee species were analyzed and compared. Clear species-specificity was found. Ethyl dodecanoate was identified as the major component from the glands of *Bombus* (*Bombus*) *hypocrita* *hypocrita* and *Bombus* (*Bombus*) *hypocrita* *sapporoensis* while dihydrofarnesol and dihydrofarnesol were the major components from *Bombus* (*Bombus*) *ignitus*. Citronellol and trans,trans-farnesol were found from *Bombus* (*Pyrobombus*) *ardens* *ardens* and *Bombus* (*Diversobombus*) *diversus* *diversus*, respectively. trans,trans-Farnesol was also found from *Bombus* (*Diversobombus*) *diversus* *tersatus* in Hokkaido. Such differences strongly suggest that these chemicals play an important role in reproductive isolation between sympatric species of Japanese bumblebees.

Ecological specialization in *Diaeretiella rapae* (Hymenoptera: Braconidae: Aphidiinae) on aphid species from wild and cultivated plants

Navasse, Y; Derocles, SAP; Plantegenest, M; Le Ralec, A. 2018

Diaeretiella rapae is an aphid parasitoid with potential for use in biological control strategies. However, several recent genetic studies have challenged the long held view that it is a generalist parasitoid. We investigated its ecological specialization and ability to use resources in cultivated and uncultivated areas. Ecological specialization would reduce its ability to exploit the diversity of aphid species, particularly in uncultivated areas, and to control pest aphids. Four *D. rapae* strains were studied, three reared on pest aphids on Brassicaceae and one strain on a non-pest aphid on Chenopodiaceae. For each strain, we performed host-switching experiments, with a total of six aphid species, five of which *D. rapae* parasitizes in France. We tested cross-breeding ability between strains to detect potential reproductive isolation linked to aphid host species in *D. rapae*. The strain reared on non-pest aphids was able to develop on aphid species from both cultivated and uncultivated plants. The strains reared on pest aphids, however, exclusively parasitized aphid species on cultivated Brassicaceae. In addition, reproductive isolation was detected between strains from uncultivated and cultivated plants. Thus, the *D. rapae* populations examined here appear to be showing ecological specialization or they may even be composed of a complex of cryptic species related to the aphid hosts. The role of *Chenopodium album* as a reservoir for *D. rapae*, by providing a habitat for non-pest aphids on which it can feed, appears to be severely limited, and thus its efficiency to maintain local populations of *D. rapae* in the vicinity of crops is questionable.

The chemical basis of mate recognition in two parasitoid wasp species of the genus *Nasonia*

Mair, MM; Kmezc, V; Huber, S; Pannebakker, BA; Ruther, J. 2017

To recognize one's mate is essential for all sexually reproducing animals. In insects, mate recognition is often based on chemical cues such as hydrocarbons which are distributed over the insect's cuticle. In the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae), interspecific mating possibly occurs in microsympatry between *Nasonia vitripennis* Walker and *Nasonia giraulti* Darling despite post-zygotic isolation mechanisms preventing **hybridization**. Males of *N.vitripennis* are known to equally court con- and heterospecific females, which they recognize by means of cuticular hydrocarbons. A recent study surprisingly showed that this might not be the case in *N.giraulti*, leaving open how males of this species achieve the recognition of mating partners. In this study, we investigated chemical mate recognition in *N.giraulti* in more detail and compared observed behaviors with behaviors of *N.vitripennis* by conducting experiments with both species concurrently

and under the same experimental conditions. We disentangled the role of female-derived non-polar cuticular lipids - i.e., cuticular hydrocarbons - and more polar cuticular lipids in the ability of males to recognize con- and heterospecific females. In addition, we tested whether females of the two species discriminate similarly between con- and heterospecific males. We demonstrate that, in contrast to *N.vitripennis*, males of *N.giraulti* prefer live conspecific females over heterospecific ones. Furthermore, in contrast to *N.vitripennis*, mate recognition in *N.giraulti* males is not based on cuticular hydrocarbons, but rather involves other chemical messengers, presumably more polar cuticular lipids. In both species, discrimination against heterospecific males decreases with female age.

Diversity of *Ooencyrtus* spp. (Hymenoptera: Encyrtidae) parasitizing the eggs of *Stenozygum coloratum* (Klug) (Hemiptera: Pentatomidae) with description of two new species

Samra, S; Cascone, P; Noyes, J; Ghanim, M; Protasov, A; Guerrieri, E; Mendel, Z. 2018

Ooencyrtus spp. (Hymenoptera, Chalcidoidea, Encyrtidae) are important natural enemies of agricultural and forest insect pests, and are distributed worldwide. Their reduced dimensions, highly variable morphological characters and possible effect of wide host range and abiotic factors, make correct identification at the species level particularly difficult. This paper combined molecular, morphological, and biological data to characterize a group of *Ooencyrtus* spp. emerging from the eggs of the variegated caper bug, *Stenozygum coloratum* in the east Mediterranean area. COI and ITS2 sequencing revealed the presence of six and five divergent clades, respectively. Three clades were identified as *Ooencyrtus telenomicida*, *Ooencyrtus pityocampae* and *O. pistaciae*. Two clades represent new species which are here described and named *Ooencyrtus zoeae* and *Ooencyrtus mevalbelus*. These features were combined with reliable morphological characters to facilitate the separation of these species. A dichotomous key and a new synonymy are proposed. *Ooencyrtus pistaciae* had two distinct COI clades but only one ITS2 Glade. Crossbreeding trials that included *Ooencyrtus telenomicida*, *Ooencyrtus melvabelus* sp. nov. and *Ooencyrtus zoeae* sp. nov. confirmed their reproductive isolation. COI sequences showed 0-0.8% and 4-9% within and between-species genetic differences, respectively. ITS2 showed 0.4-5.9% genetic differences between species, with no genetic differences within species. Haplotype diversity of Israeli and Turkish populations of the various species was 0-0.98 and was particularly low in *Ooencyrtus pityocampae*, whose Israeli population showed no diversity. The discovery of the *Ooencyrtus* spp. on the eggs of the caper bug, and their abundance support the idea that the bug can be used as an alternative host for augmentation of populations of these parasitoids in agricultural and forestry systems.

Reproductive isolation due to prezygotic isolation and postzygotic cytoplasmic incompatibility in parasitoid wasps

Koenig, K; Zundel, P; Krimmer, E; Koenig, C; Pollmann, M; Gottlieb, Y; Steidle, JLM. 2019

The reproductive barriers that prevent gene flow between closely related species are a major topic in evolutionary research. Insect clades with parasitoid lifestyle are among the most species-rich insects and new species are constantly described, indicating that speciation occurs frequently in this group. However, there are only very few studies on speciation in parasitoids. We studied reproductive barriers in two lineages of *Lariophagus distinguendus* (Chalcidoidea: Hymenoptera), a parasitoid wasp of pest beetle larvae that occur in human environments. One of the two lineages occurs in households preferably attacking larvae of the drugstore beetle *Stegobium paniceum* ("DB-lineage"), the other in grain stores with larvae of the granary weevil *Sitophilus granarius* as main host ("GW-lineage"). Between two populations of the DB-lineage, we identified slight sexual isolation as intraspecific barrier. Between populations from both lineages, we found almost complete sexual isolation caused by female mate choice, and postzygotic isolation, which is partially caused by cytoplasmic incompatibility induced by so far undescribed endosymbionts which are not *Wolbachia* or *Cardinium*. Because separation between the two lineages is almost complete, they should be considered as separate species according to the biological species concept. This demonstrates that cryptic species within parasitoid Hymenoptera also occur in Central Europe in close contact to humans.

Genome-wide MIG-seq and morphometric data reveals heterospecificity of the *Gnamptogenys taivanensis* group (Hymenoptera: Formicidae: Ectatomminae) in the northern mountainous region of Vietnam

Nguyen, DD; Oguri, E; Yamada, A; Lin, CC; Chen, ZL; Nguyen, AD; Suyama, Y; Eguchi, K. 2020

The *Gnamptogenys taivanensis* group (Hymenoptera: Formicidae: Ectatomminae) is a species-group that contains eight described species known from southern China, Taiwan, and northern Vietnam. The taxonomy of the group remains poorly understood due to limited morphological evidence (mostly relying on the morphology of the worker caste), the complete lack of molecular evidence, as well as poor sampling effort: most of the species except *G. taivanensis* have so far been known only from one or two localities in southern China each, and are allopatric to each other. However, our recent collection of many colonies of *G. taivanensis* group from three localities (Tay Con Linh, Phia Oac - Phia Den, and Hoang Lien Son) in the northern mountainous region of Vietnam showed remarkable diversity in the external morphology of workers, even within a single locality. The present study thus aims to explore species delimitation of the *G. taivanensis* group in the region using a combination of phylogenetic and morphometric analyses. Phylogenetic analyses for the genome-wide sequence datasets generated by MIG-seq and morphometric analysis using Nest-Centroid (NC) clustering consistently recovered seven major lineages of the *G. taivanensis* group (six Vietnamese and one Taiwanese lineages), with four singleton colonies for which these analyses yielded inconsistent and complex pictures. Moreover, the sympatric occurrence of the two or three lineages in each of the three localities of northern Vietnam is indirect evidence of the presence of reproductive isolation among these lineages. These lines of evidence strongly support heterospecificity of the lineages. However, phylogenetic analysis for mitochondrial COI gene showed an almost incompatible pattern with these lineages, possibly due to incomplete lineage sorting and/or introgressive hybridisation. A future comprehensive study on a larger geographic scale is needed to clarify the placement of the singleton colonies and conduct a formal taxonomic revision of the group.

"Darwin's corollary" and cytoplasmic incompatibility induced by *Cardinium* may contribute to speciation in *Encarsia* wasps (Hymenoptera: Aphelinidae)

Gebiola, M; Kelly, SE; Hammerstein, P; Giorgini, M; Hunter, MS. 2016

The potential importance of cytoplasmic incompatibility (CI)-inducing bacterial symbionts in speciation of their arthropod hosts has been debated. Theoretical advances have led to a consensus that a role is plausible when CI is combined with other isolating barriers. However, the insect model systems *Nasonia* and *Drosophila* are the only two experimental examples documented. Here, we analyzed the components of reproductive isolation between the parasitoid wasp *Encarsia suzannae*, which is infected by the CI-inducing symbiont *Cardinium*, and its uninfected sibling species *Encarsia gennaro*. Laboratory crosses demonstrated that: (1) sexual isolation is incomplete; (2) hybrid offspring production is greatly reduced in the interspecific CI cross; (3) viable hybrids may be produced by curing *E. suzannae* males of *Cardinium* with antibiotics; (4) hybrid offspring production in the reciprocal cross is greatly reduced by hybrid inviability due to genetic incompatibilities; (5) hybrid sterility is nearly complete in both directions at the F1 stage. Thus, asymmetrical hybrid incompatibilities and CI act as complementary isolating mechanisms. We propose a new model for contributions of CI symbionts to speciation, with CI reducing gene flow between species in one direction, and in the other, a symbiont sweep resulting in accelerated mtDNA evolution, negative cytonuclear interactions, and hybrid incompatibilities.

Courtship of the two female morphs of *Melittobia digitata* (Hymenoptera : Eulophidae)

Gonzalez, JM; Matthews, RW. 2005

Courtship of sib-mating *Melittobia digitata* Dahms, a parasitoid of solitary wasps and bees, is reviewed, described, and quantified for 125 virgins of the non-dispersing

brachypterous female (BF) morph paired with 24 experienced males, and for 158 virgins of the dispersing macropterous female (MF) morph paired with 21 males. Males performed 1-5 courtship bouts with both morphs; about half of all successful matings in both morphs occurred after a single bout. Depending on number of bouts performed, mean courtship durations ranged from 47-268 sec for MFs and 59-277 sec for BF. Courtship success rates were greater for BF couples (80%) than for MF couples (57%). Compared to BF couples, MF couples were more apt to undergo multiple bouts. Results are interpreted in the context of the morphs' life history and the costs/benefits of alternatives. Failure to initiate any courtship during the 15-min observation period (22% for MF pairs, 21% for BF pairs) appeared to be due to apparent lack of interest or to occasional male violence toward females. Possible explanations for the latter, including mistaken identity, odor contamination, and nutritional stress are discussed.

Robertsonian rearrangements in Neotropical Meliponini karyotype evolution (Hymenoptera: Apidae: Meliponini)

Cunha, MS; Soares, FAF; Clarindo, WR; Campos, LAO; Lopes, DM. 2021

Genome changes, evidenced through karyotype or nuclear genome size data, can result in reproductive isolation, diversification and speciation. The aim of this study was to understand how changes in the karyotype such as chromosome number and nuclear genome size accompanied the evolution of neotropical stingless bees, and to discuss these data in a phylogenetic context focusing on the karyotype evolution of this clade. We sampled 38 species representing the three Neotropical Meliponini groups; 35 for karyotype analyses and 16 for 1C value measurement. The chromosome number varied from $2n = 16$ to $2n = 34$, with distinct karyotypic formulae and the presence of a few polymorphisms, such as B chromosomes in one species and arm size differences between homologous chromosomes in two species. The mean 1C value varied from 0.31 pg to 0.92 pg. We associated empirical data on chromosome number and mean 1C value to highlight the importance of Robertsonian fusion rearrangements, leading to a decrease in chromosome number during the Neotropical Meliponini evolution. These data also allowed us to infer the independent heterochromatin amplification in several genera. Although less frequent, Melipona species with $2n = 22$ represent evidence of Robertsonian fissions. We also pointed out the importance of chromosomal rearrangements that did not alter chromosome number, such as inversions and heterochromatin amplification.

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.

The evolution of antennal courtship in diplazontine parasitoid wasps (Hymenoptera, Ichneumonidae, Diplazontinae)

Klopfstein, S; Quicke, DLJ; Kropf, C. 2010

Background: As predicted by theory, traits associated with reproduction often evolve at a comparatively high speed. This is especially the case for courtship behaviour which plays a central role in reproductive isolation. On the other hand, courtship behavioural traits often involve morphological and behavioural adaptations in both sexes; this suggests that their evolution might be under severe constraints, for instance irreversibility of character loss. Here, we use a recently proposed method to retrieve data on a peculiar courtship behavioural trait, i.e. antennal coiling, for 56 species of diplazontine parasitoid wasps. On the basis of a well-resolved phylogeny, we reconstruct the evolutionary history of antennal coiling and associated morphological modifications to study the mode of evolution of this complex character system. Results: Our study reveals a large variation in shape, location and ultra-structure of male-specific modifications on the antennae. As for antennal coiling, we find either single-coiling, double-coiling or the absence of coiling; each state is present in multiple genera. Using a model comparison approach, we show that the possession of antennal modifications is highly correlated with antennal coiling behaviour. Ancestral state reconstruction shows that both antennal modifications and antennal coiling are highly congruent with the molecular phylogeny, implying low levels of homoplasy and a comparatively low speed of evolution. Antennal coiling is lost on two independent occasions, and never reacquired. A zero rate of regaining antennal coiling is supported by maximum parsimony, maximum likelihood and Bayesian approaches. Conclusions: Our study provides the first comparative evidence for a tight correlation between male-specific antennal modifications and the use of the antennae during courtship. Antennal coiling in Diplazontinae evolved at a comparatively low rate, and was never reacquired in any of the studied taxa. This suggests that the loss of antennal coiling is irreversible on the timescale examined here, and therefore that evolutionary constraints have greatly influenced the evolution of antennal courtship in this group of parasitoid wasps. Further studies are needed to ascertain whether the loss of antennal coiling is irreversible on larger timescales, and whether evolutionary constraints have influenced courtship behavioural traits in a similar way in other groups.

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

Velthuis, BJ; Yang, WC; van Opijnen, T; Werren, JH. 2005

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

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.

A re-assessment of within-host mating behavior in the *Nasonia* species complex

Trienens, M; Giesbers, MCWG; Pannebakker, BA; van de Zande, L; Beukeboom, LW. 2021

Insects have a wide variety of mating systems. For parasitic wasps, mating sites are often confined to where their hosts occur and this mutual interaction therefore greatly affects the evolution of alternative mating strategies. Parasitoids of the genus *Nasonia* (Hymenoptera: Pteromalidae) - comprising the species *Nasonia vitripennis* (Walker), *Nasonia longicornis* Darling, *Nasonia giraulti* Darling, and *Nasonia oneida* Raychouhury & Desjardins - parasitize blowfly pupae and typically mate immediately after emergence of the females from the host puparium. However, in one *Nasonia* species, *N. giraulti*, mating predominantly occurs within the host. Here, we re-assess within-host mating (WHM) rates within the *Nasonia* genus, using laboratory lines and field-collected hosts, including *N. oneida* for which no data were available yet. We confirm that WHM rates are low in *N. vitripennis* and *N. longicornis*, but high in *N. giraulti*. WHM is rare in *N. oneida*, despite its sympatry and close phylogenetic relationship with *N. giraulti*. Multiparasitization experiments with *N. vitripennis* and *N. giraulti* resulted in lower WHM proportions of *N. giraulti*. To evaluate whether this was due to an exit hole in the host pupa created by *N. vitripennis* males - where an exit hole may result in emergence of *N. giraulti* females and possibly males prior to mating - we artificially created exit holes in the host parasitized by *N. giraulti* only. This also yielded a lower WHM proportion. Progeny analysis of interspecific crosses of *N. longicornis* and *N. oneida* with *N. giraulti*, generating **hybrid** female but **non-hybrid** male offspring, revealed that WHM is largely determined by the maternal species and, hence, by the species to which the male offspring belong. We conclude that WHM is predominantly a male-mediated trait in *N. giraulti*, resulting from males refraining from making exit holes and mating with females inside the host. We discuss how these findings can be used to evaluate whether WHM contributes to preventing species **hybridization**.

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

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

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

Courtship and mating behaviour of interspecific *Nasonia* hybrids (Hymenoptera, Pteromalidae): A grandfather effect

Beukeboom, LW; van den Assem, J. 2001

Nasonia courtship behaviour includes easily quantifiable, stereotyped components. We analysed displays of *N. vitripennis* X *N. longicornis* **hybrid** males. Most of them performed well-organised displays that were intermediate between the parental species. However, in both reciprocal crosses, a significant bias towards the behaviour of the grandpaternal species was observed. Possible explanations for this effect are a biased recovery of genotypes, either due to nucleo-cytoplasmic interaction or non-mendelian transmission, or differential activation of genes in **hybrid** females. This study is a first step towards unravelling the genetic architecture of courtship behaviour of *Nasonia*, which may provide information about factors responsible for species isolation.

