

((TS=(reproductive barriers)) OR TS=(reproductive isolation)) AND ALL=(Orthoptera)

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Allonemobius shalontaki, a new cryptic species of ground cricket (Orthoptera : Gryllidae : Nemobiinae) from the southwestern United States

Braswell, WE; Birge, LM; Howard, DJ. 2006

A new species of ground cricket in the genus *Allonemobius* (Hebard) is described from the southwestern United States. Like many singing Orthoptera, this species is morphologically cryptic and most easily distinguished from related species by using molecular characters and male calling song traits. We describe *Allonemobius shalontaki* as a new species and present data suggesting its reproductive isolation from congeneric species. *Allonemobius shalontaki* exhibits a unique male calling song and possesses a distinct allozyme genotype. Additionally, we present an electrophoretic key to the genus and propose phylogenetic relationships among species.

Reproductive isolation in the wood cricket *Gryllus vernalis* (Orthoptera : Gryllidae)

Jang, Y; Bockhorst, A; Gerhardt, HC. 2007

Two species of closely related wood crickets, *Gryllus vernalis* (Orthoptera: Gryllidae) and *G. fultoni*, occur together in the eastern USA and have a similar calling-song structure, consisting of three-pulse chirps. Previous studies revealed that male calling song and female selectivity were divergent between sympatric and far allopatric populations of *G. fultoni*, consistent with the pattern expected of reproductive character displacement. We studied the reproductive isolation of *G. vernalis* in relation to *G. fultoni* by investigating the geographic variation in calling songs of *G. vernalis* and by examining close-range mating behaviors. Neither field nor laboratory studies revealed differences in any of the calling-song characters between sympatric and allopatric populations, but this could reflect the limited sampling within the relatively small region of allopatry for *G. vernalis*. Although close-range mating trials revealed that females of both species discriminated against heterospecific mating partners, the strength of discrimination was especially strong in females of sympatric *G. vernalis* populations. Our studies of long-range and close-range mating behaviors suggest that selection pressures for reproductive isolation are exerted primarily on close-range mating behaviors in *G. vernalis* but on long-range mating behaviors in *G. fultoni*.

Reproductive costs to heterospecific mating between two hybridizing katydids (Orthoptera : Tettigoniidae)

Shapiro, LH. 2000

Animal taxa meeting in **hybrid** zones often exhibit partial reproductive isolation. This isolation may result from a variety of causes both before mating (e.g., incompatibilities in courtship behaviors) and subsequent to mating. Understanding the factors effecting reproductive isolation in **hybrid** zones can offer important insights into the process of speciation and the maintenance of species boundaries. The katydids *Orchelimum nigripes* Scudder and *O. pulchellum* Davis (Orthoptera: Tettigoniidae) form 2 **hybrid** zones in the eastern United States. I carried out breeding studies in the laboratory using animals from pure *O. nigripes* and *O. pulchellum* populations in the vicinity of one of these **hybrid** zones to examine possible modes of postmating reproductive isolation. The number of eggs produced by females mated to heterospecific males was dramatically lower than that of females mated conspecifically, but there was no evidence of any differences in hatch rate or offspring viability between egg clutches from heterospecific and conspecific crosses. Hatch rate, offspring viability, development time, and adult weight of **hybrid** progenies were all intermediate relative to corresponding values for progenies resulting from the 2 types of conspecific matings, although most of the differences between **hybrids** and each of the 2 classes of non**hybrid** progenies were not statistically significant. The reduced oviposition of heterospecifically mated females suggests that females mated to heterospecific males do not receive the necessary stimulation to trigger oocyte maturation or oviposition behavior. The results reported here show that although some females will mate with heterospecific males, these matings tend to result in substantially reduced reproductive success relative to conspecific matings.