Rhadinoceraea n sp (Hymenoptera: Tenthredinidae) from West Virginia, a second species on *Zigadenus* (Liliaceae)

Smith, DR; Barrows, EM. 1995

Rhadinoceraea sodsensis, n. sp., is described from West Virginia. Its larvae feed on inflorescences of *Zigadenus leimanthoides* (Liliaceae). This species is separated from the closely related *R. zigadenusae* from coastal Mississippi by reproductive isolation, phenology, and wing morphology. *Rhadinoceraea sodsensis* is univoltine. Adults fly in mid-July, and larvae feed in late July through mid-August.

A test of the sympatric host race formation hypothesis in *Neodiprion* (Hymenoptera: Diprionidae)

Linnen, CR; Farrell, BD. 2010

Theory suggests that sympatric speciation is possible; however, its prevalence in nature remains unknown. Because *Neodiprion* sawflies are host specialists and mate on their hosts, sympatric speciation via host shifts may be common in this genus. Here, we test this hypothesis using near-complete taxonomic sampling of a species group, comprehensive geographical and ecological data, and multiple comparative methods. Host-use data suggest that host shifts contributed to the evolution of reproductive isolation in *Neodiprion* and previous work has shown that gene flow accompanied divergence. However, geographical data provide

surprisingly little support for the hypothesis that host shifts occurred in sympatry. While these data do not rule out sympatric host race formation in *Neodiprion*, they suggest that this speciation mode is uncommon in the genus and possibly in nature.

Species status of two populations of *Encarsia sophia* (Girault & Dodd) (Hymenoptera : Aphelinidae) native to different geographic areas

Giorgini, M; Baldanza, F. 2004

Encarsia sophia is a cosmopolitan parasitoid of whitefly pests and is regarded as a potential biocontrol agent of the *Bemisia tabaci* species-complex. However.. the possibility exists that under this specific name a complex of cryptic species may be contained, whose correct identification is essential for their effective use in biological control programmes. In this paper two populations native to Pakistan and Spain, respectively, were characterized by means of morphometric analysis, crossing experiments and karyological analysis. The two populations, indistinguishable on the basis of single morphological characters, were distinguished through a canonical discriminant function analysis. Mating tests showed rare copulation attempts and no transfer of spermatozoa into the spermatheca in heterogamic crosses, thus suggesting that the Pakistani and Spanish populations are reproductively isolated. Although the two populations shared the same karyotype formula ($2n = 2m + 2sm + 6a$), consistent differences were found in their chromosome sets. In the Spanish population the metacentric chromosome was almost one half of the whole genome set ($rl = 44.23$), while in the Pakistani one, it was shorter ($rl = 29.55$) and as long as the sub-metacentric chromosome. Moreover, silver staining provided evidence of a different localization of the active nucleolus organizer regions (NORs). Results obtained in this study suggest that the Pakistani and Spanish populations are distinct cryptic species. (C) 2003 Elsevier Inc. All rights reserved.

Hybridization in the European carpenter ants *Camponotus herculeanus* and *C. ligniperda* (Hymenoptera: Formicidae)

Seifert, B. 2019

The first case of **hybridization** between the large European carpenter ants *Camponotus herculeanus* (Linnaeus 1758) and *C. ligniperda* (Latreille 1802) is demonstrated by means of exploratory and hypothesis-driven data analyses of standardized phenotypic characters. The strong signal separating the parental species allows the identification of **hybrid** workers on the individual level, based on only seven characters. The frequency of **hybridization** between the two species is estimated for Central Europe as 0.2-1.0%. This low ratio indicates strong reproductive barriers considering syntopic occurrence at about 10% of the observation sites, a nearly complete overlap of swarming times and basically equal meteorological conditions to release swarming. The presented case increases the known ratio of **hybridizing** species within the 178 ant species of Central Europe to 19.1%. This figure dramatically contrasts the known **hybridization** ratio of 0.55% within an estimated number of 2000 ant species from the Holarctic outside Central Europe. This 30-fold lower discovery rate of **hybrids** may be explained by the predominance of idiosyncratic species delimitation methods in morphology-based taxonomy in combination with the psychology of human decision-making. A neotype of *Camponotus ligniperda* is fixed in a specimen from the terra typica and comments on the Latin naming are given.

FORMICA-NIGRICANS EMERY, 1909 - AN ECOMORPH OF FORMICA-PRATENSIS RETZIUS, 1783 (HYMENOPTERA, FORMICIDAE)

SEIFERT, B. 1991

Workers and queens from 224 nest samples of *Formica pratensis* Retzius originating from all over Europe, but mainly from Germany were investigated for several morphological characters, particularly pilosity. Statistic differences between the hairy N morph (= *F. nigricans* Emery 1909) and the less hairy P morph in body size, pilosity, geographic frequency, habitat selection and mound construction could be shown but other aspects of external biology coincide. There are no suggestions of reproductive isolation of the morphs which are interpreted as different genotypes of the same population and represent different ecological adaptations. The strong decrease of N morph frequency in *pratensis* populations from S to N Europe, its higher frequency in more xerothermous habitats in Germany, and its well-documented peculiarity of constructing higher mounds than the P morph for conditions of equal sun-exposure 11hb167003.reich characterize the N morph as a genotype adapted to higher temperatures. In Germany, as much as 16% of *pratensis* nests investigated contained both morphs. Polycalic colonies are found in both morphs but isolated nests predominate. *Formica minor pratensisoides* Gosswald 1951 is a synonym of *pratensis* and refers to polycalic colonies of the P morph which occasionally occur inside more mesophilic, less sun-exposed forests.

TAXONOMIC STUDY OF A POPULATION OF TRICHOGRAMMA-TURKESTANICA DISCOVERED IN SOUTHERN PORTUGAL (HYMENOPTERA, TRICHOGRAMMATIDAE)

NETO, L; PINTUREAU, B. 1995

Based on morphological characters, *Trichogramma* strains collected in southern Portugal were determined as *T. turkestanica* Meyer. A biochemical characterisation by electrophoresis of these strains concerned 2 enzymatic systems, esterases and superoxide dismutases. The identified alleles show that *T. turkestanica* belongs to the *evanescens* group. Some interspecific compatibilities were revealed from crossing experiments between species of that group. Based either on allele frequencies of loci coding for studied enzymes or on data issued from crossing experiments, Nei genetic distances and reproductive isolation indices were respectively calculated. These values allowed to draw 2 dendrograms suggesting that *T. brassicae* and *T. voegelei* are the closest species in the *evanescens* group. Among two hypothetical cladograms based on morphological characters, one suggests that *T. evanescens* and *T. brassicae* are the closest species, and the other is consistent with the preceding constructions. Thus, it is likely that the cladogram where *T. voegelei* and *T. brassicae* are the closest, corresponds to the phylogeny of the *evanescens* group. Synonymy between *T. turkestanica* and *T. meyeri* Sorokina is discussed.

Evolution of reproductive structures for in-flight mating in thynnine wasps (Hymenoptera: Thynnidae: Thynninae)

Semple, TL; Vidal-Garcia, M; Tatarinic, NJ; Peakall, R. 2021

Thynnine wasps have an unusual mating system that involves concurrent in-flight copulation and nuptial feeding of wingless females by alate males. Consequently, thynnine genitalia play a multifunctional role and have likely been subject to various different selective pressures for both reproductive success and food provisioning. Here, we present a new molecular phylogeny for the Australian Thynninae and use 3D-geometric morphometrics and comparative methods to investigate the morphological evolution of select genital structures across the group. We found significant morphological integration between all male and female structures analysed, which is likely influenced by sexual selection, but also reproductive isolation requirements and mechanical constraints. The morphology of the primary male and female coupling structures was correlated with female body size, and female genitalia exhibited strong negative size allometry. Those male and female coupling structures have evolved at similar evolutionary rates, whereas female structures appear to have evolved a higher degree of morphological novelty over time. We conclude that the unique reproductive strategies of thynnine wasps have resulted in complex evolutionary patterns in their genital morphology, which has likely played a central role in the extensive diversification of the subfamily across Australasia and South America. Our study reinforces the need to treat composite characters such as genitalia by their component parts, and to consider the roles of both male and female reproductive structures in evolutionary studies.

Interspecific hybridisations in natural populations of ants by example of a regional fauna (Hymenoptera, Formicidae)

Seifert, B. 1999

The identification of **hybrids** by high-precision stereomicroscopy and chorological data is described in detail by example of the ant species *Formica bruni* and *pressilabris*. In a synopsis of heterogeneous data, the overall **hybrid** frequency in the ant fauna of Central Europe is estimated. 17 of the 164 ant species of this region are demonstrated and further 2 species strongly suspected to **hybridise**. The low-frequency **hybridisers**, showing overall **hybrid** ratios < 3%, were native elements of the Central European fauna before the onset of human cultivation and experienced direct interspecific contact for longer periods of their natural history. They developed more effective mechanisms of reproductive isolation beginning at the prezygotic level. Extremely high (12-31%) local **hybridisation** ratios occurred in species that invaded the area after anthropogenic changes in landscape structure. The segregated distribution of invaders and autochthonous species in the precultural period apparently did not impose the need to evolve more effective mechanisms for reproductive isolation. Prezygotic mechanisms in particular are deficient. In local situations, 19% of *Lasius jensi* matings and 44% of *Leptothorax albipennis* matings leading to successful nest foundations were matings with heterospecific partners. Signs for a dissolution of interspecific phenotypic differences are not detectable in most of the species. The dispersal of **hybrid** genotypes is apparently inhibited. Factors that probably stabilise the genomic integrity of **hybridizing** parent species are: (a) inability to produce **hybrid** queens (*L. jensi* x *umbratus*), (b) aneuploidy of F(1) females with inability to perform a balanced meiosis in **hybrids** of *L. albipennis* with 3 other species), and (c) selection against **hybrids** in the epigenetic environment of alleles (*Leptothorax nylanderi* x *slavonicus*). Extreme ratios of heterospecific matings would mean a dangerous drain of genetic material. A mechanism to reduce these losses is postulated consisting of a 'cleptogamy' (theft of heterospecific sperm) by queens that missed a conspecific male, an establishment of a functioning colony of F(1) **hybrid** workers, a depression of the rearing or fertility of **hybrid** queens, and a maximum production of own sons by the queen. This mechanism could explain the persistence of rare species (*Lasius jensi* or *Leptothorax albipennis*) in an environment of more populous heterospecific **hybridisation** partners.

Honeybees, *Apis mellifera* Linnaeus (Hymenoptera : Apidae), of the Drakensberg Mountains in relation to neighbouring populations

Radloff, SE; Hepburn, HR. 1999

Multivariate morphometric analyses were performed on measurements of nine characteristics of 4011 worker bees from natural populations of the Drakensberg mountains and surrounding areas in southern Africa between 28 degrees and 34 degrees S latitude and 25 degrees and 31 degrees E longitude. A discriminant function analysis yielded three morphoclusters: group 1 comprising an unnamed population at higher than 1500 m altitude in the mountains; group 2 consisting of bees considered to be *A. m. scutellata* x *A. capensis* **hybrids**, and group 3 consisting of *A. m. scutellata* surrounding the mountains at lower (less than 1500 m) altitudes. The bees of group 1 show size similarities to disjunct populations of other large Afri-montane bees, *A. m. monticola*. Group 2 **hybrid** bees occur in an area of marked climatic variability and manifest significantly high variances in their morphometrics, pheromones and DNA characteristics. It is suggested that differences in swarming seasons among these populations effectively cause temporal reproductive isolation between the group 1 mountain bees and those *A. m. scutellata* below the escarpment.

DIFFERENCES IN LIFE-HISTORY TRAITS BETWEEN ISOFEMALE LINES OF THE APHID PARASITOID APHELINUS-ABDOMINALIS (HYMENOPTERA, APHELINIDAE)

HAARDT, H; HOLLER, C. 1992

Six isofemale lines of the aphid parasitoid *Aphelinus abdominalis* (Dalman) were compared for life history traits related to parasitism of three cereal aphid species. The lines differed most in the number of hosts parasitized in 24 h and the developmental times of females and males. Cluster and discriminant analysis showed that these differences were pronounced between line I, line II and lines III-VI, but not between lines III, IV, V and VI. Cross-breeding experiments revealed reproductive barriers between the lines which proved to differ most in life history traits, indicating the possible existence of morphologically similar species. Given the variability in life history traits between lines of *A. abdominalis* recorded in this study, we suggest a careful selection of candidate strains for biological control will be rewarding.

Genetic analyses reveal cryptic diversity in the native North American fire ants (Hymenoptera: Formicidae: Solenopsis)

Chialvo, P; Gotzek, DA; Shoemaker, D; Ross, KG. NA

The native North American fire ants (*Solenopsis* Westwood) comprise a difficult group taxonomically that has undergone multiple revisions in the past century yet remains in a state of taxonomic uncertainty. In the present study, we utilised a set of 59 microsatellite markers analysed in 238 specimens to conduct the first robust genetic analysis of the four nominal species. Our approach used a variety of methods to test operational criteria commonly employed in species delimitation, including genotypic clustering, reproductive isolation/cohesion and monophyly. We conclude from our results that the recognised North American fire ant species represent evolutionarily independent entities and, moreover, we confirm the presumed sister status of the desert fire ants, *S. aurea* Wheeler and *S. amblychila* Wheeler. However, the presence of at least two genetically divergent populations within the nominal species boundaries, including a western form of *S. xyloni* and a distinct population of *S. aurea* endemic to the Salton Trough, suggests that the current taxonomy does not fully capture the species-level diversity in this group. Our study provides the molecular foundation for future integrated studies of the taxonomy and evolution of this scientifically and economically important group of insects.

Species status of two host-associated populations of *Aphytis lingnanensis* (Hymenoptera: Aphelinidae) in citrus

Fernando, LCP; Walter, GH. 1997

Although *Aphytis lingnanensis* Compere is an important parasitoid of California red scale *Aonidiella aurantii* Maskell, several 'races' of *A. lingnanensis* exist which parasitize white louse scale *Unaspis citri* Comstock. The reproductive status of a white louse scale 'race' of *A. lingnanensis* that originated in Thailand was examined in relation to several populations (from Queensland, California and the Philippines) that parasitize California red scale. None of the wasps from red scale mated with individuals from white louse scale in small cages within 10 min, whereas control crosses all mated in that time. The two 'races' would therefore constitute independent reproductive entities (species) in sympatry in the field, although no consistent anatomical differences could be found between them, even with the aid of discriminant function analysis on the number of setae on the delta region of the forewing and the mesoscutum. *Aphytis lingnanensis* from California red scale in Queensland mated readily with wasps derived from the same host species in California and the Philippines, and mating took place at random among individuals in mate choice tests. Although such results are equivocal, there are no reasons for suspecting that these different populations of *A. lingnanensis* from California red scale also comprise more than one species. The results obtained indicate that cross-mating tests designed to quantify reproductive isolation between sexual populations are inappropriate. They should rather be designed to establish whether individuals recognize one another as potential mates. The design of cross-mating tests should therefore consider the usual time to mating, of known conspecifics, under the experimental conditions to be used.

Host plants impact courtship vibration transmission and mating success of a parasitoid wasp, *Cotesia flavipes* (Hymenoptera: Braconidae)

Joyce, AL; White, WH; Medina, RF. 2014

Host plants provide food, shelter, and mating habitats for herbivorous and parasitoid insects. Yet each plant species is a microhabitat with distinct chemical and physical attributes that may drive selection and diversification of insects. This study examines differences in courtship vibration signal transmission through leaves of three host plant species that vary in physical characteristics (leaf tissue density, leaf width and percent moisture) and how signal transmission relates to mating success for a parasitoid wasp. The vibration signals transmit with a longer duration and a higher relative amplitude in the host plant leaves of the species with the lowest leaf tissue density, which was also the plant type with the highest mating frequency. Host plants may be a selective force on courtship vibration signals and may contribute to the behavioral or genetic divergence of populations on different host plant species.

Host selection and reproductive success of French and Moroccan populations of the parasitoid, *Microctonus aethiopoides* (Hymenoptera : Braconidae)

Sundaralingam, S; Hower, AA; Kim, KC. 2001

French and Moroccan populations of the parasitoid *Microctonus aethiopoides* Loan were studied in the laboratory for their host selection, mating behavior, and reproductive success. The French strain, collected on *Hypera postica* (Gyllenhal), although capable of parasitizing and producing viable offspring on *Sitona* weevils, preferred *Hypera* weevils, its known target host. The Moroccan strain, collected on *Sitona discoideus* Gyllenhal, exhibited host specificity for *Sitona*. A partial reproductive isolation was observed between the two strains. Moroccan females mated more frequently with French males than did French females with Moroccan males. The pre-copulation time for mating pairs of opposite strains was significantly longer than that for mating pairs of the same strain. There was no significant difference in copulation time nor in larval and pupal duration between French and Moroccan strains. In summary, the French and Moroccan strains of *M. aethiopoides* are clearly separable by biological, behavioral, and morphometric traits and the preferred host for *Hypera* is the French strain and *Sitona* for the Moroccan strain. Consequently, geographic location and host source become important when considering this parasitoid as a potential biological control agent.

A first demonstration of interspecific hybridization in *Myrmica* ants by geometric morphometrics (Hymenoptera: Formicidae)

Yazdi, AB; Munch, W; Seifert, B. 2012

A case of **hybridization** between *Myrmica scabrinodis* NYLANDER, 1846 and *M. vandeli* BONDROIT, 1920 is demonstrated by means of geometric morphometrics in a nest sample found in Baden-Wurttemberg/Germany. 41 land marks and 252 semilandmarks were fixed in four anatomical aspects in 299 worker ants - dorsal head, frontodorsal clypeus, dorsal mesosoma and lateral petiole. 316 relative warps (RWs) were extracted from the shape variables. Shape differences between these very similar species are visualized by mean deformation grids exaggerated by the factor of three. Among a number of differences already known, geometric morphometrics revealed also differences not discovered so far by conventional morphometrics or subjective character assessment. A full separation of the parental species was provided by the first two RWs of the head and clypeus aspect by this approach was not sufficient to reliably demonstrate **hybrid** identities on individual level. A stepwise linear discriminant analysis (LDA) using the RWs as characters and reducing character number from 316 to 25, offered very powerful separation of the 291 workers of the parental species ($F = 6735.1$, ANOVA) and placed the eight workers of the **hybrid** sample in a coherent cluster exactly in the empty space between the parental species. Basically similar results were achieved with conventional linear morphometrics considering 16 characters but this system was less powerful ($F = 3108.0$, ANOVA) and placed two specimens of the parental species close to the **hybrids**. There was no directional asymmetry detectable in the whole material and the **hybrid** sample did not show increased fluctuating asymmetry. With the current methodology applied, data acquisition time was 48 minutes per specimen in conventional linear morphometrics but as much as 160 minutes in geometric morphometrics. The main problems of the latter method are the slowness of the automatic z-stack imaging methods and the absent software assisting landmark fixation. A considerable reduction of total data acquisition time to 84 (one-fold digitizing) or to 108 minutes (two-fold digitizing) per specimen is predicted if a grid-and-sector system assisting landmark fixation is automatically projected on the screen. The overall **hybridization** frequency for the region of Baden-Wurttemberg is estimated as 0.44% from the side of *M. vandeli* and 0.03% from the side of *M. scabrinodis*. Despite apparently strong reproductive barriers between *M. scabrinodis* and *M. vandeli*, rare **hybridization** may occur because of the constant and extremely close spatial association, largely overlapping swarming times and some aspects of male mating behavior. *Myrmica vandeli* is very likely a facultative temporary social parasite of *M. scabrinodis*. As much as 17% of the 225 *M. vandeli* nests found in Baden-Wurttemberg still contained *M. scabrinodis* workers.