First evidence of Wolbachia infection in populations of grasshopper *Podisma sapporensis* (Orthoptera: Acrididae)

Bugrov, AG; Ilinsky, YY; Strunov, A; Zhukova, M; Kiseleva, E; Akimoto, S; Tatsuta, H. 2016

The brachypterous grasshopper *Podisma sapporensis* (Orthoptera: Acrididae) is distributed throughout the Sakhalin, Kunashir and Hokkaido Islands. Karyotypes of this species consist of two major chromosomal races with different sex chromosome systems, XO/XX and XY/XX. Molecular phylogeographic analysis of the chromosome races and subraces confirms the genetic divergence of the races and subraces in *P. sapporensis*. Here we first report that *P. sapporensis* is infected with *Wolbachia* consisting of three variants on *wsp* locus, while *gatB* locus was monomorphic. Furthermore, observation of cell tissue of *P. sapporensis* using electron microscopy confirmed the infection of *Wolbachia* that was inferred from polymerase chain reaction and revealed the distribution of the bacteria in the head, thorax and abdomen of *P. sapporensis* embryos. Our finding may shed new light on *Wolbachia* as a possible agent causing **hybrid** dysfunction resulting from experimental crosses between chromosome races or subraces of *P. sapporensis*.

Premating barriers to gene exchange and their implications for the structure of a mosaic hybrid zone between *Chorthippus brunneus* and *C. jacobsi* (Orthoptera : Acrididae)

Bailey, RI; Thomas, CD; Butlin, RK. 2004

Many **hybrid** zones contain a deficit of **hybrid** genotypes relative to expectations from tension zone models. This is often associated with separation of parental genotypes into distinct habitats (mosaicism), but sometimes parentals can be found co-occurring in the same local population (bimodality). In both cases, prezygotic isolation may play an important role in determining the genotypic composition of the zone. *Chorthippus brunneus* and *C. jacobsi* (Orthoptera: Acrididae) meet and form a complex **hybrid** zone in northern Spain. Analysis of stridulatory peg numbers reveals partial spatial and seasonal isolation in a 25 km² area of the zone: *C. jacobsi* phenotypes predominate in June and July and are present in both valley and mountain habitats; *C. brunneus* phenotypes predominate in August and are restricted to valley habitats, always in sympatry with *C. jacobsi*. Strong assortative mating was observed in laboratory mating experiments. Spatial, seasonal and behavioural isolation combine to produce strong premating isolation in the study area. These results suggest a role for premating isolation in maintaining both the mosaic structure and bimodality of this **hybrid** zone.

Reproductive Interference between the Common Ground-hopper *Tetrix undulata* and the Slender Ground-hopper *Tetrix subulata* (Orthoptera, Tetrigidae)

Hochkirch, A; Bucker, A; Groning, J. 2008

The coexistence of closely related species is often hampered by resource competition or reproductive interference (interspecific sexual interactions). Species utilising similar signal channels might face Substantial problems when they cooccur. It has, therefore, been suggested that reinforcement might drive signal evolution in narrow suture zones of secondary contact. However, species with large overlapping ranges are usually not believed to interact sexually. The Slender Ground-hopper, *Tetrix subulata*, and the Common Ground-hopper, *Tetrix undulata* (Orthoptera: Tettigidae) are sister species, which occur sympatrically in large parts of western and Central Europe, but rarely share the same habitat. It has been hypothesized that reproductive interference might account for their missing coexistence. Here, we test experimentally whether these two species interact sexually. Our results suggest an incomplete premating isolation of these groundhoppers, as we recorded heterospecific courtship, mating attempts and mating. The number of conspecific copulations and mating attempts of *T. subulata* decreased substantially in the presence of *T. undulata*, whereas the latter species was not negatively affected. Males of both species preferred to approach females of *T. undulata*, whereas females of both species did not discriminate against heterospecific males. Further studies on the reproductive success are needed to clarify whether reproductive interference might influence habitat partitioning between these species.