A taxonomic issue of two species of *Trissolcus* (Hymenoptera: Platygasteridae) parasitic on eggs of the brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae): resurrection of *T. plautiae*, a cryptic species of *T. japonicus* revealed by morphology, reproductive isolation and molecular evidence

Matsuo, K; Hirose, Y; Johnson, NF. 2014

Trissolcus plautiae (Watanabe) is known as a major egg parasitoid of the brown-winged green bug *Plautia stali* Scott, which is a serious pest of various fruit trees in Japan. Although *T. plautiae* was synonymized with *T. japonicus* (Ashmead) in 1981, both scientific names have been used for the same egg parasitoid of *P. stali* for about the past 30 years because of their taxonomic confusion. To promote an effective IPM program for *P. stali* using its egg parasitoids, we attempted to resolve the confusion by the use of a variety of methods. On detailed observation of adult morphology, we found that sublateral setae on the T1 are present in *T. plautiae* and absent in *T. japonicus*, and that this morphological difference is corroborated by more subtle differences between the two species. This finding supports the view that they are different species. The view was also supported by the results of mating experiments to determine the reproductive isolation of *T. plautiae* from *T. japonicus* and DNA analysis of these two species. We conclude that *T. plautiae* is a cryptic species of *T. japonicus* and resurrect *T. plautiae* from *T. japonicus* stat. rev.

FERTILIZATION OF HETERO-SPECIFIC INSECT EGGS BY SPERM INJECTION

SAWA, M. 1991

Mature eggs dissected from the ovary of unmated sawflies (Tenthredinidae, Hymenoptera) can be activated to develop (to haploid males), simply by placing on a filter paper wet with distilled water. These unfertilized eggs may be injected with sperm, and some, successfully completing fertilization, develop as diploid females. Premating reproductive isolation exists between two sympatric species of this family, *Athalia rosae ruficornis* Jokovlev and *Athalia infumata* Marlatt. Taking advantage of the difference in karyotypes, it is shown that hetero-specific sperm injection results in successful fertilization and that the **hybrid** survives at least through the middle stage of embryogenesis.

Reconstructing the relatedness of cooperatively breeding queens in the Panamanian leaf-cutting ant *Acromyrmex echinator* (Hymenoptera: Formicidae)

Nehring, V; Dijkstra, MB; Sumner, S; Hughes, WOH; Boomsma, JJ. 2018

The evolution of permanent inquiline social parasites in ants has been conjectured to be facilitated by secondary polygyny, that is, the re-adoption of new queens into existing mature colonies. This idea was first formulated by Wasmann, Wheeler, and Emery more than a century ago. Emery predicted that inquilines should be the sister-lineages of their hosts, which prompted Alfred Buschinger to propose that they evolve by sympatric speciation. However, these scenarios hinge on two vital conditions that have not been quantitatively documented: 1. That host sister species are secondarily polygynous and primarily recruit close kin, and 2. That such adoptions are prone to occasional mistakes that would select for the condition-dependent expression of exploitative traits and reproductive isolation by disruptive selection. Here, we use a long-term data set on the leaf-cutting ant *Acromyrmex echinator* (FOREL, 1899), known to have a closely related inquiline social parasite *A. insinuator* SCHULTZ, BEKKEVOLD & BOOMSMA, 1998, to address the first of these conditions. We estimate the frequency of secondary polygyny and the degree to

which cooperatively breeding queens are related. We find that the overall frequency of polygynous colonies is ca. 8% and that polygynous colonies typically have two queens. Most queen pairs are first-degree relatives, consistent with colonies adopting one or two daughters either before or just after becoming orphaned. However, we also document a few pairs of cooperatively breeding queens that are unrelated and estimate that this social structure may apply to ca. 20% of the polygynous colonies, and thus ca. 1% of all colonies. Our findings show that the breeding system of *A. echinator* matches the polygyny characteristics that are believed to facilitate the emergence of socially parasitic queen morphs.

Asymmetric Assortative Mating Behaviour Reflects Incomplete Pre-zygotic Isolation in the *Nasonia* Species Complex

Buellesbach, J; Greim, C; Raychoudhury, R; Schmitt, T. 2014

Preference of con- over heterospecific mates leading to assortative mating can substantially contribute to pre-zygotic reproductive isolation and prevent fitness losses if post-zygotic **hybridization** barriers already exist. The jewel wasp genus *Nasonia* displays quite strong and well-studied post-zygotic reproductive isolation due to a ubiquitous *Wolbachia* infection causing cytoplasmic incompatibility between different species. Pre-zygotic isolation, however, has received far less research attention in this model organism, especially concerning the mechanisms and criteria of mate choice. In the present study, we analysed mate rejection and mate acceptance rates in cross-comparisons between all four *Nasonia* species. We put emphasis on observing which sex is more likely to interrupt interspecific matings and how discriminatory behaviour varies across the different species in all possible combinations. We found an asymmetric distribution of assortative mating among the four *Nasonia* species that appears to be highly influenced by the respective combinations of sex and species. Females appeared to be the main discriminators against heterospecific mating partners, but interestingly, we could also detect mate discrimination and rejection behaviour in males, a widely neglected factor in research on mating behaviour in general and on *Nasonia* in particular. Moreover, the asymmetry in the assortative mating behaviour was partially reflective of sym- or allopatric distributions of natural *Nasonia* populations.

Divergent strategies in pre- and postzygotic reproductive isolation between two closely related *Dianthus* species

Cahenzli, F; Bonetti, C; Erhardt, A. 2018

Quantifying the relative contribution of multiple isolation barriers to gene flow between recently diverged species is essential for understanding speciation processes. In parapatric populations, local adaptation is thought to be a major contributor to the evolution of reproductive isolation. However, extrinsic postzygotic barriers assessed in reciprocal transplant experiments are often neglected in empirical assessments of multiple isolation barriers. We analyzed multiple isolation barriers between two closely related species of the plant genus *Dianthus*, a genus characterized by the most rapid species diversification in plants reported so far. Although *D. carthusianorum* L. and *D. sylvestris* Wulf. can easily be **hybridized** in crossing experiments, natural **hybrids** are rare. We found that in parapatry, pollinator-mediated prezygotic reproductive isolation barriers are important for both *D. carthusianorum* (0.761) and *D. sylvestris* (0.468). In contrast to *D. carthusianorum*, high **hybrid** viability in *D. sylvestris* (-0.491) was counteracted by strong extrinsic postzygotic isolation (0.900). Our study highlights the importance of including reciprocal transplant experiments for documenting extrinsic postzygotic isolation and demonstrates clearly divergent strategies and hence asymmetric pre- and postzygotic reproductive isolation between closely related species. It also suggests that pollinator-mediated and ecological isolation could have interacted in synergistic ways, further stimulating rapid speciation in *Dianthus*.

Cascading reproductive isolation: Plant phenology drives temporal isolation among populations of a host-specific herbivore

Hood, GR; Zhang, LY; Hu, EG; Ott, JR; Egan, SP. 2019

All organisms exist within a complex network of interacting species, thus evolutionary change may have reciprocal effects on multiple taxa. Here, we demonstrate "cascading reproductive isolation," whereby ecological differences that reduce gene flow between populations at one trophic level affect reproductive isolation (RI) among interacting species at the next trophic level. Using a combination of field, laboratory and common-garden studies and long-term herbaria records, we estimate and evaluate the relative contribution of temporal RI to overall prezygotic RI between populations of *Belonocnema treatae*, a specialist gall-forming wasp adapted to sister species of live oak (*Quercus virginiana* and *Q. geminata*). We link strong temporal RI between host-associated insect populations to differences between host plant budbreak phenology. Budbreak initiates flowering and the production of new leaves, which are an ephemeral resource critical to insect reproduction. As flowering time is implicated in RI between plant species, budbreak acts as a "multitrophic multi-effect trait," whereby differences in budbreak phenology contribute to RI in plants and insects. These sister oak species share a diverse community of host-specific gall-formers and insect natural enemies similarly dependent on ephemeral plant tissues. Thus, our results set the stage for testing for parallelism in a role of plant phenology in driving temporal cascading RI across multiple species and trophic levels.

Intraspecific Geographic Variation of Fragrances Acquired by Orchid Bees in Native and Introduced Populations

Ramirez, SR; Eltz, T; Fritzsche, F; Pemberton, R; Pringle, EG; Tsutsui, ND. 2010

Male orchid bees collect volatiles, from both floral and non-floral sources, that they expose as pheromone analogues (perfumes) during courtship display. The chemical profile of these perfumes, which includes terpenes and aromatic compounds, is both species-specific and divergent among closely related lineages. Thus, fragrance composition is thought to play an important role in prezygotic reproductive isolation in euglossine bees. However, because orchid bees acquire fragrances entirely from exogenous sources, the chemical composition of male perfumes is prone to variation due to environmental heterogeneity across habitats. We used Gas Chromatography/Mass Spectrometry (GC/MS) to characterize the perfumes of 114 individuals of the green orchid bee (*Euglossa* aff. *viridissima*) sampled from five native populations in Mesoamerica and two naturalized populations in the southeastern United States. We recorded a total of 292 fragrance compounds from hind-leg extracts, and found that overall perfume composition was different for each population. We detected a pronounced chemical dissimilarity between native (Mesoamerica) and naturalized (U.S.) populations that was driven both by proportional differences of common compounds as well as the presence of a few chemicals unique to each population group. Despite these differences, our data also revealed remarkable qualitative consistency in the presence of several major fragrance compounds across distant populations from dissimilar habitats. In addition, we demonstrate that naturalized bees are attracted to and collect large quantities of tricloro 2-butoxyethyl ester, the active ingredient of several commercially available herbicides. By comparing incidence values and consistency indices across populations, we identify putative functional compounds that may play an important role in courtship signaling in this species of orchid bee.

Reproductive isolation between *Pogonomyrmex rugosus* and two lineages with genetic caste determination

Cahan, SH; Julian, GE; Schwander, T; Keller, L. 2006

Hybrid speciation occurs when combination of two interspecific genomes results in individuals that are of high fitness but reproductively incompatible with the parental species. Although **hybrid** speciation is a relatively common source of new species in plants, it appears to be a much rarer occurrence in animal taxa. Here we report on reproductive isolation and range overlap between the rough harvester ant *Pogonomyrmex rugosus* and two lineages with **hybrid** genotypes (H1 and H2). Both lineages obligately interbreed and produce genetically distinct queen and worker offspring, a phenomenon referred to as genetic caste determination (GCD). Diploid offspring produced by gametes of the same lineage develop only into queens, whereas diploid offspring derived from gametes of distinct lineages develop

into workers. We investigated small-scale patterns of gene flow between the parent and the two H lineages by sampling along an 80-km transect between a pure *P. rugosus* population and a two-lineage population. Microsatellite and mitochondrial markers both indicated virtually no gene flow between the parent species and either lineage even at sites where parental and H-lineage colonies co-occurred. The geographic ranges of the parental species and the two-lineage population were essentially parapatric, with a surprisingly narrow band of overlap and evidence of spatial structuring even at microgeographic scales within the transition zone. This suggests that ecological competition with the parent species plays a significant role in determining the evolutionary persistence and current distribution of the **hybrid** lineages and the genetic caste system.

Electrophoretic study of five aphid parasitoid species of the genus *Aphidius* (Hymenoptera : Braconidae), including evidence for reproductively isolated sympatric populations and a cryptic species

Atanassova, P; Brookes, CP; Loxdale, HD; Powell, W. 1998

Four polymorphic enzymes (PEP, PGI, PGM and IDH) were separated from adult individuals of five aphid parasitoid species of the genus *Aphidius* Nees (*A. ervi* Haliday, *A. microlophii* Pennacchio & Tremblay, *A. eadyi* Stary, Gonzalez & Hall, *A. picipes* Nees and *A. urticae* Haliday) using horizontal cellulose acetate plate electrophoresis. These markers were used to investigate the genetic relationships, including reproductive isolation and host adaptation/specificity, in laboratory and field populations. Samples were collected from the pea aphid, *Acyrtosiphon pisum* (Harris) and/or the nettle aphid, *Microlophium carnosum* (Buckton) in the UK and Bulgaria between 1991 and 1994. Whilst all loci discriminated between some species, PGM discriminated all five species, one species (*A. eadyi*) bearing two unique alleles (PGM(a) and PGM(c)). *Aphidius microlophii* (from nettle aphid) and *A. ervi* (from pea aphid), which are difficult to separate morphologically, possessed unique PGM alleles - PGM(b) and PGM(e), respectively. Both parasitoids occur sympatrically, and whilst **hybrids** heterozygous for PGM were produced in the laboratory (pGM(b,e)), such genotypes were not observed in the field populations sampled. Hence, the species appear to be reproductively isolated. Most parasitoid populations studied showed mean heterozygote deficiencies per locus (homozygote excess) compared with Hardy-Weinberg expectations. In particular, *A. eadyi* bearing PGM(a) alleles were always homozygous whilst additionally, many were homozygous for another allele, PGI(b). This is evidence for the existence of one or more morphologically-indistinguishable 'cryptic' species occurring sympatrically within European field populations. A dendrogram of relatedness was produced following calculation of Nei's genetic identity coefficient, *I* from the parasitoid population allele frequency data. All species showed very high similarity between populations at the intraspecific level (>0.9), but fewer interspecific similarities (0.23-0.63). These values compare well with previously published values for *Aphidius* populations and for other insects.

Labial gland marking secretion in males of two Scandinavian cuckoo bumblebee species (genus *Psithyrus*)

Bergman, P; Bergstrom, G; Appelgren, M. 1996

Chemical components of the cephalic labial gland secretion, which most likely is used as a precopulatory attracting signal, were identified in males of the cuckoo bumblebees *Psithyrus vestalis* (Geoffroy) and *P. bohemicus* (Seidl.) by gas chromatography-mass spectrometry (GC-MS). The former species has geranylcytronellyl acetate as main component, plus in decreasing amounts geranylcytronellol, an eicosadienal, an eicosenol, and an eicosenal. Another 17 fatty acid derivatives are present in minor amounts. The labial secretion of *P. vestalis* differs distinctly in its chemistry from that of all of the 8 other Scandinavian *Psithyrus* species. In the taxonomically closely related (same subgenus) *P. bohemicus*, the labial gland secretion contains 16 fatty acid derivatives, of which 12 (75%) are present also in the secretion of *P. vestalis*. Thus, both the labial gland secretions, and morphological traits lend support to the hypothesis that the two species share a common ancestor. The reproductive isolation between them is, at least in part, supported by the inclusion of a second biosynthetic pathway (the mevalonic acid pathway producing terpenoids) in *P. vestalis*, in addition to the pathway common to both species (the polyketide pathway producing fatty acid derivatives).

Between- and within-host species selection on cytoplasmic incompatibility-inducing *Wolbachia* in haplodiploids

Vavre, F; Fouillet, P; Fleury, F. 2003

The most common effect of the endosymbiont *Wolbachia* is cytoplasmic incompatibility (CI), a form of postzygotic reproductive isolation that occurs in crosses where the male is infected by at least one *Wolbachia* strain that the female lacks. We revisited two puzzling features of *Wolbachia* biology: how *Wolbachia* can invade a new species and spread among populations, and how the association, once established in a host species, can evolve, with emphasis on the possible process of infection loss. These questions are particularly relevant in haplodiploid species, where males develop from unfertilized eggs, and females from fertilized eggs. When CI occurs in such species, fertilized eggs either die (female mortality type: FM), or develop into males (male development type: MD), raising one more question: how transition among CI types is possible. We reached the following conclusions: (1) the FM type is a better invader and should be retained preferentially after a new host is captured; (2) given the assumptions of the models, FM and MD types are selected on neither the bacterial side nor the host side; (3) selective pressures acting on both partners are more or less congruent in the FM type, but divergent in the MD type; (4) host and symbiont evolution can drive infection to extinction for all CI types, but the MD type is more susceptible to the phenomenon; and (5) under realistic conditions, transition from MD to FM type is possible. Finally, all these results suggest that the FM type should be more frequent than the MD type, which is consistent with the results obtained so far in haplodiploids.

Pollinator-mediated isolation in sympatric milkweeds (*Asclepias*): do floral morphology and insect behavior influence species boundaries?

Kephart, S; Theiss, K. 2004

We explored whether mechanical or ethological differences provide pollinator-mediated floral isolation capable of reinforcing existing species barriers among sympatric *Asclepias* with divergent floral morphologies: *A. incarnata*, *A. verticillata* and *A. syriaca*. In a common garden, we quantified pollinator visitation and flight patterns, differences in corporal attachment of pollinia to insects, and the potential outcome of putative floral barriers for interspecific pollination and fruit set. We detected significant variation in the importance, constancy, and behavior of major pollinators on sympatric *asclepiads*, including *Bombus*, *Xylocopa* and large sphecids wasps. Pollinia attach differentially to the arolium on insect legs for *A. syriaca*, but to the tarsal hairs in other *asclepiads*. Fruit-set was lower in mixed than unispecific patches of *Asclepias*. We detected mechanical isolation between *A. syriaca* and its congeners and a tendency toward wasp pollination in *A. verticillata*. All three species appear to show some specialization for long-tongued hymenoptera and lepidopterans. Pre-mating barriers provide a potentially effective means of reducing interspecific pollination, but more study is needed in species visited by generalists.