Divergence in the calling songs between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera : Gryllidae)

Jang, Y; Gerhardt, HC. 2006

In the eastern United States the wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae) occurs in sympatry with *G. vernalis* in an area between eastern Kansas and west of the Appalachian Mountains. Calling songs were recorded from 13 sympatric and allopatric localities. Both field and laboratory recordings showed that chirp rate (CR) and pulse rate (PR) overlapped extensively between allopatric populations of *G. fultoni* and sympatric populations of *G. vernalis*; by contrast, there was little or no overlap in these variables between sympatric populations of these two species. Divergence in PR and CR between the two species was thus greater in areas of sympatry than in areas of allopatry. Our field and laboratory studies of *G. fultoni* calling songs thus demonstrate the pattern expected of character displacement and support the genetic assumptions of this hypothesis. Other possible explanations for the sympatric divergence such as ecological character displacement and clinal variation are discussed.

Molecular phylogeny of *Banza* (Orthoptera : Tettigoniidae), the endemic katydids of the Hawaiian Archipelago

Shapiro, LH; Strazanac, JS; Roderick, GK. 2006

The extant endemic katydids (Orthoptera: Tettigoniidae) of the Hawaiian Archipelago include one to three species per high island and a single species on Nihoa, all currently placed in the genus *Banza*. These acoustic insects provide an excellent opportunity for investigating the evolution of reproductive isolation and speciation, but such studies require an understanding of phylogenetic relationships within the group. We use maximum parsimony, likelihood-based Bayesian inference, and maximum likelihood to infer phylogenetic relationships among these taxa, based on similar to 2 kb of mitochondrial cytochrome oxidase I and cytochrome b. Our results strongly support two distinct high island clades: one clade ("Clade I") composed of species from Kauai, Oahu, Molokai, and Lanai and another clade ("Clade II") composed of species from Maui and Hawaii (*Banza unica*, from Oahu, may be basal to both these clades, but its placement is not well resolved). Within these clades, some inferred relationships are strongly supported, such as the sister status of *B. kauaiensis* (Kauai) and *B. parvula* (Oahu) within Clade 1, but other relationships remain more ambiguous, such as the relative position of *B. brunnea* (Maui) within Clade II. Although a detailed reconstruction of the historical biogeography of the Hawaiian katydids is difficult, we use our genetic data combined with the known geological history of the Hawaiian Islands to set limits on plausible historical scenarios for diversification of this group. Beyond these historical biogeographic inferences, our results indicate possible cryptic speciation on both Oahu and Hawaii, as well as what may be unusually high average rates of nucleotide substitution. The present work sets the stage for future genetic and experimental investigations of this group. (c) 2006 Elsevier Inc. All rights reserved.

Geographical patterns of chromosomal differentiation in the brachypterous grasshopper *Podisma sapporensis* (Orthoptera : Acrididae)

Warchalowska-Sliwa, E; Tatsuta, H; Akimoto, SI; Maryanska-Nadachowska, A; Kowalczyk, M; Bugrov, A. 2008

The distribution patterns of the X0/XX and neo-XY/neo-XX chromosome races, subraces, and "hybrids" between subraces of the grasshopper *P. sapporensis* were analyzed. The origin of the observed variation is Robertsonian translocations between a sex chromosome and an autosome, and chromosome rearrangements. The fixation levels of inversions varied depending on geographic regions. No **hybrid** population is known implying that a strong reproductive isolation system exists in **hybrids** between the different chromosomal races. The probable reasons for the purity of X0 and neo-XY chromosome races and high chromosome polymorphism in contact zones between chromosomal subraces are discussed. The presence of isolating barriers between chromosome races indicates a review of the taxonomic structure of *P. sapporensis* is required. It is proposed to divide *P. sapporensis* into two sibling species, which differ in the chromosome mechanisms of the sex determination system. The analysis of the distribution of chromosomal races and subraces of *P. sapporensis* allows a reconstruction of the history of this species in the Okhotsk sea region.

Hybrids between *Chorthippus brunneus* and *C. jacobsi* (Orthoptera : Acrididae) do not show endogenous postzygotic isolation

Saldamando, CI; Tatsuta, H; Butlin, RK. 2005

Studies of prezygotic and postzygotic isolation in *Drosophila* have shown in general that species in sympatry tend to evolve prezygotic barriers earlier than do species in allopatry. However, postzygotic barriers tend to evolve at the same evolutionary rate in both sympatric and allopatric species. In contrast to these observations, the grasshoppers *Chorthippus parallelus parallelus* and *C. p. erythropus* show complete **hybrid** male sterility but only limited prezygotic isolation after an estimated 0.5 millions years of divergence. Like their congeners, *C. brunneus* and *C. jacobsi* form a **hybrid** zone where their ranges meet in northern Spain. However, the **hybrid** zone is mosaic and bimodal and, in contrast to the high levels of postzygotic isolation between *C. parallelus* subspecies, these two species showed no significant reduction in **hybrid** fitness in F₁ or backcross generations relative to the parental generations. The level of prezygotic isolation in laboratory tests was comparable to that between *C. parallelus* subspecies. These results suggest that endogenous postzygotic isolation does not play an important role in the reproductive isolation between *C. brunneus* and *C. jacobsi*, or in determining the structure of the **hybrid** zone. Exogenous postzygotic isolation may be present and should be tested in future studies. (C) 2005 The Linnean Society of London.

Relative effects of juvenile and adult environmental factors on mate attraction and recognition in the cricket, *Allonemobius socius*

Olvido, AE; Fernandes, PR; Mousseau, TA. 2010

Finding a mate is a fundamental aspect of sexual reproduction. To this end, specific-mate recognition systems (SMRS) have evolved that facilitate copulation between producers of the mating signal and their opposite-sex responders. Environmental variation, however, may compromise the efficiency with which SMRS operate. In this study, the degree to which seasonal climate experienced during juvenile and adult life-cycle stages affects the SMRS of a cricket, *Allonemobius socius* (Scudder) (Orthoptera: Gryllidae) was assessed. Results from two-choice behavioral trials suggest that adult ambient temperature, along with population and family origins, mediate variation in male mating call, and to a lesser extent directional response of females for those calls. Restricted maximum-likelihood estimates of heritability for male mating call components and for female response to mating call appeared statistically nonsignificant. However, appreciable "maternal genetic effects"

suggest that maternal egg provisioning and other indirect maternal determinants of the embryonic environment significantly contributed to variation in male mating call and female response to mating calls. Thus, environmental factors can generate substantial variation in *A. socius* mating call, and, more importantly, their marginal effect on female responses to either fast-chirp or long-chirp mating calls suggest negative fitness consequences to males producing alternative types of calls. Future studies of sexual selection and SMRS evolution, particularly those focused on **hybrid** zone dynamics, should take explicit account of the loose concordance between signal producers and responders suggested by the current findings.

LABORATORY HYBRIDIZATION STUDIES OF ALLONEMOBIUS-FASCIATUS AND A-SOCIUS (ORTHOPTERA, GRYLLIDAE)

GREGORY, PG; HOWARD, DJ. 1993

Allonemobius fasciatus (De Geer) and *A. socius* (Scudder) are small ground-dwelling crickets that meet and **hybridize**, to a limited extent, in a mosaic contact zone stretching from New Jersey to at least as far west as Illinois. To determine whether there is selection against **hybridization**, we compared heterospecific and **hybrid** crosses with conspecific crosses with regard to offspring production, viability, and development. Parental, F1, and F2 heterospecific and **hybrid** crosses produced considerable numbers of offspring, although some cross classes produced significantly fewer offspring than conspecific crosses. Differences in the number of offspring produced between heterospecific or **hybrid** crosses and conspecific crosses could be attributed to differences in the number of eggs laid rather than to differences in the percentage hatching. The time of hatching, the time required to reach adulthood, and hatching-to-adult survival were similar in the various cross classes. Postzygotic barriers to gene exchange do not appear to be strong enough to account for the low number of **hybrids** found in mixed populations.