Mechanisms of prezygotic reproductive isolation between two sympatric species, *Gelsemium rankinii* and *G. sempervirens* (Gelsemiaceae), in the southeastern United States

Pascarella, JB. 2007

Natural **hybridization** plays a critical role in speciation, the maintenance of reproductive isolation, and genetic introgression. While many plant species have **hybrid** swarms in areas of sympatry, the lack of **hybrids** among closely related sympatrically distributed species suggests that strong pre- and/or postzygotic barriers exist to **hybridization**. *Gelsemium sempervirens* and *G. rankinii* (Gelsemiaceae) are sympatrically distributed southeastern sister taxa that have strong postzygotic barriers to **hybrid** formation and high levels of genetic differentiation. In this study, two sympatric populations in Lowndes County, Georgia were surveyed from 1999-2005 to

assess the role of temporal and pollinator isolation as potential prezygotic barriers. The populations had mostly non-overlapping flowering periods in 2003-2005, with significant differences in time of peak flowering and length of flowering. Both species shared a similar community of flower visitors, with the apid bee *Habropoda laboriosa* the dominant visitor to both species. A choice experiment found that *H. laboriosa* visited both species but preferred *G. sempervirens*. The primary prezygotic barrier is temporal isolation preventing **hybridization** in spite of the shared pollinators. This study suggests that reliance on a shared pollinator during speciation may limit opportunity for divergent selection on flowering time.

Ecological Niche Models and Coalescent Analysis of Gene Flow Support Recent Allopatric Isolation of Parasitoid Wasp Populations in the Mediterranean

Lozier, JD; Mills, NJ. 2009

Background: The integration of multiple complementary approaches is a powerful way to understand the processes of diversification and speciation. The parasitoid wasp *Aphidius transcaspicus* Telenga (Hymenoptera: Braconidae) is a parasitoid of *Hyalopterus* aphids across a wide geographic range. This species shows a remarkable degree of genetic structure among western, central, and eastern Mediterranean population clusters. In this paper we attempt to better characterize this genetic structure. Methodology/Principal Findings: We use a Bayesian coalescent analysis of gene flow under the Isolation with Migration model using mitochondrial and microsatellite markers together with climate-based ecological niche models to better understand the genetic structure of *A. transcaspicus* in the Mediterranean. The coalescent analysis revealed low levels of migration among western and eastern Mediterranean populations ($N_m < 1$) that were not statistically distinguishable from zero. Niche models showed that localities within population clusters each occupy areas of continuously high environmental suitability, but are separated from each other by large regions of completely unsuitable habitat that could limit dispersal. Overall, environmental characteristics were similar among the population clusters, though significant differences did emerge. Conclusions/Significance: These results support contemporary allopatric isolation of Mediterranean populations of *A. transcaspicus*, which together with previous analyses indicating partial behaviorally mediated reproductive isolation, suggest that the early stages of cryptic speciation may be in progress.

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

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

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

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

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

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

Genetic and hybridization evidence confirms that a geographic population of *Gonatocerus morrilli* (Hymenoptera : Mymaridae) from California is a new species: Egg parasitoids of the glassy-winged sharpshooter *Homalodisca coagulata* (Homoptera : Cicadellidae)

de Leon, JH; Jones, WA; Samou, M; Morgan, DJW. 2006

We investigated the differentiation and reproductive isolation among different geographic populations of *Gonatocerus morrilli*, egg parasitoids of the glassy-winged sharpshooter (*Homalodisca coagulata*), to confirm previous observations that there may exist a cryptic species complex or a new species. Two mitochondrial genes [cytochrome oxidase subunits I (COI) and II (COII)] and the internal transcribed spacer region 2 (ITS2) of several individuals per population were sequenced. *G. morrilli* populations from Texas (TX), Florida (FL), California (CA), and an outgroup (*G. ashmeadi*) were analyzed. For comparison, a population from Argentina (*G. annulicornis*) morphologically similar to *G. morrilli* was also included. For all three sequence fragments, percentage sequence divergence (%D) demonstrated that both the TX and FL populations (TX/FL) were closely related and therefore determined to be the same species; in contrast, the %D between TX/FL and CA fell within the range of the outgroup, making the CA population a new species or sp. n. Neighbor-joining distance trees also clustered the TX/FL and CA populations or species into two well supported distinctive clades. The near *G. morrilli* sp. n. was more closely related to *G. annulicornis* than to the TX/FL species. Mating studies demonstrated that the populations or species from CA and TX were reproductively incompatible, producing no female offspring in both direct and reciprocal crosses; whereas, the heterogamic crosses between TX and FL produced fertile offspring and relative compatibility indices similar to the homogamic crosses. Unidirectional cytoplasmic incompatibility was ruled out as a cause for the lack of reproduction since both males and females were infected in equal portions with *Wolbachia*. (c) 2006 Elsevier Inc. All rights reserved.

Intraspecific variability in the parasitoid wasp *Trichogramma chilonis*: can we predict the outcome of hybridization?

Benvenuto, C; Tabone, E; Vercken, E; Sorbier, N; Colombel, E; Warot, S; Fauvergue, X; Ris, N. 2012

In the framework of biological control, the selection of effective natural enemies determines the final pest control. Thus, the genetic improvement of biocontrol agents could enhance the efficiency of biocontrol programs. Although promising, this approach has rarely been applied in this field. At the intraspecific level, **hybridization** between divergent populations of biocontrol agents is expected to promote **hybrid** vigor (heterosis), but it is not clear to what extent. An even more difficult task is the ability to predict the fitness of **hybrids** from the biological characteristics of their parents. We investigated these general questions by crossing seven populations of the parasitoid wasp *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Our results show different levels of mating compatibilities among populations, including asymmetric or almost complete reproductive isolation. **Hybrids'** performance (fitness of the F1 generation) ranges from inbreeding depression to heterosis. It was possible, to some extent, to predict **hybrid** fitness from pairwise genetic and phenotypic distances among parents, in accordance with the dominance hypothesis. This may provide general guidelines for the genetic improvement of biological control agents.

EVIDENCE FOR THE EXISTENCE OF A SPECIES CLOSELY RELATED TO THE CEREAL APHID PARASITOID *APHIDIUS RHOPALOSIPHII* DESTEFANI-PEREZ BASED ON HOST RANGES, MORPHOLOGICAL CHARACTERS, ISOELECTRIC-FOCUSING BANDING-PATTERNS, CROSS-BREEDING EXPERIMENTS AND SEX-PHEROMONE SPECIFICITIES (HYMENOPTERA, BRACONIDAE, APHIDIINAE)

HOLLER, C. 1991

Host transfer trials with six strains of the cereal aphid parasitoid *Aphidius rhopalosiphii* De Stefani-Perez revealed the presence of two differing host ranges: three strains parasitized *Sitobion avenae* (F.) and *S. fragariae* (Wlk.) exclusively, whereas the others successfully developed in both *Sitobion* species and also in *Metopolophium dirhodum* (Wlk.), *M. festucae cerealium* Stroyan, *Rhopalosiphum padi* (L.) and *Diuraphis muehleii* Börner. The two host range groups were poorly distinguishable by means of morphological characters. In contrast, isoelectric focusing proved to be a much better tool for discrimination. Reproductive isolation between the two host range groups was clear but not complete, as evidenced by crossing experiments with laboratory-bred and field-collected material. Under field conditions, males showed a specific response to virgin females of the two groups. Hence, the sex pheromones of the females were obviously different. Considering these results, a species (*Aphidius* sp. A) closely related to *A. rhopalosiphii* seems to occur. As a detailed study of morphological characters is still lacking, *aphidius* sp. A is not yet formally established here. Due to its dependence upon *Sitobion* spp., *Aphidius* sp. A may be an economically important parasitoid of the pest aphid *S. avenae*.

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

Goldblatt, P; Manning, JC. 2006

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

Oviposition traits generate extrinsic postzygotic isolation between two pine sawfly species

Bendall, EE; Vertacnik, KL; Linnen, CR. 2017

Background: Although empirical data indicate that ecological speciation is prevalent in nature, the relative importance of different forms of reproductive isolation and the traits generating reproductive isolation remain unclear. To address these questions, we examined a pair of ecologically divergent pine-sawfly species: while *Neodiprion pinetum* specializes on a thin-needled pine (*Pinus strobus*), *N. lecontei* utilizes thicker-needled pines. We hypothesized that extrinsic postzygotic isolation is generated by oviposition traits. To test this hypothesis, we assayed ovipositor morphology, oviposition behavior, and host-dependent oviposition success in both species and in F1 and backcross females. Results: Compared to *N. lecontei*, *N. pinetum* females preferred *P. strobus* more strongly, had smaller ovipositors, and laid fewer eggs per needle. Additionally, we observed host- and trait-dependent reductions in oviposition success in F1 and backcross females. **Hybrid** females that had *pinetum*-like host preference (*P. strobus*) and *lecontei*-like oviposition traits (morphology and egg pattern) fared especially poorly. Conclusions: Together, these data indicate that maladaptive combinations of oviposition traits in **hybrids** contribute to extrinsic postzygotic isolation between *N. lecontei* and *N. pinetum*, suggesting that oviposition traits may be an important driver of divergence in phytophagous insects.

A gene-based SNP resource and linkage map for the copepod *Tigriopus californicus*

Foley, BR; Rose, CG; Rundle, DE; Leong, W; Moy, GW; Burton, RS; Edmands, S. 2011

Background: As yet, few genomic resources have been developed in crustaceans. This lack is particularly evident in Copepoda, given the extraordinary numerical abundance, and taxonomic and ecological diversity of this group. *Tigriopus californicus* is ideally suited to serve as a genetic model copepod and has been the subject of extensive work in environmental stress and reproductive isolation. Accordingly, we set out to develop a broadly-useful panel of genetic markers and to construct a linkage map dense enough for quantitative trait locus detection in an interval mapping framework for *T. californicus*—a first for copepods. Results: One hundred and ninety Single Nucleotide Polymorphisms (SNPs) were used to genotype our mapping population of 250 F2 larvae. We were able to construct a linkage map with an average intermarker distance of 1.8 cM, and a maximum intermarker distance of 10.3 cM. All markers were assembled into linkage groups, and the 12 linkage groups corresponded to the 12 known chromosomes of *T. californicus*. We estimate a total genome size of 401.0 cM, and a total coverage of 73.7%. Seventy five percent of the mapped markers were detected in 9 additional populations of *T. californicus*. Of available model arthropod genomes, we were able to show more colocalized pairs of homologues between *T. californicus* and the honeybee *Apis mellifera*, than expected by chance, suggesting preserved macrosynteny between Hymenoptera and Copepoda. Conclusions: Our study provides an abundance of linked markers spanning all chromosomes. Many of these markers are also found in multiple populations of *T. californicus*, and in two other species in the genus. The genomic resource we have developed will enable mapping throughout the geographical range of this species and in closely related species. This linkage map will facilitate genome sequencing, mapping and assembly in an ecologically and taxonomically interesting group for which genomic resources are currently under development.

Pollinator isolation in Louisiana iris: legitimacy and pollen transfer

Shaw, JP; Taylor, SJ; Dobson, MC; Martin, NH. 2017

Background: Iris brevicaulis and Iris fulva can be found in sympatric populations in southern Louisiana. Ethological pollinator isolation is an important reproductive barrier, as the pollination syndromes of Iris brevicaulis flowers result in primarily bumblebee visits, while the pollination syndromes of Iris fulva primarily result in hummingbird visits. Other potentially important components of pollinator isolation - namely, 'legitimacy of visitation' (i.e. whether or not a floral visitor physically interacts with a flower in such a way that it can pick up pollen from an anther or transfer pollen to a stigma) and 'pollen transfer success' (i.e. whether or not a 'legitimate' floral visitor successfully transfers pollen from one flower to the stigma of another) - have not been explored. Hypotheses: Hummingbirds, the primary floral visitors of I. fulva, are more likely to visit I fulva pollination units legitimately than they are the pollination units of I brevicaulis. Furthermore, hummingbirds are more likely to successfully transfer pollen in I. fulva intraspecific visitation bouts compared with either interspecific or I. brevicaulis intraspecific floral visits. Bumblebees, the primary floral visitors of I. brevicaulis, are more likely to visit I brevicaulis pollination units legitimately than they are those of I. fulva. In addition, bumblebees are more likely to successfully transfer pollen in I. brevicaulis intraspecific visitation bouts compared with interspecific or I. fulva intraspecific floral visits. Methods: We recorded the proportion of legitimate floral visits made by bumblebees and hummingbirds to both I. fulva and I. brevicaulis flowers. We also assayed intraspecific and interspecific pollen transfer effectiveness among bumblebees and hummingbirds visiting I. fulva and I. brevicaulis flowers using powdered fluorescent dye as a pollen analogue. Results: The proportion of legitimate hummingbird visits was higher in I. fulva flowers than in I. brevicaulis flowers, and hummingbirds were more effective at transferring pollen among I. fulva flowers than between I. fulva and I. brevicaulis flowers or among I. brevicaulis flowers. Conversely, the proportion of bumblebee visits that were legitimate was higher in I. brevicaulis than in I. fulva, and bumblebees were more effective at transferring pollen among I. brevicaulis flowers than between I. brevicaulis and I. fulva flowers or among I. fulva flowers. Calculations of reproductive isolation (RI) were quite high such that reproductive isolation due to hummingbird visitation legitimacy was calculated as $RI = 0.892$, and that due to bumblebee visitation legitimacy as $RI = 0.557$. Isolation due to hummingbird pollen transfer efficiency was calculated as $RI = 0.747$, and that of bumblebees as $RI = 0.711$.

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

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

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

Disentangling a Holobiont - Recent Advances and Perspectives in Nasonia Wasps

Dittmer, J; van Opstal, EJ; Shropshire, JD; Bordenstein, SR; Hurst, GDD; Brucker, RM. 2016

The parasitoid wasp genus Nasonia (Hymenoptera: Chalcidoidea) is a well-established model organism for insect development, evolutionary genetics, speciation, and symbiosis. The host microbiota assemblage which constitutes the Nasonia holobiont (a host together with all of its associated microbes) consists of viruses, two heritable bacterial symbionts and a bacterial community dominated in abundance by a few taxa in the gut. In the wild, all four Nasonia species are systematically infected with the obligate intracellular bacterium Wolbachia and can additionally be co infected with Arsenophonus nasoniae. These two reproductive parasites have different transmission modes and host manipulations (cytoplasmic incompatibility vs. male killing, respectively). Pioneering studies on Wolbachia in Nasonia demonstrated that closely related Nasonia species harbor multiple and mutually incompatible Wolbachia strains, resulting in strong symbiont-mediated reproductive barriers that evolved early in the speciation process. Moreover, research on host symbiont interactions and speciation has recently broadened from its historical focus on heritable symbionts to the entire microbial community. In this context, each Nasonia species hosts a distinguishable community of gut bacteria that experiences a temporal succession during host development and members of this bacterial community cause strong **hybrid** lethality during larval development. In this review, we present the Nasonia species complex as a model system to experimentally investigate questions regarding: (i) the impact of different microbes, including (but not limited to) heritable endosymbionts, on the extended phenotype of the holobiont, (ii) the establishment and regulation of a species-specific microbiota, (iii) the role of the microbiota in speciation, and (iv) the resilience and adaptability of the microbiota in wild populations subjected to different environmental pressures. We discuss the potential for easy microbiota manipulations in Nasonia as a promising experimental approach to address these fundamental aspects.

A Social Parasite Evolved Reproductive Isolation from Its Fungus-Growing Ant Host in Sympatry

Rabeling, C; Schultz, TR; Pierce, NE; Bacci, M. 2014

Inquiline social parasitic ant species exploit colonies of other ant species mainly by producing sexual offspring that are raised by the host. Ant social parasites and their hosts are often close relatives (Emery's rule), and two main hypotheses compete to explain the parasites' evolutionary origins: (1) the interspecific hypothesis proposes an allopatric speciation scenario for the parasite, whereas (2) the intraspecific hypothesis postulates that the parasite evolves directly from its host in sympatry [1-10]. Evidence in support of the intraspecific hypothesis has been accumulating for ants [3, 5, 7, 9-12], but sympatric speciation remains controversial as a general speciation mechanism for inquiline parasites. Here we use molecular phylogenetics to assess whether the socially parasitic fungus-growing ant Mycocepurus castrator speciated from its host Mycocepurus goeldii in sympatry. Based on differing patterns of relationship in mitochondrial and individual nuclear genes, we conclude that host and parasite occupy a temporal window in which lineage sorting has taken place in the mitochondrial genes but not yet in the nuclear alleles. We infer that the host originated first and that the parasite originated subsequently from a subset of the host species' populations, providing empirical support for the hypothesis that inquiline parasites can evolve reproductive isolation while living sympatrically with their hosts.

Species delimitation in native South American fire ants

Ross, KG; Shoemaker, DD. 2005

The taxonomy of fire ants has been plagued by difficulties in recognizing species on the basis of morphological characters. We surveyed allozyme markers and sequences of the mtDNA COI gene in several closely related nominal species from two areas of sympatry in the native ranges to learn whether the morphology-based

delimitation of these species is supported by genetic data. We found that *Solenopsis invicta* and *Solenopsis richteri*, pest species whose distinctiveness has been debated, appear to be fully reproductively isolated at both study sites. This isolation contrasts with the extensive **hybridization** occurring between them in the USA, where both have been introduced. We also found strong genetic differentiation consistent with barriers to gene flow between *Solenopsis quinquecupis* and the other two species. However, several lines of evidence suggest that nuclear and mitochondrial genes of *S. invicta* and *S. richteri* are introgressing into *S. quinquecupis*. The latter apparently is a recently derived member of the clade that includes all three species, suggesting that there has been insufficient time for its full development of intrinsic isolating mechanisms. Finally, our discovery of genetically distinct populations within both *S. invicta* and *S. richteri* suggests the presence of previously unrecognized (cryptic) species. Their existence, together with the difficulties in developing diagnostic morphological characters for described species, imply that the group is actively radiating species and that morphological divergence generally does not keep pace with the development of reproductive isolation and neutral genetic divergence in this process.

Four new species of *Luciuranus* fireflies from the Brazilian Atlantic Rainforest (Coleoptera: Lampyridae)

Da Silva, LFL; Souto, PM; Mermudes, JRM. 2018

Luciuranus Silveira, Khattar & Mermudes, 2016 is a firefly genus whose species bear an intricate, species-specific lock-and-key mechanism of reproductive isolation. Here we propose four new species, *Luciuranus magnoculus* sp. nov., *L. desideratus* sp. nov., *L. takiyai* sp. nov. and *L. carioca* sp. nov., and provide illustrations of their diagnostic features and an updated key to species. As previously reported for their congeners, each of the four new species have stereotypical morphology of both male and female terminalia, and are regarded as *prima facie* endemics of single massifs of the Serra da Mantiqueira and Serra do Mar, in the Brazilian Atlantic Rainforest.

Cryptic diversity, reproductive isolation and cytoplasmic incompatibility in a classic biological control success story

Gebiola, M; White, JA; Cass, BN; Kozuch, A; Harris, LR; Kelly, SE; Karimi, J; Giorgini, M; Perlman, SJ; Hunter, MS. NA

Molecular genetics and symbiont diagnostics have revolutionized our understanding of insect species diversity, and the transformative effects of bacterial symbionts on host life history. *Encarsia inaron* is a parasitoid wasp that has been shown to harbour two bacterial endosymbionts, *Wolbachia* and *Cardinium*. Known then as *E. partenopea*, it was introduced to the USA in the late 1980s from populations collected in Italy and Israel for the biological control of an ornamental tree pest, the ash whitefly, *Siphoninus phillyreae*. We studied natural populations from sites in the USA, the Mediterranean and the Middle East as well as from a *Cardinium*-infected laboratory culture established from Italy, with the aims of characterizing these populations genetically, testing reproductive isolation, determining symbiont infection status in their native and introduced range, and determining symbiont role. The results showed that the two *Encarsia* populations introduced to the USA are genetically distinct, reproductively isolated, have different symbionts and different host-symbiont interactions, and can be considered different biological species. One (*E. inaron*) is doubly infected by *Wolbachia* and *Cardinium*, while only *Cardinium* is present in the other (*E. partenopea*). The *Cardinium* strains in the two species are distinct, although closely related, and crossing tests indicate that the *Cardinium* infecting *E. partenopea* induces cytoplasmic incompatibility. The frequency of symbiont infection found in the native and introduced range of these wasps was similar, unlike the pattern seen in some other systems. These results also lead to a retelling of a successful biological control story, with several more characters than had been initially described. (C) 2015 The Linnean Society of London

Sex Attractant Pheromones of Virgin Queens of Sympatric Slave-Making Ant Species in the Genus *Polyergus*, and their Possible Roles in Reproductive Isolation

Greenberg, L; Johnson, CA; Trager, JC; McElfresh, JS; Rodstein, J; Millar, JG. 2018

Species of the ant genus *Polyergus* are social parasites that steal brood from colonies of their hosts in the closely related genus *Formica*. Upon emergence as adults in a mixed population, host *Formica* workers carry out all the normal worker functions within the *Polyergus* colony, including foraging, feeding, grooming, and rearing brood of the parasitic *Polyergus* ants. Some unmated *Polyergus* gynes (queens) run in the raiding columns of their colonies and attract males by releasing a pheromone from their mandibular glands. There are two *Polyergus* species groups in North America: an eastern *P. lucidus* group and a western *P. breviceps* group. One species of each of these groups, *P. lucidus* Mayr and *P. mexicanus* Emery, are sympatric in Missouri. In this study, we characterized the sex pheromones of virgin queens of two species of the *P. lucidus* group (*P. lucidus sensu stricto* and *P. sanwaldi*) and one species of the *P. breviceps* group (*P. mexicanus*), and compared these with the previously identified sex pheromone of *P. topoffi* of the *P. breviceps* group. We then used sex pheromone blends reconstructed from synthesized components of the two groups to test their efficacy at reproductively isolating these species. We found that methyl 6-methylsalicylate is conserved as the major component of the pheromone blends for both *Polyergus* species groups; however, methyl (R)-3-ethyl-4-methylpentanoate is the species-specific minor component produced by *P. lucidus* group queens, and (R)-3-ethyl-4-methylpentan-1-ol is the crucial minor component for *P. breviceps* group queens. The optimal ratio of the major and minor components for *P. lucidus* group queens was about 100:1 salicylate to ester. In concurrent field trials in Missouri, males of *P. lucidus sensu stricto* and *P. mexicanus* (a member of the *P. breviceps* group) were attracted almost exclusively to their particular blends of sex pheromone components. To our knowledge, this is the first example of a possible sex-pheromone-based reproductive isolating mechanism in ants.

Lack of floral constancy by bee fly pollinators: implications for ethological isolation in an African daisy

Ellis, AG; Johnson, SD. 2012

Although divergent floral preferences of pollinating species is a likely mechanism maintaining integrity of co-occurring floral ecotypes or incipient species, reproductive isolation of floral morphs could also result from temporary floral constancy of shared pollinators. Context-dependent floral preferences have been demonstrated for many pollinating insects, suggesting that this behavior might be widespread. However, it is not yet known whether 2 plant species or morphotypes that share a single pollinator could be reproductively isolated through assortative visitation. We investigated the foraging behavior of the bee fly, *Megapalpus capensis*, in experimental arrays of distinct floral morphotypes of *Gorteria diffusa*, a South African daisy which is pollinated almost exclusively by *M. capensis*. Flies exhibited no intrinsic or context-dependent (according to prevailing morph) preference for floral variants nor do they display floral constancy behavior during visitation bouts. Thus, pollinator isolation through assortative visitation is an unlikely explanation for the maintenance of floral integrity across the narrow contact zones encountered in *Gorteria*. Despite random visitation patterns on arrays, flies exhibited different behaviors on different floral morphotypes, suggesting intrinsic responses to morphotype-specific floral cues. Our findings suggest that results of previous studies demonstrating labile preferences and constancy in insects such as bees and butterflies cannot necessarily be extrapolated to other important groups of pollinators. In pollinators, such as *Megapalpus*, which use flowers as arenas for multiple behaviors, including mating, and feed during long stationary bouts, learning constraints on flower handling time, the most widely accepted explanation for floral constancy, is unlikely to drive constant foraging behavior.

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.

Mechanisms of reproductive isolation between an ant species of hybrid origin and one of its parents

Schwander, T; Suni, SS; Cahan, SH; Keller, L. 2008

The establishment of new species by **hybridization** is difficult because it requires the development of reproductive isolation (111) in sympatry to escape the homogenizing effects of gene flow from the parental species. Here we investigated the role of two pre- and two postzygotic mechanisms of RI in a system comprising two interdependent *Pogonomyrmex* harvester ant lineages (the H1 and H2 lineages) of **hybrid** origin and one of their parental species (*P. rugosus*). Similar to most other ants, *P. rugosus* is characterized by an environmental system of caste determination with female brood developing either into queens or workers depending on nongenetic factors. By contrast, there is a strong genetic component to caste determination in the H1 and H2 lineages because the developmental fate of female brood depends on the genetic origin of the parents, with interlineage eggs developing into workers and intralineage eggs developing into queens. The study of a mixed mating aggregation revealed strong differences in mating flight timing between *P. rugosus* and the two lineages as a first mechanism of RI. A second important prezygotic mechanism was assortative mating. Laboratory experiments also provided support for one of the two investigated mechanisms of postzygotic isolation. The majority of offspring produced from the few matings between *P. rugosus* and the lineages aborted at the egg stage. This **hybrid** inviability was under maternal influence, with **hybrids** produced by *P. rugosus* queens being always inviable whereas a small proportion of H2 lineage queens produced large numbers of adult **hybrid** offspring. Finally, we found no evidence that genetic caste determination acted as a second postzygotic mechanism reducing gene flow between *P. rugosus* and the H lineages. The few viable *P. rugosus*-H **hybrids** were not preferentially shunted into functionally sterile workers but developed into both workers and queens. Overall, these results reveal that the nearly complete (99.5%) RI between *P. rugosus* and the two **hybrid** lineages stems from the combination of two typical prezygotic mechanisms (mating time divergence and assortative mating) and one postzygotic mechanism (**hybrid** inviability).

Co-occurrence of ecologically equivalent cryptic species of spider wasps

Kurushima, H; Yoshimura, J; Kim, JK; Kim, JK; Nishimoto, Y; Sayama, K; Kato, M; Watanabe, K; Hasegawa, E; Roff, DA; Shimizu, A. 2016

Many cryptic species have been discovered in various taxonomic groups based on molecular phylogenetic analyses and mating experiments. Some sympatric cryptic species share equivalent resources, which contradicts the competitive exclusion principle. Two major theories have been proposed to explain the apparent lack of competitive exclusion, i.e. niche-based coexistence and neutral model, but a conclusive explanation is lacking. Here, we report the co-occurrence of cryptic spider wasp species appearing to be ecologically equivalent. Molecular phylogenetic analyses and mating experiments revealed that three phylogenetically closely related species are found sympatrically in Japan. These species share the same resources for larval food, and two of the species have the same niche for nesting sites, indicating a lack of competitive exclusion. This evidence may suggest that ecologically equivalent species can co-occur stably if their shared resources are sufficiently abundant that they cannot be over-exploited.

PERIODICITY AND DIVERSITY IN ANT MATING FLIGHTS

MCCLUSKEY, ES. 1992

1. Flight hours and seasons are alike within species, but differ among species and among genera. 2. Laboratory studies indicate internal circadian control of the phase relation to the light-dark cycle, with field evidence for modification by temperature (or other environmental variables). 3. In at least two species there is abrupt loss of obvious rhythm after mating, suggesting some special function before loss, e.g. to facilitate cross-breeding within species or reproductive isolation between species.
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A comparison between two strains from Japan and Europe of *Aphidius ervi*

Takada, H; Tada, E. 2000

A European strain of *Aphidius ervi* shall be introduced into Japan for the control of *Macrosiphum euphorbiae* on greenhouse tomatoes. We compared this strain, which is originating from The Netherlands (EVP), with a native strain from Hokkaido, Northern Japan (SPR) in several characteristics. The percentage of parasitism of SPR and EVP was significantly different, when attacking three aphid species: 13% and 30% on *M. euphorbiae* on tomato, 68% and 93% on *Acyrtosiphon pisum* on broad bean and 10% and 42% on *Aulacorthum solani* on sweet pepper, respectively. SPR and EVP differed morphologically (in the colouration of the petiole) and in the esterase banding patterns. Furthermore, cross mating experiments showed that SPR and EVP are partially reproductively isolated from each other. Complete reproductive isolation is evident in the one direction cross (EVP females and SPR males).

Two distinct morphs in the wood ant *Formica polyctena* in Finland: a result of hybridization?

Sorvari, J. 2006

Wide overlaps in morphological characters among species in mound building wood ants of the *Formica rufa*-group have been a long-standing problem. Previous Studies Suggest that *Formica polyctena* samples From Finland may represent two different types based on queen morphology. However, worker caste has not been studied completely. I found that hairiness of workers from different colonies was bimodally distributed. Morphs (based on bimodality) differed in the abundance of erect hairs on eight out of 11 studied body parts. The hairier morph may be result of incomplete reproductive isolation between closely related species.

Effects of social organization on gene flow in the fire ant *Solenopsis invicta*

Shoemaker, DD; Ross, KG. 1996

A CONTROVERSIAL model of speciation proposes that the development of alternative social organizations within populations of group-living animals may drive the inception of reproductive isolation(1-3). The alternative social behaviours, which are selectively favoured in some social or ecological contexts, may be correlated with distinctive reproductive traits such that significant barriers to interbreeding emerge between coexisting social variants. Evidence for this mode of speciation is

almost non-existent(3-8), but it provides one of the most compelling mechanisms for sympatric speciation(3,8) and could conceivably explain many species origins. Here we examine variation in mitochondrial DNA and two unique nuclear genes to demonstrate that gene flow between sympatric social forms of the fire ant *Solenopsis invicta* is restricted to only one of four possible routes. The loss of the other routes results from incompatibilities in the social systems of the two forms, demonstrating the potential for social selection to generate significant barriers to gene flow and to initiate 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.

Molecular phylogeny of fire ants of the *Solenopsis saevissima* species-group based on mtDNA sequences

Shoemaker, DD; Ahrens, ME; Ross, KG. 2006

The systematics of South American fire ants (*Solenopsis saevissima* species-group) has been plagued by difficulties in recognizing species and their relationships on the basis of morphological characters. We surveyed mtDNA sequences from 623 individuals representing 13 described and undescribed species within the species-group and 18 individuals representing other major *Solenopsis* lineages to generate a phylogeny of the mitochondrial genome. Our analyses support the monophyly of the *S. saevissima* species-group, consistent with a single Neotropical origin and radiation of this important group of ants, as well as the monophyly of the socially polymorphic species within the group, consistent with a single origin of polygyny (multiple queens per colony) as a derived form of social organization. The mtDNA sequences of the inquiline social parasite *S. daguerrei* form a clade that appears to be distantly related to sequences from the several host species, consistent with the view that advanced social parasitism did not evolve via sympatric speciation of intraspecific parasites. An important general finding is that species-level polyphyly of the mtDNA appears to be the rule in this group of ants. The existence of multiple divergent mtDNA lineages within several nominal species (including the pest *S. invicta*) suggests that the pattern of widespread polyphyly often stems from morphological delimitation that overcircumscribes species. However, in two cases the mtDNA polyphyly likely results from recent interspecific hybridization. While resolving species boundaries and relationships is important for understanding general patterns of diversification of South American fire ants, these issues are of added importance because invasive fire ants are emerging as global pests and becoming important model organisms for evolutionary research. (c) 2005 Elsevier Inc. All rights reserved.

Recent speciation and secondary contact in endemic ants

Jowers, MJ; Amor, F; Ortega, P; Lenoir, A; Boulay, RR; Cerda, X; Galarza, JA. 2014

Gene flow is the main force opposing divergent selection, and its effects are greater in populations in close proximity. Thus, complete reproductive isolation between parapatric populations is not expected, particularly in the absence of ecological adaptation and sharp environmental differences. Here, we explore the biogeographical patterns of an endemic ant species, *Cataglyphis floricola*, for which two colour morphs (black and bicolour) coexist in parapatry throughout continuous sandy habitat in southern Spain. Discriminant analyses of six biometric measurements of male genitalia and 27 cuticular hydrocarbons reveal high differentiation between morphs. Furthermore, the low number of shared alleles derived from nuclear markers (microsatellites) between the morphs at their contact zone suggests the absence of recent gene flow. Mitochondrial DNA (COI) phylogenetic analysis and median-joining networks show that the black morph is basal to the bicolour morph, with unique haplotypes recovered for each morph. Mismatch distribution analysis and Bayesian skyline plots suggest that they are undergoing different demographic changes, with the bicolour and black morphs at demographic equilibrium and expansion, respectively. Thus, our results show complete reproductive isolation between the two colour morphs as evidenced from genetic, chemical and morphological data. We suggest that these divergence events could be explained by historical vicariance during the Pleistocene, in which reproductive traits experienced strong divergent selection between the morphs initiating or culminating speciation.

Mating isolation between the ant *Myrmica rubra* and its microgynous social parasite

Leppanen, J; Seppa, P; Vepsalainen, K; Savolainen, R. 2016

Sympatric speciation has been suggested for several pairs of social parasites and their hosts. Little is known, however, about how they have become reproductively isolated from each other. The ant *Myrmica rubra* has an intraspecific microgynous social parasite, which is probably speciating from its macrogynous host morph. Mating of the host and parasite is suggested to be spatially isolated, microgynes mating in the nest and macrogynes usually joining mating swarms. The mating biology of the microgyne is, however, still poorly known. We studied mating isolation and potential gene flow between these morphs by assessing whether host males are produced in naturally parasitized nests, and by testing experimentally cross-mating of the morphs when brought together. Both parasite and host males mated with both female morphs, but host males seemed to mate more often than the parasite males with their own kind. Although most males in the parasitized nests were produced by the parasites, the host queens and workers also produced some males, thus providing the morphs an opportunity for cross-mating. Previous studies have shown, however, that genetic divergence between sympatric parasite and host populations is substantial, which indicates that hybridization is rare. This implies that even if cross-mating between the morphs took place in the wild, introgression would be weak. Our results, together with earlier studies, indicate that the parasite and host of *M. rubra* are not fully reproductively isolated, even though gene flow between the morphs is restricted enough to allow ongoing speciation of the parasite.

Prevalence of legitimate pollinators and nectar robbers and the consequences for fruit set in an *Antirrhinum majus* hybrid zone

Andalo, C; Burrus, M; Paute, S; Lauzeral, C; Field, DL. 2019

Pollinators display a remarkable diversity of foraging strategies with flowering plants, from primarily mutualistic interactions to cheating through nectar robbery. Despite numerous studies on the effect of nectar robbing on components of plant fitness, its contribution to reproductive isolation is unclear. We experimentally tested the impact of different pollinator strategies in a natural hybrid zone between two subspecies of *Antirrhinum majus* with alternate flower colour guides. On either side of a steep cline in flower colour between *Antirrhinum majus pseudomajus* (magenta) and *A. m. striatum* (yellow), we quantified the behaviour of all floral visitors at different time points during the flowering season. Using long-run camera surveys, we quantify the impact of nectar robbing on the number of flowers visited per inflorescence and the flower probing time. We further experimentally tested the effect of nectar robbing on female reproductive success by manipulating the intensity of robbing. While robbing increased over time the number of legitimate visitors tended to decrease concomitantly. We found that the number of flowers pollinated on a focal inflorescence decreased with the number of prior robbing events. However, in the manipulative experiment, fruit set and fruit volume did not vary

significantly between low robbing and control treatments. Our findings challenge the idea that robbers have a negative impact on plant fitness through female function. This study also adds to our understanding of the components of pollinator-mediated reproductive isolation and the maintenance of *Antirrhinum* **hybrid** zones.

Population differentiation in female sex pheromone and male preferences in a solitary bee

Vereecken, NJ; Mant, J; Schiestl, FP. 2007

Population differentiation in female mating signals and associated male preferences can drive reproductive isolation among segregated populations. We tested this assumption by investigating intraspecific variation in female sex pheromone and associated male odour preferences among distant populations in the solitary bee *Colletes cunicularius* (L.) by using quantitative gas chromatography and by performing field bioassays with synthetic blends of key sex pheromone compounds. We found significant differences in sex pheromone blends among the bee populations, and the divergence in odour blends correlated positively with geographic distance, suggesting that genetic divergence among distant populations can affect sex pheromone chemistry. Our behavioural experiments, however, demonstrate that synthetic copies of allopatric female sex pheromones were cross-attractive to patrolling males from distant populations, making reproductive isolation by non-recognition of mating signals among populations unlikely. Our data also show that patrolling male bees from different populations preferred odour types from allopatric populations at the two sites of bioassays. These male preferences are not expected to select for changes in the female sex pheromone, but may influence the evolution of floral odour in sexually deceptive orchids of the genus *Ophrys* that are pollinated by *C. cunicularius* males.