Divergence in female calling song discrimination between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera : Gryllidae)

Jang, Y; Gerhardt, HC. 2006

Two species of closely related wood cricket, *Gryllus fultoni* (Orthoptera: Gryllidae) and *Gryllus vernalis*, occur together in some parts of the eastern United States and have a similar calling song structure, consisting of three-pulse chirps. A previous study revealed that chirp rate in *G. fultoni* was highest (greatest difference vis-a-vis chirp rate in *G. vernalis*) in sympatric populations, intermediate in near allopatric populations that were located close to the sympatric zone, and lowest in allopatric populations. A similar trend was observed in pulse rate, but the mean values of this trait showed much more convergence than chirp rate at the low end of the range of calling temperatures. In this study, we investigated the song discrimination of females from sympatric and allopatric populations of *G. fultoni* at about 23 degrees C, which is near the middle of the normal range of calling temperatures. We used both single-stimulus and two-stimulus playback experiments to learn if geographical differences in song preferences paralleled those in calling songs. Stimuli presented were representative of calling songs in three classes of *G. fultoni* populations (sympatric, near allopatric, and far allopatric), a calling song of *G. vernalis*, and three calling songs with parameter values that were intermediate with respect to those of the songs of far allopatric *G. fultoni* and *G. vernalis*. In the single-stimulus playbacks, females of all *G. fultoni* populations responded poorly if at all to the heterospecific stimulus. Females of sympatric and near allopatric populations responded poorly to all intermediate stimuli, but females of far allopatric populations frequently responded to these sounds. In the two-stimulus playbacks, females of sympatric and near allopatric populations generally discriminated against intermediate and heterospecific stimuli. However, females of far allopatric populations often did not discriminate against intermediate stimuli, whose characteristics resembled the calling songs of *G. vernalis*. The divergent pattern of female phonotactic discrimination between sympatric and far allopatric populations was thus generally congruent with the pattern of divergence in chirp and pulse rates and would be expected to significantly reduce heterospecific mating in sympatry. These geographical patterns of female song discrimination and male calling songs conform to a commonly used definition of reproductive character displacement.

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

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

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

Isolation mechanisms in the closely related grasshopper species, *Chorthippus albomarginatus* and *Ch. oschei* (Orthoptera, Acrididae)

Vedenina, VY; Kulygina, NK; Panyutin, AK. 2007

Two closely related grasshopper species, *Chorthippus albomarginatus* and *Ch. oschei*, are known to **hybridize** at a narrow contact zone in the territory of Ukraine and Moldova. Different isolation mechanisms providing a reproductive isolation of these species were studied. In the mating experiments, females of both species demonstrated a strong assortative mating (80-90% preference for conspecific males). The comparison of the parental and **hybrid** viability revealed a reduced hatching and an increased larval mortality in F-1 and F-2 **hybrids**. In the choice mating experiments, the females mated less assortatively than parental females. The data obtained demonstrated the existence of pre- and post-mating isolation in *Ch. albomarginatus* and *Ch. oschei*. A possible fate of the **hybrid** zone is discussed.

GENETIC DIFFERENTIATION, HYBRIDIZATION AND REPRODUCTIVE ISOLATION IN MYGALOPSIS-MARKI BAILEY (ORTHOPTERA, TETTIGONIIDAE)

DADOUR, IR; JOHNSON, MS. 1983

Dominance effects strengthen premating hybridization barriers between sympatric species of grasshoppers (Acrididae, Orthoptera)

Gottsberger, B; Mayer, F. 2019

Sexual selection can lead to the rapid evolution of premating **hybridization** barriers and allows accelerated diversification and speciation within an evolutionary lineage. Especially during early stages of divergence, **hybridization** may impede further divergence, which strongly depends on the reproductive success of **hybrids**. Behavioural sterility of **hybrids** can limit or even prevent homogenizing gene flow. In this study, we investigated the attractiveness of male courtship songs for

females of the grasshopper species *Chorthippus biguttulus* and *C. brunneus* and their interspecific F1 and F2 **hybrids**. Song preferences of females of both species are highly species specific and differ in three parameters: shape of the preference function, preference for syllable pattern and phrase duration. F1 **hybrid** females of both reciprocal crosses as well as F2 **hybrid** females resembled closely pure *C. biguttulus* females in respect of shape of the preference function and preference for syllable pattern, while preference for phrase duration showed an intermediate expression. This resulted in song preferences of **hybrid** females that closely resembled those of one parental species, that is *C. biguttulus* females. Such strong dominance effects were rarely reported so far. They represent an effective barrier limiting gene flow between the two species, since **hybrid** females will backcross to only one parental species and discriminate against **hybrid** males, which are behaviourally sterile. Such taxon-specific modes of inheritance may have facilitated the rapid divergence of acoustically communicating grasshoppers of the species group of *Chorthippus biguttulus*. Our findings have novel implications on the expression of neuronal filters and the evolution of complex courtship signals.

Sound production in *Calliptamus barbarus* Costa 1836 (Orthoptera : Acrididae : Catantopinae)

Larrosa, E; Garcia, MD; Clemente, ME; Presa, JJ. 2008

Calliptamus barbarus Costa 1836 is an acridid species whose hind femora display chromatic polymorphism that may be of ruby colour with three bold and separate femoral spots, or pale orange, with only one large femoral spot. Since both bio forms (either with three (3S) or with one (1S) spot) appear to be segregated, and their sounds constitute an intra-specific recognition system during mating, the sounds emitted by both groups were studied in order to identify any differences between them. It was observed that the sound is produced by mandible friction in both males and females. The temporal characteristics of the acoustic emissions of the males of both bio forms showed significant differences under all conditions. In females, some differences in frequency and in temporal characteristics were also detected. Likewise males, the syllable length and the number of emitted pulses are greater in the 1S than in the 3S form. Together with other differences, these observations suggest a true separation between these bio forms, with a reproductive isolation that will probably lead to a speciation process.

COURTSHIP SONG ANALYSIS IN TWO HYBRID ZONES BETWEEN SIBLING SPECIES OF THE CHORTHIPPUS ALBOMARGINATUS GROUP (ORTHOPTERA, GOMPHOCERINAE)

Vedenina, VY. 2015

Two new **hybrid** zones between sibling species of the *Chorthippus albomarginatus* group were described on the basis of the courtship song analysis. Not only sound but also underlying stridulatory movements of hind legs were analyzed, which allowed classifying temporal parameters and conducting comparative analysis more reliably. One **hybrid** zone, between *Ch. albomarginatus* and *Ch. karelini*, was found in Uryanovsk and Samara regions of Russia. Another **hybrid** zone was found in Kherson region of Ukraine, around the reserved steppe Askaniya-Nova, presumably between *Ch. karelini* and *Ch. oschei*. A comparison of the natural and laboratory **hybrids** allowed suggesting the structure and fate of the **hybrid** zones.

Identification and comparative analysis of accessory gland proteins in Orthoptera

Braswell, WE; Andres, JA; Maroja, LS; Harrison, RG; Howard, DJ; Swanson, WJ. 2006