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.

The phenology of a Neotropical ant assemblage: evidence for continuous and overlapping reproduction

Kaspari, M; Pickering, J; Longino, JT; Windsor, D. 2001

Reproductive phenologies reflect the interaction between the mating system of a taxon and the local environment. Ant colonies reproduce and disperse via the flights of winged alates. Few data exist on the reproductive phenologies of ant assemblages. Here we analyze the reproductive phenologies of 81 common ant species from 23,182 individuals collected over 3 years on Barro Colorado Island, Panama (BCI). Species ranged from highly synchronous to continuous fliers, but showed a median flight duration of at least 8 of 13 lunar months. In two statistical analyses (variance ratio test and Spearman rank correlations), 84% (16 of 19) of ant genera had species trending toward positively associated phenologies, more than expected by chance ($P < 0.00036$ by a binomial test). Thus, there was little evidence for the hypothesis that competition for limiting resources staggers congeneric flights and ultimately promotes reproductive isolation. On the contrary, the timing of reproduction, and its synchrony, tended to be conserved within genera and subfamilies. These results closely match phenological studies of plant assemblages. The continuous reproduction and small colony size of many species in this study suggest that the female calling syndrome, a poorly documented mating system in ants, may be common on BCI.

The Potential for Gene Flow in a Dependent Lineage System of a Harvester Ant: Fair Meiosis in the F-1 Generation

Curry, MM; Wheeler, DE; Yang, K; Anderson, KE. 2010

We investigated the potential for gene flow in a dependent lineage (DL) system of the harvester ant *Pogonomyrmex*. Each DL system is composed of 2 reproductively isolated lineages that are locked in an obligate mutualism. The genetic components that produce the worker phenotype are acquired by **hybridizing** with the partner lineage. In the mating flight, queens of both lineages mate with multiple males from each lineage. During colony growth and reproduction, eggs fertilized by partner-lineage sperm produce F-1 **hybrid** workers with interlineage genomes, whereas eggs fertilized by same-lineage sperm result in the development of new queens with intralineage genomes. New males are typically produced from unfertilized eggs laid by the pure-lineage queen but in her absence may be produced by interlineage F-1 workers. We investigated the potential for interlineage gene flow in this system using 2 classes of lineage-specific nuclear markers to identify **hybrid** genome combinations. We confirmed the production of viable interlineage F-1 reproductive females in field colonies, the occurrence of which is associated with the relative frequencies of each lineage in the population: interlineage F-1 queens occurred only in the rare lineage of the population with dramatically skewed lineage frequencies. In laboratory colonies, we detected fair meiosis in interlineage F-1 workers leading to the production of viable and haploid interlineage F-2 males. We conclude that the genomes of each lineage recombine freely, suggesting that extrinsic postzygotic selection maintains the integrity of each lineage genome. We compare our findings with those of the H1/H2 DL system.

Genome-wide sequence data show no evidence of hybridization and introgression among pollinator wasps associated with a community of Panamanian strangler figs

Satler, JD; Herre, EA; Heath, TA; Machado, CA; Zuniga, AG; Nason, JD. 2022

The specificity of pollinator host choice influences opportunities for reproductive isolation in their host plants. Similarly, host plants can influence opportunities for reproductive isolation in their pollinators. For example, in the fig and fig wasp mutualism, offspring of fig pollinator wasps mate inside the inflorescence that the mothers pollinate. Although often host specific, multiple fig pollinator species are sometimes associated with the same fig species, potentially enabling **hybridization** between wasp species. Here, we study the 19 pollinator species (*Pegoscopus* spp.) associated with an entire community of 16 Panamanian strangler fig species (*Ficus* subgenus *Urostigma*, section *Americanae*) to determine whether the previously documented history of pollinator host switching and current host sharing predicts genetic admixture among the pollinator species, as has been observed in their host figs. Specifically, we use genome-wide ultraconserved element (UCE) loci to estimate phylogenetic relationships and test for **hybridization** and introgression among the pollinator species. In all cases, we recover well-delimited pollinator

species that contain high interspecific divergence. Even among pairs of pollinator species that currently reproduce within syconia of shared host fig species, we found no evidence of **hybridization** or introgression. This is in contrast to their host figs, where **hybridization** and introgression have been detected within this community, and more generally, within figs worldwide. Consistent with general patterns recovered among other obligate pollination mutualisms (e.g. yucca moths and yuccas), our results suggest that while **hybridization** and introgression are processes operating within the host plants, these processes are relatively unimportant within their associated insect pollinators.

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.

Supercolonies of billions in an invasive ant: What is a society?

Moffett, MW. 2012

All societies are characterized by the capacity of their members to distinguish one another from outsiders. Ants are among the species that form "anonymous societies": members are not required to tell each other apart as individuals for the group to remain unified. Rather, each society depends on shared cues recognized by all its members. These cues permit societies to reach populations in the low millions in certain ant and termite species, and to grow indefinitely populous, expansive, and possibly long lasting in a few other ant species, which are described as having supercolonies. Anonymous societies are contrasted with "individual recognition societies" such as those of most vertebrates, which are limited to a few individuals by the necessity that the members individually recognize each other. The shared recognition cues of ants provide clear criteria for defining colonies and are what enables a supercolony to remain a single society no matter how large it becomes. I examine the often conflicting ideas about the best studied ant with supercolonies, the Argentine ant (*Linepithema humile*). Its invasive supercolonies, containing in some cases billions of workers and queens spread over hundreds of square kilometers, can be most parsimoniously understood as single colonies that have had an opportunity to expand across regions of suitable habitat because of a lack of well-matched competitors. This capacity for unrestricted growth is the defining characteristic of supercolonies. There is no evidence that the local patchiness of nests and patterns of worker and food traffic within these wide-ranging populations are so invariant that supercolonies do not exist but instead are collections of numerous independent nest clusters that should be called "colonies." Nor is there evidence for the hypothesis that invasive supercolonies have been able to grow large and successful overseas only as a result of evolving through genetic drift or selection to become fundamentally different from the smaller colonies typical of the species' region of origin around northern Argentina. The most unique feature of the Argentine ant, however, is not that its colonies are anonymous or that they can grow indefinitely large-though the last trait is found only in a few ant species and humans. Rather, it is that Argentine ant colonies do not interbreed. Indeed, the only fighting among Argentine ants occurs along colony borders, which even reproductives seldom, if ever, cross and survive. For this reason, each Argentine ant supercolony acts as virtually a sibling species.

Cloning and sequencing of wsp encoding gene fragments reveals a diversity of co-infecting *Wolbachia* strains in *Acromyrmex* leafcutter ants

Van Borm, S; Wenseleers, T; Billen, J; Boomsma, JJ. 2003

By sequencing part of the wsp gene of a series of clones, we detected an unusually high diversity of nine *Wolbachia* strains in queens of three species of leafcutter ants. Up to four strains co-occurred in a single ant. Most strains occurred in two clusters (InvA and InvB), but the social parasite *Acromyrmex insinuator* hosted two additional infections. The multiple *Wolbachia* strains may influence the expression of reproductive conflicts in leafcutter ants, but the expected turnover of infections may make the cumulative effects on host ant reproduction complex. The additional *Wolbachia* infections of the social parasite *A. insinuator* were almost certainly acquired by horizontal transmission, but may have facilitated reproductive isolation from its closely related host. (C) 2002 Elsevier Science (USA). All rights reserved.

Scent of a break-up: phylogeography and reproductive trait divergences in the red-tailed bumblebee (*Bombus lapidarius*)

Lecocq, T; Dellicour, S; Michez, D; Lhomme, P; Vanderplanck, M; Valterova, I; Rasplus, JY; Rasmont, P. 2013

Background: The Pleistocene climatic oscillations are considered as a major driving force of intraspecific divergence and speciation. During Ice Ages, populations isolated in allopatric glacial refugia can experience differentiation in reproductive traits through divergence in selection regimes. This phenomenon may lead to reproductive isolation and dramatically accentuates the consequences of the climatic oscillations on species. Alternatively, when reproductive isolation is incomplete and populations are expanding again, further mating between the formerly isolated populations can result in the formation of a **hybrid** zone, genetic introgression or reinforcement speciation through reproductive trait displacements. Therefore changes in reproductive traits driven by population movements during climatic oscillations can act as an important force in promoting pre-zygotic isolation. Notwithstanding, divergence of reproductive traits has not been approached in the context of climatic oscillations. Here we investigate the impact of population movements driven by climatic oscillations on a reproductive trait of a bumblebee species (*Bombus lapidarius*). We characterise the pattern of variation and differentiation across the species distribution (i) with five genes (nuclear and mitochondrial), and (ii) in the chemical composition of male marking secretions (MMS), a key trait for mate attraction in bumblebees. Results: Our results provide evidence that populations have experienced a genetic allopatric differentiation, in at least three main refugia (the Balkans, Centre-Eastern Europe, and Southern Italy) during Quaternary glaciations. The comparative chemical analyses show that populations from the Southern Italian refugium have experienced MMS differentiation and an incipient speciation process from another refugium. The meeting of Southern Italian populations with other populations as a result of range expansion at a secondary contact zone seems to have led to a reinforcement process on local MMS patterns. Conclusions: This study suggests that population movement during Quaternary climatic oscillations can lead to divergence in reproductive traits by allopatric differentiation during Ice Ages and by reinforcement during post-glacial recolonization.

Molecular genetic analysis and ecological evidence reveals multiple cryptic species among thynnine wasp pollinators of sexually deceptive orchids

Griffiths, KE; Trueman, JWH; Brown, GR; Peakall, R. 2011

Sexually deceptive *Chiloglottis* orchids lure their male thynnine wasp pollinators to the flower by emitting semiochemicals that mimic the specific sex pheromone of

the wasp. Sexual deception is possible because chemical rather than visual cues play the key role in wasp mate search, suggesting that cryptic wasp species may be frequent. We investigated this prospect among *Neozeleboria* wasp pollinators of *Chiloglottis* orchids, drawing on evidence from molecular phylogenetic analysis at three genes (CO1, rhodopsin and wingless), population genetic and statistical parsimony analysis at CO1, orchid associations and their semiochemicals, and geographic ranges. We found a compelling relationship between genetically defined wasp groups, orchid associations, semiochemicals and geographic range, despite a frequent lack of detectable morphological differences. Our findings reveal multiple cryptic species among orchid pollinators and indicate that chemical changes are important for wasp reproductive isolation and speciation. The diversity of *Neozeleboria* may have enabled, rather than constrained, pollinator-driven speciation in these orchids. (C) 2011 Elsevier Inc. All rights reserved.

Phylogeography of social polymorphism in a boreo-montane ant

Trettin, J; Agrawal, S; Heinze, J. 2016

Background: The disjunct distribution of several Palearctic species has been widely shaped by the changes in climatic conditions during the Quaternary. The observed genetic differentiation or reproductive isolation between extant populations may be the outcome of their contemporary geographic separation or reproductive incompatibility due to differences in phenotypic traits which have evolved in isolated refugia. In the boreal ant *Leptothorax acervorum*, colonies from central and peripheral populations differ in social structure: colonies from Central and Northern Europe may contain several equally reproductive queens (facultative polygyny), while in colonies from peripheral populations in Spain only one the most dominant of several queens lays eggs (functional monogyny). By reconstructing the specie's evolutionary and demographic history in Southwestern Europe we examine whether variation in social organization is associated with restricted gene flow between the two social forms. Results: We show that multi-queen colonies from all so far known inner Iberian populations of *L. acervorum* are functionally monogynous, whereas multi-queen colonies from all Pyrenean populations are polygynous, like those from other previously studied areas in Europe. Our analyses revealed complex spatial-genetic structure, but no association between spatial-genetic structure and social organization in SW-Europe. The population in the western Pyrenees diverged most strongly from other Iberian populations. Moreover, microsatellite data suggest the occurrence of recent bottlenecks in Pyrenean and inner Iberian populations. Conclusions: Our study shows a lack of reproductive isolation between the two social forms in SW-Europe. This in turn suggests that demographic and spatial patterns in genetic variation as well as the distribution of social phenotypes are better explained by co-variation with climatic, ecological, and historical factors. Moreover, we for the first time show the existence of substantial spatial-genetic structure in *L. acervorum*, suggesting the existence of multiple refugia in SW-Europe, including two extra-Mediterranean refugia in France. While gene flow among inner Iberian refugia may have been larger during the late glacial, extra-Mediterranean refugia in southern France may have contributed to the post-glacial recolonization of W-Europe.

Wolbachia infections in native and introduced populations of fire ants (*Solenopsis* spp.)

Shoemaker, DD; Ross, KG; Keller, L; Vargo, EL; Werren, JH. 2000

Wolbachia are cytoplasmically inherited bacteria that induce a variety of effects with fitness consequences on host arthropods, including cytoplasmic incompatibility, parthenogenesis, male-killing and feminization. We report here the presence of Wolbachia in native South American populations of the fire ant *Solenopsis invicta*, but the apparent absence of the bacteria in introduced populations of this pest species in the USA. The Wolbachia strains in native *S. invicta* are of two divergent types (A and B), and the frequency of infection varies dramatically between geographical regions and social forms of this host. Survey data reveal that Wolbachia also are found in other native fire ant species within the *Solenopsis saevissima* species complex from South America, including *S. richteri*. This latter species also has been introduced in the USA, where it lacks Wolbachia. Sequence data reveal complete phylogenetic concordance between mtDNA haplotype in *S. invicta* and Wolbachia infection type (A or B). In addition, the mtDNA and associated group A Wolbachia strain in *S. invicta* are more closely related to the mtDNA and Wolbachia strain found in *S. richteri* than they are to the mtDNA and associated group B Wolbachia in *S. invicta*. These data are consistent with historical introgression of *S. richteri* cytoplasmic elements into *S. invicta* populations, resulting in enhanced infection and mtDNA polymorphisms in *S. invicta*. Wolbachia may have significant fitness effects on these hosts (either directly or by cytoplasmic incompatibility) and therefore these microbes potentially could be used in biological control programmes to suppress introduced fire ant populations.

Strong, but incomplete, mate choice discrimination between two closely related species of paper wasp

Miller, SE; Legan, AW; Flores, ZA; Ng, HY; Sheehan, MJ. 2019

Paper wasps (genus *Polistes*) are one of the most species-rich genera of social insect. Prior studies have found that male coloration, male colour pattern, territory choice and female caste are potential drivers of intraspecific mate choice in paper wasps. However, there has been no formal assessment of interspecific mate choice in this group; therefore, the mechanism driving diversification in paper wasps remains an open question. In this study, we measured interspecific and intraspecific mating behaviour between two closely related species of paper wasps, *Polistes fuscatus* and *Polistes metricus*. These two species have ample opportunity to interbreed because *P. fuscatus* and *P. metricus* forage, nest and mate in the same habitats. We tested the strength of reproductive isolation between these species using no-choice and choice mating trials. Our results show strong, symmetric, prezygotic isolation between *P. fuscatus* and *P. metricus*. Males discriminated between conspecifics and heterospecifics but attempted to mate with females of the other species in similar to 10% of heterospecific mating trials. Female wasps were more discriminating than males and probably evaluated species identity and male quality through visual or olfactory cues. We additionally report sexual dimorphism in *P. metricus* body size.

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

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

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

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

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

The tea geometrid (*Ectropis obliqua* Prout, Lepidoptera: Geometridae) is a dominant chewing insect endemic in most tea-growing areas in China. Recently some *E. obliqua* populations have been found to be resistant to the nucleopolyhedrovirus (EoNPV), a host-specific virus that has so far been found only in *E. obliqua*. Although the resistant populations are morphologically indistinguishable from susceptible populations, we conducted a nationwide collection and examined the genetic

divergence in the COI region of the mtDNA in *E. obliqua*. Phylogenetic analyses of mtDNA in 17 populations revealed two divergent clades with genetic distance greater than 3.7% between clades and less than 0.7% within clades. Therefore, we suggest that *E. obliqua* falls into two distinct groups. Further inheritance analyses using reciprocal single-pair mating showed an abnormal F-1 generation with an unbalanced sex ratio and the inability to produce fertile eggs (or any eggs) through F1 self-crossing. These data revealed a potential cryptic species complex with deep divergence and reproductive isolation within *E. obliqua*. Uneven distribution of the groups suggests a possible geographic effect on the divergence. Future investigations will be conducted to examine whether EoNPV selection or other factors prompted the evolution of resistance.

Composition of cuticular lipids in the pteromalid wasp *Lariophagus distinguendus* is host dependent

Kuhbandner, S; Hacker, K; Niedermayer, S; Steidle, JLM; Ruther, J. 2012

The insect cuticle is covered by a thin layer of hydrocarbons not only preventing desiccation but also playing an important role in the sexual communication of several species. In the pteromalid wasp *Lariophagus distinguendus*, a parasitoid of grain infesting beetles, female cuticular hydrocarbons (CHCs) elicit male courtship behaviour. We analyzed the CHC profiles of male and female *L. distinguendus* wasps reared on different beetle hosts by coupled gas chromatography- mass spectrometry (GC-MS). Statistical analysis of the data revealed significant differences between strains reared on different hosts, while spatially isolated strains reared on the same host produced similar profiles. CHC profiles of parasitoids reared on *Stegobium paniceum* were statistically distinguishable from those of wasps reared on all other hosts. A host shift from *Sitophilus granarius* to *S. paniceum* resulted in distinguishable CHC profiles of *L. distinguendus* females after only one generation. Considering the role of CHCs as contact sex pheromones, our data suggest that host shifts in parasitic wasps might lead to reproductive isolation of host races due to the modification of the cuticular semiochemistry.

Genetic differentiation in sympatric wood ants, *Formica rufa* and *F. polyctena*

Gyllenstrand, N; Seppa, P; Pamilo, P. 2004

Direct observations have suggested that the closely related wood ants *Formica polyctena* and *F. rufa* represent different social organizations, with high queen number in *F. polyctena* and a high frequency of monogynous nests in *F. rufa*. We examined social organization and genetic population structure in a setup where populations of the two species are sympatric and gene flow between the species is possible. Our aim was to compare social organization in the species, and study evolutionary relationships between them. The observed relatedness among colony workers suggested that the difference in the level of polygyny is quantitative rather than qualitative, with a higher queen number in *F. polyctena*. The observed difference in polygyny was not accompanied by a difference in spatial genetic differentiation which was weak in both species. The genetic distance between the species is consistent with limited interspecific gene flow. Identification of a few possible *F. rufa* migrants in *F. polyctena* populations suggests potential interspecific gene flow. Thus, reproductive isolation of the species may not be complete when they are sympatric.

Dynamics of the evolution of Batesian mimicry: molecular phylogenetic analysis of ant-mimicking *Myrmarachne* (Araneae : Salticidae) species and their ant models

Ceccarelli, FS; Crozier, RH. 2007

Batesian mimicry is seen as an example of evolution by natural selection, with predation as the main driving force. The mimic is under selective pressure to resemble its model, whereas it is disadvantageous for the model to be associated with the palatable mimic. In consequence one might expect there to be an evolutionary arms race, similar to the one involving host-parasite coevolution. In this study, the evolutionary dynamics of a Batesian mimicry system of model ants and ant-mimicking salticids is investigated by comparing the phylogenies of the two groups. Although Batesian mimics are expected to coevolve with their models, we found the phylogenetic patterns of the models and the mimics to be indicative of adaptive radiation by the mimic rather than co-speciation between the mimic and the model. This shows that there is strong selection pressure on *Myrmarachne*, leading to a high degree of polymorphism. There is also evidence of sympatric speciation in *Myrmarachne*, the reproductive isolation possibly driven by female mate choice in polymorphic species.

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

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

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

Genetics of postzygotic isolation and Haldane's rule in haplodiploids

Koevoets, T; Beukeboom, LW. 2009

The process of speciation has puzzled scientists for decades, but only recently they have been able to reveal the genetic basis of reproductive isolation. Much emphasis has been on Haldane's rule, the observation that the heterogametic sex often suffers more from hybridization than the homogametic sex. Most research on Haldane's rule has focused on diploid organisms with chromosomal sex determination. We argue that species lacking chromosomal sex determination, such as haplodiploids, also follow Haldane's rule and thus should be included in the definition of this rule. We provide evidence for Haldane's rule in *Nasonia* wasps and describe how haplodiploids can be used to test the different theories that have been proposed to explain Haldane's rule. We discuss how the faster-male and faster-X theories can shape speciation differently in haplodiploids compared to diploids.

Phylogenetic Relationships Among Populations of *Vollenhovia* ants, With Particular Focus on the Evolution of Wing Morphology

Kobayashi, K; Tamura, K; Okamoto, M; Hasegawa, E; Ohkawara, K. 2012

Queen polymorphism in wing morphology and thoracic structure provides the opportunity to test hypotheses about mating strategies and colony founding modes. Some studies indicate that the difference in mating behavior between winged and wingless queens may promote genetic isolation, possibly leading to speciation. However, the knowledge about genetic differences and phylogenetic relationships among polymorphic queens is limited. Queens of the myrmicine ant *Vollenhovia emeryi* Wheeler exhibit two morphs: a long-winged (L-queen) and a short-winged (S-queen) morph. We analyzed the phylogenetic relationship among populations of L- and S-queens in *V. emeryi* and the congeneric species *V. nipponica*, *V. benzai*, *V. okinawana*, and *V. yambaru*. The molecular phylogeny inferred from mtDNA (approximate to 2,200 nt) showed that S-queens formed a monophyletic clade and that L- and S-queens sampled from the same location did not group together. The phylogeny indicates that wing reduction occurred only once and that S-queen populations are genetically differentiated from L-queen populations, at least in their maternal genomes. The phylogeny is consistent with the hypothesis that wing reduction leads to reproductive isolation in *V. emeryi*.

Characterization and distribution of *Pogonomyrmex* harvester ant lineages with genetic caste determination

Schwander, T; Cahan, SH; Keller, L. 2007

Genetic caste determination has been described in two populations of *Pogonomyrmex* harvester ants, each comprising a pair of interbreeding lineages. Queens mate with males of their own and of the alternate lineage and produce two types of diploid offspring, those fertilized by males of the queens' lineage which develop into queens and those fertilized by males of the other lineage which develop into workers. Each of the lineages has been shown to be itself of **hybrid** origin between the species *Pogonomyrmex barbatus* and *Pogonomyrmex rugosus*, which both have typical, environmentally determined caste differentiation. In a large scale genetic survey across 35 sites in Arizona, New Mexico and Texas, we found that genetic caste determination associated with pairs of interbreeding lineages occurred frequently (in 26 out of the 35 sites). Overall, we identified eight lineages with genetic caste determination that always co-occurred in the same complementary lineage pairs. Three of the four lineage pairs appear to have a common origin while their relationship with the fourth remains unclear. The level of genetic differentiation among these eight lineages was significantly higher than the differentiation between *P. rugosus* and *P. barbatus*, which questions the appropriate taxonomic status of these genetic lineages. In addition to being genetically isolated from one another, all lineages with genetic caste determination were genetically distinct from *P. rugosus* and *P. barbatus*, even when colonies of interbreeding lineages co-occurred with colonies of either putative parent at the same site. Such nearly complete reproductive isolation between the lineages and the species with environmental caste determination might prevent the genetic caste determination system to be swept away by gene flow.

Ultraconserved yet informative for species delimitation: Ultraconserved elements resolve long-standing systematic enigma in Central European bees

Gueuning, M; Frey, JE; Praz, C. 2020

Accurate and testable species hypotheses are essential for measuring, surveying and managing biodiversity. Taxonomists often rely on mitochondrial DNA barcoding to complement morphological species delimitations. Although COI-barcoding has largely proven successful in assisting identifications for most animal taxa, there are nevertheless numerous cases where mitochondrial barcodes do not reflect species hypotheses. For instance, what is regarded as a single species can be associated with two distinct DNA barcodes, which can point either to cryptic diversity or to within-species mitochondrial divergences without reproductive isolation. In contrast, two or more species can share barcodes, for instance due to mitochondrial introgression. These intrinsic limitations of DNA barcoding are commonly addressed with nuclear genomic markers, which are expensive, may have low repeatability and often require high-quality DNA. To overcome these limitations, we examined the use of ultraconserved elements (UCEs) as a quick and robust genomic approach to address such problematic cases of species delimitation in bees. This genomic method was assessed using six different species complexes suspected to harbour cryptic diversity, mitochondrial introgression or mitochondrial paraphyly. The sequencing of UCEs recovered between 686 and 1,860 homologous nuclear loci and provided explicit species delimitation in all investigated species complexes. These results provide strong evidence for the suitability of UCEs as a fast method for species delimitation even in recently diverged lineages. Furthermore, we provide the first evidence for both mitochondrial introgression among distinct bee species, and mitochondrial paraphyly within a single bee species.

Ant Species Differences Determined by Epistasis between Brood and Worker Genomes

Linksvayer, TA. 2007

Epistasis arising from physiological interactions between gene products often contributes to species differences, particularly those involved in reproductive isolation. In social organisms, phenotypes are influenced by the genotypes of multiple interacting individuals. In theory, social interactions can give rise to an additional type of epistasis between the genomes of social partners that can contribute to species differences. Using a full-factorial cross-fostering design with three species of closely related *Temnothorax* ants, I found that adult worker size was determined by an interaction between the genotypes of developing brood and care-giving workers, i.e. intergenomic epistasis. Such intergenomic social epistasis provides a strong signature of coevolution between social partners. These results demonstrate that just as physiologically interacting genes coevolve, diverge, and contribute to species differences, so do socially interacting genes. Coevolution and conflict between social partners, especially relatives such as parents and offspring, has long been recognized as having widespread evolutionary effects. This coevolutionary process may often result in coevolved socially-interacting gene complexes that contribute to species differences.

Allodiplogaster josephi n. sp and *A-seani* n. sp (Nematoda: Diplogasteridae), associates of soil-dwelling bees in the eastern USA

Kanzaki, N; Giblin-Davis, RM; Ragsdale, EJ. 2015

Two commensal associates of bees, *Allodiplogaster josephi* n. sp. from the Dufour's gland of a cellophane bee (*Colletes thoracicus*) from Maryland, USA, and *A. seani* n. sp. from the abdominal glands of an andrenid bee (*Andrena alleghaniensis*) from New York, USA, are described and illustrated. Both species were collected as dauers from their respective hosts and cultured on bacteria on tryptic soy broth (TSB) or NGM agar. *Allodiplogaster josephi* n. sp. and *A. seani* n. sp. are morphologically closer to each other than to other species of *Allodiplogaster*, which was recently revised to include 37 valid species. However, the two new species are distinguished by reproductive isolation, shape of the spicule manubrium, host associations and molecular characters, the latter in sequences of the near-full length small subunit (SSU) rRNA gene, D2-D3 expansion segments of the large subunit (LSU) rRNA gene and partial mitochondrial COI. Morphological characterisation was supplemented by scanning electron microscopy (SEM), which revealed furcation of both v5 and v6 male genital papillae, consistent with previous reports for species of the *henrichiae* group of *Allodiplogaster*.

Revision of the Palaearctic *Trachusa interrupta* species complex (Apoidea: Anthidiini) with description of four new species

Kasperek, M. 2020

Trachusa interrupta (Fabricius, 1781) s.l. has so far been regarded as a widespread resin bee in the tribe Anthidiini, whose range extends from northwest Africa and

the Iberian Peninsula in the west to Central Asia and China in the east. It was thought that the pattern of yellow maculations on head, meso- and metasoma was quite variable. Several forms which were described since the 18th century have been regarded as synonyms. A comprehensive analysis of hundreds of specimens from all parts of its distribution found that *T. interrupta* s. l. actually represents a complex of closely related species. The study which included the examination of type material and the analyses of morphometric data of 15 measurements of the head, antennae and wings through multivariate statistical methods showed that there was relatively little variation in the colour pattern. Different colour patterns mostly represent different taxa which form distinctive clusters in Discriminant Function Analysis of morphometric data. The complex hereby consists of three widespread species. *Trachusa interrupta* (Fabricius, 1781) s.str., *T. integra* (Eversmann, 1852) stat. resurr. and *T. anatolica* sp. n., whose combined range extends from the Western Mediterranean to Central Asia and China, and five further species with restricted ranges in the southern part of the overall distribution: *T. varia* (Olivier, 1789) stat. resurr. and *T. maghrebensis* sp. n. in Spain and north-western Africa, and *T. heinzi* Dubitzky, 2007, *T. grandicornis* sp. n., and *T. taurica* sp. n. in Turkey and Iran. Additionally, some populations mainly of *T. interrupta* s. str. show in the southern part of its distribution (e.g. Spain, Italy, Greece) distinctive features in the colour pattern or morphological traits such as antennal length. As these characters are widely overlapping between populations and seem to follow geographic clines, these differences do not seem to reflect taxonomically relevant units. It was thought that they represent populations with reduced but still not ceased reproductive isolation and hence species in statu nascendi. All species of the *T. interrupta* complex as here defined have clearly delineated distributional areas. There is a little overlap in the distribution areas of the species and even the two most widespread species, *T. interrupta* s. str. and *T. integra*, which both occur widely in the West Palaearctic show mutually exclusive patchy distribution patterns, i.e. the two species normally do not occur in the same region. Nevertheless, a few cases were found where two species occur in sympatry, and a few cases (less than 1% of all specimens) where specimens had characters of two species. The latter may indicate that **hybridisation** occasionally occurs in parapatric contact zones.

Genetic differentiation across the social transition in a socially polymorphic sweat bee, *Halictus rubicundus*

Soro, A; Field, J; Bridge, C; Cardinal, SC; Paxton, RJ. 2010

Eusociality is widely considered a major evolutionary transition. The socially polymorphic sweat bee *Halictus rubicundus*, solitary in cooler regions of its Holarctic range and eusocial in warmer parts, is an excellent model organism to address this transition, and specifically the question of whether sociality is associated with a strong barrier to gene flow between phenotypically divergent populations. Mitochondrial DNA (COI) from specimens collected across the British Isles, where both solitary and social phenotypes are represented, displayed limited variation, but placed all specimens in the same European lineage; haplotype network analysis failed to differentiate solitary and social lineages. Microsatellite genetic variability was high and enabled us to quantify genetic differentiation among populations and social phenotypes across Great Britain and Ireland. Results from conceptually different analyses consistently showed greater genetic differentiation between geographically distant populations, independently of their social phenotype, suggesting that the two social forms are not reproductively isolated. A landscape genetic approach revealed significant isolation by distance (Mantel test $r = 0.622$, $P < 0.001$). The Irish Sea acts as physical barrier to gene flow (partial Mantel test $r = 0.453$, $P < 0.01$), indicating that geography, rather than expression of solitary or social behaviour (partial Mantel test $r = -0.238$, $P = 0.053$), had a significant effect on the genetic structure of *H. rubicundus* across the British Isles. Although we cannot reject the hypothesis of a genetic underpinning to differences in solitary and eusocial phenotypes, our data clearly demonstrate a lack of reproductive isolation between the two social forms.

Comparisons of host mitochondrial, nuclear and endosymbiont bacterial genes reveal cryptic fig wasp species and the effects of *Wolbachia* on host mtDNA evolution and diversity

Sun, XJ; Xiao, JH; Cook, JM; Feng, G; Huang, DW. 2011

Background: Figs and fig-pollinating wasp species usually display a highly specific one-to-one association. However, more and more studies have revealed that the "one-to-one" rule has been broken. Co-pollinators have been reported, but we do not yet know how they evolve. They may evolve from insect speciation induced or facilitated by *Wolbachia* which can manipulate host reproduction and induce reproductive isolation. In addition, *Wolbachia* can affect host mitochondrial DNA evolution, because of the linkage between *Wolbachia* and associated mitochondrial haplotypes, and thus confound host phylogeny based on mtDNA. Previous research has shown that fig wasps have the highest incidence of *Wolbachia* infection in all insect taxa, and *Wolbachia* may have great influence on fig wasp biology. Therefore, we look forward to understanding the influence of *Wolbachia* on mitochondrial DNA evolution and speciation in fig wasps. Results: We surveyed 76 pollinator wasp specimens from nine *Ficus microcarpa* trees each growing at a different location in Hainan and Fujian Provinces, China. We found that all wasps were morphologically identified as *Eupristina verticillata*, but diverged into three clades with 4.22-5.28% mtDNA divergence and 2.29-20.72% nuclear gene divergence. We also found very strong concordance between *E. verticillata* clades and *Wolbachia* infection status, and the predicted effects of *Wolbachia* on both mtDNA diversity and evolution by decreasing mitochondrial haplotypes. Conclusions: Our study reveals that the pollinating wasp *E. verticillata* on *F. microcarpa* has diverged into three cryptic species, and *Wolbachia* may have a role in this divergence. The results also indicate that *Wolbachia* strains infecting *E. verticillata* have likely resulted in selective sweeps on host mitochondrial DNA.

Genome and cuticular hydrocarbon-based species delimitation shed light on potential drivers of speciation in a Neotropical ant species complex

Meza-Lazaro, RN; Pena-Carrillo, KI; Poteaux, C; Lorenzi, MC; Wetterer, JK; Zaldivar-Riveron, A. 2022

Geographic separation that leads to the evolution of reproductive isolation between populations generally is considered the most common form of speciation. However, speciation may also occur in the absence of geographic barriers due to phenotypic and genotypic factors such as chemical cue divergence, mating signal divergence, and mitonuclear conflict. Here, we performed an integrative study based on two genome-wide techniques (3RAD and ultraconserved elements) coupled with cuticular hydrocarbon (CHC) and mitochondrial (mt) DNA sequence data, to assess the species limits within the *Ectatomma ruidum* species complex, a widespread and conspicuous group of Neotropical ants for which heteroplasmy (i.e., presence of multiple mtDNA variants in an individual) has been recently discovered in some populations from southeast Mexico. Our analyses indicate the existence of at least five distinct species in this complex: two widely distributed across the Neotropics, and three that are restricted to southeast Mexico and that apparently have high levels of heteroplasmy. We found that species boundaries in the complex did not coincide with geographic barriers. We therefore consider possible roles of alternative drivers that may have promoted the observed patterns of speciation, including mitonuclear incompatibility, CHC differentiation, and colony structure. Our study highlights the importance of simultaneously assessing different sources of evidence to disentangle the species limits of taxa with complicated evolutionary histories.

PLANT-POLLINATOR INTERACTIONS FROM FLOWER TO LANDSCAPE The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation

Armbruster, WS. 2017

1. Specialization in plant-pollinator relationships is a core concept in discussions of plant evolution and ecology; it is central to our thinking, not just about the ecology of plant-pollinator interactions and pollinator services, but also about reproductive isolation, speciation, extinction and assembly of communities. However, as reviewed here, the concept 'plant-pollinator specialization' has multiple definitions and uses, and these disparate uses have engendered confusion in the literature. Organizing these disparate uses into a comprehensive framework is an overdue task, prior efforts notwithstanding. 2. This contribution attempts to make clear the variation in meaning and usage of plant-pollinator specialization, including distinguishing between ecological specialization (interacting with few partners or resources), evolutionary specialization (genetic change associated with increased specialization) and phenotypic specialization (having specialized or derived phenotypic traits), with application of all three concepts to both plants and flower-visiting animals. These variations in interpretation of specialization affect how we view evolutionary and biogeographical trends, as well as extinction risk. 3. In the light of this

conceptual diversity, I evaluate the relationships between specialization and possible trends in floral evolution and rates of speciation and extinction. I also address several implications of specialization for community ecology and resilience of pollination services in the face of environmental disturbance.

Reproductive interference between honeybee species in artificial sympatry

Remnant, EJ; Koetz, A; Tan, K; Hinson, E; Beekman, M; Oldroyd, BP. 2014

Reproductive isolation between closely related species is often incomplete. The Western honeybee, *Apis mellifera*, and the Eastern hive bee, *Apis cerana*, have been allopatric for millions of years, but are nonetheless similar in morphology and behaviour. During the last century, the two species were brought into contact anthropogenically, providing potential opportunities for interspecific matings. **Hybrids** between *A. mellifera* and *A. cerana* are inviable, so natural interspecific matings are of concern because they may reduce the viability of *A. cerana* and *A. mellifera* populations - two of the world's most important pollinators. We examined the mating behaviour of *A. mellifera* and *A. cerana* queens and drones from Caoba Basin, China and Cairns, Australia. Drone mating flight times overlap in both areas. Analysis of the spermathecal contents of queens with species-specific genetic markers indicated that in Caoba Basin, 14% of *A. mellifera* queens mated with at least one *A. cerana* male, but we detected no *A. cerana* queens that had mated with *A. mellifera* males. Similarly, in Cairns, no *A. cerana* queens carried *A. mellifera* sperm, but one-third of *A. mellifera* queens had mated with at least one *A. cerana* male. No **hybrid** embryos were detected in eggs laid by interspecifically mated *A. mellifera* queens in either location. However, *A. mellifera* queens artificially inseminated with *A. cerana* sperm produced inviable **hybrid** eggs or unfertilized drones. This suggests that reproductive interference will impact the viability of honeybee populations wherever *A. cerana* and *A. mellifera* are in contact.