Accessory reproductive gland proteins (Acps) in *Drosophila* evolve quickly and appear to play an important role in ensuring the fertilization success of males. Moreover, Acps are thought to be involved in establishing barriers to fertilization between closely related species. While accessory glands are known to occur in the males of many insect groups, the proteins that are passed on to females by males during mating have not been well characterized outside of *Drosophila*. To gain a better understanding of these proteins, we characterized ESTs from the accessory glands of two cricket species, *Allonemobius fasciatus* and *Gryllus firmus*. Using an expressed sequence tag (EST) approach, followed by bioinformatic and evolutionary analyses, we found that many proteins are secreted and, therefore, available for transfer to the female during mating. Further, we found that most ESTs are novel, showing little sequence similarity between taxa. Evolutionary analyses suggest that cricket proteins are subject to diversifying selection and indicate that *Allonemobius* is much less polymorphic than *Gryllus*. Despite rapid nucleotide sequence divergence, there appears to be functional conservation of protein classes among *Drosophila* and cricket taxa.

HIGH ULTRASONIC AND TREMULATION SIGNALS IN NEOTROPICAL KATYDIDS (ORTHOPTERA, TETTIGONIIDAE)

MORRIS, GK; MASON, AC; WALL, P; BELWOOD, JJ. 1994

Myopophyllum speciosum is a pseudophylline katydid (Tettigoniidae) from the neotropics that generates unusually high ultrasonic frequencies as the dominant carrier in its calling song. Male calls average only 148 ms duration and are given at long intervals: 8.7 s. Fairing is completed with vibrational signals, generated at closer range by body oscillation (tremulation). Two distinctive vibrational motor patterns, short and long, are produced by both sexes. Physical parameters of the sound and vibratory signals of this species are described. The relatively high-Q carrier frequency (mean = 81 kHz) varies between males over a range of 20 kHz but does not predict a singer's body size. Short tremulations are much more intense than long as measured by acceleration. Descriptions of the songs of three other pseudophylline species with unusually high principal carriers (65-105 kHz) are also presented. Eavesdropping by predatory bats offers the most plausible selective explanation for the features of *M. speciosum*'s signal system. This hypothesis is supported by the species' sexually dimorphic defensive spination: males, the sound-signalling sex, have metafemoral spines of greater size and distinctive orientation. Evidence for eavesdropping and for alternative hypotheses is assessed. Other neotropical tettigoniids in rainforest understorey also employ elaborate vibratory signals (species of *Choeroparnops*, *Schedocentrus*, *Docidocercus*, *Copiphora*) and some show a trend to reduce or even to eliminate their use of airborne sound. Some rainforest tettigoniids may have replaced acoustic with vibrational signalling as a response to bat eavesdropping.

The mosaic distribution pattern of two sister bush-cricket species and the possible role of reproductive interference

Dorkova, M; Kristin, A; Jarcuska, B; Kanuch, P. 2020

Reproductive interference can shape regional distribution patterns in closely related species, if prezygotic isolation barriers are weak. The study of such interaction could be more challenging in nuptial gift-giving species due to the direct nutritional effects on both sexes of both species during copulation. We mapped the distribution of two sister bush-cricket species, *Pholidoptera aptera* and *Pholidoptera transsylvanica*, at the northern margin of their overlapping ranges in Europe, and with a behavioral experiment, we tested the possibility of heterospecific mating. We found a very rare coexistence of species locally (0.5%, n = 391 sites) with mostly mutually exclusive distribution patterns, resulting in a mosaic pattern of sympatry, whereas they occupied the same climate niche in forest-dominated mountain landscape. Over 14 days of a mating experiment with seven mixed groups of conspecifics and heterospecifics (n = 56 individuals in total), the number of received spermatophores per female was 3-6 in *P. aptera* and 1-7 in *P. transsylvanica*. In total, we found 8.1% of heterospecific copulations (n = 99 transferred spermatophores with genetic identification of the donor species), while we also confirmed successful transfer of heterospecific sperms into a female's reproductive system. Because bush-cricket females also obtain required nutrition from a heterospecific spermatophylax what should increase their fitness and fecundity, we suggest that their flexibility to mate with heterospecifics is beneficial and drives reproductive interference. This may substantially limit the reproductive success of the less frequent species (*P. transsylvanica*), coupled with eventual detrimental effects from **hybridization**, and result in the competitive exclusion of that species from their areas of coexistence.