Adaptive radiation and coevolution - pollination biology case studies

Lunau, K. 2004

The impact of coevolutionary interaction between species on adaptive radiation processes is analysed with reference to pollination biology case studies. Occasional colonization of archipelagos can bring together coevolving partners and cause coradiation of the colonizing species, e.g. the drepanidids and the lobelioids on Hawaii. Permanent reciprocal selective pressure between pairs of coevolving species can lead to a coevolutionary race and rapid evolutionary change. This is exemplified by spurred flowers and long-tongued flower-visitors. The geographic patterning of diffuse coevolution systems can lead to dramatic changes in species interactions. In different populations, interaction between pollinating and seed-parasitizing *Greya* moths and their host plants varies from mutualism to commensalism and antagonism, depending on the presence of copollinators. Asymmetrical coevolution between angiosperms and oligolectic flower-visitors may facilitate rapid reproductive isolation of populations following a food-plant switch, if the oligolectes use their specific food plants as the rendezvous sites. Diffuse coevolution between angiosperm species and pollinating insects may cause frequent convergent evolution of floral traits such as nectar reward instead of pollen reward, floral guides, zygomorphic flowers, or mimicry of pollen signals, since the multiple plant species experience similar selective pressures via the coevolving partners. Patterns of angiosperm adaptive radiation are highlighted in the context of coevolution with pollinators. (C) 2004 Elsevier GmbH. All rights reserved.

Evolutionary history of nematodes associated with sweat bees

McFrederick, QS; Taylor, DR. 2013

Organisms that live in close association with other organisms make up a large part of the world's diversity. One driver of this diversity is the evolution of host-species specificity, which can occur via reproductive isolation following a host-switch or, given the correct circumstances, via cospeciation. In this study, we explored the diversity and evolutionary history of *Acrostichus* nematodes that are associated with halictid bees in North America. First, we conducted surveys of bees in Virginia, and found six halictid species that host *Acrostichus*. To test the hypothesis of cospeciation, we constructed phylogenetic hypotheses of *Acrostichus* based on three genes. We found *Acrostichus* purl and *Acrostichus* halicti to be species complexes comprising cryptic, host-specific species. Although several nodes in the host and symbiont phylogenies were congruent and tests for cospeciation were significant, the host's biogeography, the apparent patchiness of the association across the host's phylogeny, and the amount of evolution in the nematode sequence suggested a mixture of cospeciation, host switching, and extinction events instead of strict cospeciation. Cospeciation can explain the relationships between *Ac. purl* and its augochlorine hosts, but colonization of *Halictus* hosts is more likely than cospeciation. The nematodes are vertically transmitted, but sexual transmission is also likely. Both of these transmission modes may explain host-species specificity and congruent bee and nematode phylogenies. Additionally, all halictid hosts come from eusocial or socially polymorphic lineages, suggesting that sociality may be a factor in the suitability of hosts for *Acrostichus*. (C) 2012 Elsevier Inc. All rights reserved.

Behavioral and spermatogenic hybrid male breakdown in *Nasonia*

Clark, ME; O'Hara, FP; Chawla, A; Werren, JH. 2010

Several reproductive barriers exist within the *Nasonia* species complex, including allopatry, premating behavioral isolation, postzygotic inviability and Wolbachia-induced cytoplasmic incompatibility. Here we show that **hybrid** males suffer two additional reproductive disadvantages, an inability to properly court females and decreased sperm production. **Hybrid** behavioral sterility, characterized by a reduced ability of **hybrids** to perform necessary courtship behaviors, occurs in **hybrids** between two species of *Nasonia*. **Hybrid** males produced in crosses between *N. vitripennis* and *N. giraulti* courted females at a reduced frequency (23-69%), compared with wild-type *N. vitripennis* and *N. giraulti* males (> 93%). Reduced courtship frequency was not a simple function of inactivity among **hybrids**. A strong effect of cytoplasmic (mitochondrial) background was also found in *N. vitripennis* and *N. giraulti* crosses; F2 **hybrids** with *giraulti* cytoplasm showing reduced ability at most stages of courtship. **Hybrids** produced between a younger species pair, *N. giraulti* and *N. longicornis*, were behaviorally fertile. All males possessed motile sperm, but sperm production is greatly reduced in **hybrids** between the older species pair, *N. vitripennis* and *N. giraulti*. This effect on **hybrid** males, lowered sperm counts rather than nonfunctional sperm, is different from most described cases of **hybrid** male sterility, and may represent an earlier stage of **hybrid** sperm breakdown. The results add to previous studies of F2 **hybrid** inviability and behavioral sterility, and indicate that Wolbachia-induced **hybrid** incompatibility has arisen early in species divergence, relative to behavioral sterility and spermatogenic infertility. *Heredity* (2010) 104, 289-301; doi:10.1038/hdy.2009.152; published online 20 January 2010

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

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

The plausibility of sympatric speciation is still debated despite increasing evidence, such as host races in insects. This speciation process may be occurring in the case of the two phenological forms of the obligatorily myrmecophilous *Phengaris arion*. The main goal of our research was to study the nature and causes of difference between these forms focusing primarily on the incipient speciation via host races. Molecular analyses based on highly variable microsatellites together with Wolbachia screening, male genitalia morphometrics and host ant studies were carried out on four syntopic sample pairs. Our results show that the two phenological forms of *P. arion* may meet the criteria for host plant races. They coexist in sympatry in certain parts of the species range which is allowed by the adaptation to the distinct phenology of the host plants. Negative selection acts against the intermediate individuals which are on the wing in the inappropriate time frame. Thus, disruptive selection affects and produces bimodal distributions of phenotypes. However, the phenology of food plants is not entirely distinct and

fluctuates year by year. Therefore, the two forms can exchange genes occasionally depending on the length of the time slot when they can meet with each other. Consequently, the reproductive isolation could not be completed and the existence of the two arion forms may represent only an incipient stage of sympatric speciation. It is also clear that *Wolbachia* is likely not a driver of sympatric speciation in this case.

Genetic and chemical divergence among host races of a socially parasitic ant

Torres, CW; Tonione, MA; Ramirez, SR; Sapp, JR; Tsutsui, ND. 2018

Host-parasite associations facilitate the action of reciprocal selection and can drive rapid evolutionary change. When multiple host species are available to a single parasite, parallel specialization on different hosts may promote the action of diversifying natural selection and divergence via host race formation. Here, we examine a population of the kidnapper ant (*Polyergus mexicanus*) that is an obligate social parasite of three sympatric ant species: *Formica accreta*, *F. argentea*, and *F. subaenescens* (formerly *F. fusca*). Behavioral and ecological observations of *P. mexicanus* have shown that individual colonies parasitize only one species of host and that new *Polyergus* queens maintain host fidelity when establishing new colonies. To successfully adapt to a particular host, *Polyergus* ants may mimic or camouflage themselves with the species-specific chemical cues (cuticular hydrocarbons) that their hosts use to ascertain colony membership. To investigate the extent of host specialization, we collected both genetic and chemical data from *P. mexicanus* that parasitize each of the three different *Formica* species in sympatry. We show that host-associated genetic structure exists for both maternally inherited mitochondrial DNA data and biparentally inherited microsatellite markers. We also show that *P. mexicanus* can be distinguished by chemical profile according to host due to partial matching with their host. Our results support the hypothesis that host race formation is occurring among lineages of *P. mexicanus* that use different *Formica* hosts. Thus, this system may represent a promising model for illuminating the early steps of divergence, accumulation of reproductive isolation, and speciation.

EFFECTS OF FLORAL METAL ACCUMULATION ON FLORAL VISITOR COMMUNITIES: INTRODUCING THE ELEMENTAL FILTER HYPOTHESIS

Meindl, GA; Ashman, TL. 2015

Premise of the study: For plant species that occur in heavy-metal-rich soil, floral metal accumulation may produce an "elemental filter" that reduces pollinator visitation rate and species richness and changes pollinator species composition relative to closely related species growing on normal soils. Consequently, metal hyperaccumulation may contribute to pollinator-mediated reproductive isolation between closely related plant species that differ in metal accumulation. Methods: To test these ideas, we characterized plant-pollinator interactions in a sympatric pair of species that differ in metal accumulation (*Streptanthus polygaloides*, a nickel (Ni) hyperaccumulator, and *S. tortuosus*, a nonaccumulator). To test the elemental filter hypothesis, we presented arrays of *Streptanthus polygaloides* that were grown in either Ni-treated or control soils to insects at both *Streptanthus polygaloides* and *S. tortuosus* sites and recorded visitation. Key results: Naturally occurring *Streptanthus polygaloides* hyperaccumulated Ni in anthers and accumulated Ni in nectar, while *S. tortuosus* did not. Floral visitation rates in natural populations were higher to *S. tortuosus* than *Streptanthus polygaloides*. In addition, while floral visitor richness was similar, few pollinator taxa were shared between the two plant species. Nickel-treatment of *Streptanthus polygaloides* reduced visits by bees, but only for arrays presented at *S. tortuosus* sites. Conclusions: We show that the Ni hyperaccumulator *S. polygaloides* hosts a distinct floral visitor community, indicating that metal accumulation creates a filter for pollinators, similar to that documented for herbivores. Our study highlights a novel mechanism by which the abiotic environment can alter plant-pollinator interactions, and consequently plant reproduction and speciation.

Speciation and hybridization in invasive fire ants

Cohen, P; Privman, E. NA

BackgroundA major focus of evolutionary biology is the formation of reproductive barriers leading to divergence and ultimately, speciation. Often, it is not clear whether the separation of populations is complete or if there still is ongoing gene flow in the form of rare cases of admixture, known as isolation with migration. Here, we studied the speciation of two fire ant species, *Solenopsis invicta* and *Solenopsis richteri*, both native to South America, both inadvertently introduced to North America in the early twentieth century. While the two species are known to admix in the introduced range, in the native range no **hybrids** were found. ResultsWe conducted a population genomic survey of native and introduced populations of the two species using reduced representation genomic sequencing of 337 samples. Using maximum likelihood analysis over native range samples, we found no evidence of any gene flow between the species since they diverged. We estimated their time of divergence to 190,000 (100,000-350,000) generations ago. Modelling the demographic history of native and introduced *S. invicta* populations, we evaluated their divergence times and historic and contemporary population sizes, including the original founder population in North America, which was estimated at 26 (10-93) unrelated singly-mated queens. ConclusionsWe provide evidence for complete genetic isolation maintained between two invasive species in their native range, based, for the first time, on large scale genomic data analysis. The results lay the foundations for further studies into different stages in the formation of genetic barriers in dynamic, invasive populations.

Recent Speciation in Three Closely Related Sympatric Specialists: Inferences Using Multi-Locus Sequence, Post-Mating Isolation and Endosymbiont Data

Xue, HJ; Li, WZ; Nie, RE; Yang, XK. 2011

Shifting between unrelated host plants is relatively rare for phytophagous insects, and distinct host specificity may play crucial roles in reproductive isolation. However, the isolation status and the relationship between parental divergence and post-mating isolation among closely related sympatric specialists are still poorly understood. Here, multi-locus sequence were used to estimate the relationship among three host plant-specific closely related flea beetles, *Altica cirsiicola*, *A. fragariae* and *A. viridicyanea* (abbreviated as AC, AF and AV respectively). The tree topologies were inconsistent using different gene or different combinations of gene fragments. The relationship of AF+(AC+AV) was supported, however, by both gene tree and species tree based on concatenated data. Post-mating reproductive data on the results of crossing these three species are best interpreted in the light of a well established phylogeny. Nuclear-induced but not *Wolbachia*-induced unidirectional cytoplasmic incompatibility, which was detected in AC-AF and AF-AV but not in AC-AV, may also suggest more close genetic affinity between AC and AV. Prevalence of *Wolbachia* in these three beetles, and the endosymbiont in most individuals of AV and AC sharing a same wsp haplotype may give another evidence of AF+(AC+AV). Our study also suggested that these three flea beetles diverged in a relative short time (0.94 My), which may be the result of shifting between unrelated host plants and distinct host specificity. Incomplete post-mating isolation while almost complete lineage sorting indicated that effective pre-mating isolation among these three species should have evolved.

Species Delimitation: A Case Study in a Problematic Ant Taxon

Ross, KG; Gotzek, D; Ascunce, MS; Shoemaker, DD. 2010

Species delimitation has been invigorated as a discipline in systematics by an influx of new character sets, analytical methods, and conceptual advances. We use genetic data from 68 markers, combined with distributional, bioclimatic, and coloration information, to hypothesize boundaries of evolutionarily independent lineages (species) within the widespread and highly variable nominal fire ant species *Solenopsis saevissima*, a member of a species group containing invasive pests as well as species that are models for ecological and evolutionary research. Our integrated approach uses diverse methods of analysis to sequentially test whether populations

meet specific operational criteria (contingent properties) for candidacy as morphologically cryptic species, including genetic clustering, monophyly, reproductive isolation, and occupation of distinctive niche space. We hypothesize that nominal *S. saevissima* comprises at least 4-6 previously unrecognized species, including several pairs whose parapatric distributions implicate the development of intrinsic premating or postmating barriers to gene flow. Our genetic data further suggest that regional genetic differentiation in *S. saevissima* has been influenced by **hybridization** with other nominal species occurring in sympatry or parapatry, including the quite distantly related *Solenopsis geminata*. The results of this study illustrate the importance of employing different classes of genetic data (coding and noncoding regions and nuclear and mitochondrial DNA [mtDNA] markers), different methods of genetic data analysis (tree-based and non-tree based methods), and different sources of data (genetic, morphological, and ecological data) to explicitly test various operational criteria for species boundaries in clades of recently diverged lineages, while warning against over reliance on any single data type (e.g., mtDNA sequence variation) when drawing inferences.

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., xanthones 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 inquilinous 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 Calophya Lbw 1879 associated with *M. indica*. Due to insufficient information this proposition remains unverified.

Maintenance of adaptive differentiation by *Wolbachia* induced bidirectional cytoplasmic incompatibility: the importance of sib-mating and genetic systems

Branca, A; Vavre, F; Silvain, JF; Dupas, S. 2009

Background: Bacteria of the genus *Wolbachia* are reproductive parasites widespread among arthropods. The most common effect arising from the presence of *Wolbachia* in a population is Cytoplasmic Incompatibility (CI), whereby postmating reproductive isolation occurs in crosses between an infected male and an uninfected female, or when a male is infected with a different strain of *Wolbachia* to that of the female (bidirectional CI). Previous theoretical models have demonstrated that bidirectional CI can contribute to the genetic divergence of populations in haploid and diploid organisms. However, haplodiploid organisms were not considered in these models even though they include *Nasonia* parasitoid wasps - the best example of the implication of *Wolbachia* in ongoing speciation. Moreover, previous work did not investigate inbreeding mating systems, which are frequently observed in arthropod species. Results: We developed a stochastic two-island model which simulated three genetic scenarios, diploidy, haploidy, and haplodiploidy, with two CI phenotypes being considered for the latter: (1) male development of female progeny; and (2) mortality of fertilized eggs. We also investigated the effect of varying the proportion of sib mating. In the model each allopatric population was initially fixed for a single allele at a nuclear locus under positive selection and infected with one strain of *Wolbachia*. Each simulation presupposed that the two populations were fixed for a different allele and a different strain of *Wolbachia*. The degree of genetic differentiation observed in the locus under selection due to bidirectional CI was much lower for the two haplodiploid phenotypes than for either diploids or haploids. Furthermore, we demonstrated that sib-mating may compensate for the lower efficiency of bidirectional CI in haplodiploids by maintaining genetic divergence. Conclusion: Our model suggests that maintenance of genetic differentiation facilitated by *Wolbachia* is more likely to occur in diploids and haploids than in haplodiploids. However, increasing the level of sib-mating may compensate for the weak effect of bidirectional CI in haplodiploids. Our work therefore gives a potential explanation for why the haplodiploid *Nasonia* species, which are infected with bidirectionally incompatible *Wolbachia* strains and undergo sib-mating, have differentiated genetically and maintained this differentiation without premating isolation.

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; Driscue, 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*.

Dispersal and mating in a size-dimorphic ant

Wolf, JI; Seppa, P. 2016

Alternative reproductive strategies linked to size are often connected with differences in dispersal and mating behaviour. Temporal and spatial mating isolation of different sized individuals can lead to size-assortative mating, which may maintain intraspecific size polymorphism. In the size-dimorphic ant *Myrmica ruginodis*, newly produced sexuals of the larger macrogyne morph join nuptial flights to mate, but mating behaviour of the smaller microgyna females remains elusive and even less is known about males. In this study, we examined the differences in dispersal strategies of the two size morphs as a possible mechanism causing reproductive isolation between them. We developed a new method for sampling sexuals dispersing from their nests, and sampled also mating couples and random males from a nearby mating site. We studied propensity and timing of dispersal and assortative mating of the size morphs based on their expected and observed participation in the nuptial flight. Our study shows that predominantly, macrogynes participate in the nuptial flight, while microgynes stay close to their natal nest. However, dispersal is not associated with male size, and larger males, preferred by all females, are available for both morphs. Size-assortative mating, mainly caused by macrogynes mating with large males, exists at the nuptial flight and should promote mating isolation of the size morphs, but microgynes counterbalance this to a certain degree by mating with large males. Size-associated alternative dispersal strategies in size polymorphic species provide insight into the mechanisms enhancing genetic divergence, a potential early step in the speciation process. We studied the association between size and dispersal in the size-dimorphic ant *Myrmica ruginodis*, to evaluate potential premating isolation between the morphs. To date, there is a shortage of studies on mating and dispersal behaviour of ants in natural settings. We captured dispersing sexuals from their natal nests, separating those dispersing on foot or by flight-a technique never applied to dispersing ants before-and assessed mate selection at the mating swarm. We found that dispersal propensity depends on the size of the females (but not males) so that the larger macrogyne morph joins a nuptial flight and the smaller microgyne morph rarely does. Furthermore, macrogyna females actively choose to mate with large males causing size-assortative mating, creating partial mating isolation between the size morphs and opening a possibility for genetic divergence between the morphs.

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 Volcanico 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.