Evolution of Novel Signal Traits in the Absence of Female Preferences in *Neoconocephalus* Katyids (Orthoptera, Tettigoniidae)

Bush, SL; Schul, J. 2010

Background Significance: Communication signals that function to bring together the sexes are important for maintaining reproductive isolation in many taxa. Changes in male calls are often attributed to sexual selection, in which female preferences initiate signal divergence. Natural selection can also influence signal traits if calls attract predators or parasitoids, or if calling is energetically costly. Neutral evolution is often neglected in the context of acoustic communication. **Methodology/Principal Findings:** We describe a signal trait that appears to have evolved in the absence of either sexual or natural selection. In the katydid genus *Neoconocephalus*, calls with a derived pattern in which pulses are grouped into pairs have evolved five times independently. We have previously shown that in three of these species, females require the double pulse pattern for call recognition, and hence the recognition system of the females is also in a derived state. Here we describe the remaining two species and find that although males produce the derived call pattern, females use the ancestral recognition mechanism in which no pulse pattern is required. Females respond equally well to the single and double pulse calls, indicating that the derived trait is selectively neutral in the context of mate recognition. **Conclusions/Significance:** These results suggest that 1) neutral changes in signal traits could be important in the diversification of communication systems, and 2) males rather than females may be responsible for initiating signal divergence.

Experimental hybridization between X0 and XY chromosome races in the grasshopper *Podisma sapporensis* (Orthoptera : Acrididae). II. Cytological analysis of embryos and adults of F-1 and F-2 generations

Warchalowska-Sliwa, E; Bugrov, AG; Sugano, Y; Maryaska-Nadachowska, A; Akimoto, S. 2008

Experimental **hybridization** of X0 and XY chromosome races of the brachypterous grasshopper *P. sapporensis* did not reveal pre-zygotic reproductive isolation. However, a partial zygotic barrier was found between the X0-standard race from Shimokawa and XY-standard chromosome race from Akan. Approximately 40% of embryos from females crossed with males from other chromosome races developed parthenogenetically, the remaining embryos were normal heterozygotes. Adult F₁ males and females from crosses of this type had properly developed testes and ovaries. Non-sister associations and other irregularities in meiosis were not observed in male meiosis. Crossing experiments demonstrated that **hybrids** between X0 and XY races occur to some extent. The absence of a **hybrid** zone between the X0 and XY chromosome races may be the result of selection against heterozygotes. Crosses between the XY-Tanno and X0-standard (Teine) subraces resulted in F-1 and F-2 generations in spite of the many chromosome differences between them such as a X-A translocation and fixed pericentric inversions in four pairs of autosomes. These results do not support the hypothesis that chromosomal differences play a key role in restricting gene flow between the X0 and XY races of *P. sapporensis*.

Impact of Limited Dispersion Capacity and Natural Barriers on the Population Structure of the Grasshopper *Ommexecha virens* (Orthoptera: Ommexechidae)

de Souza, TE; Cruz, GAD; de Moura, RD. 2021

The grasshopper *Ommexecha virens* Serville has low dispersion capacity, and it is regarded as a specialist, only being found in sandy, dry environments with high incidence of sunlight. Considering these aspects, we evaluated the diversity and genetic structure of *O. virens* natural populations using ISSR (Inter Simple Sequence Repeat) markers. The data pointed to low expected heterozygosity for some populations ($H-E = 0.06-0.09$), probably a consequence of positive inbreeding, which is typical of species showing low or null dispersion indices. Moreover, significant genetic differentiation was observed ($F_{ST} = 0.50$ and $G_{ST} = 0.51$), as well as low number of migrants ($N_m = 0.47$), indicating that the populations are genetically differentiated. This is likely related to the limitation in dispersing and fragmentation of suitable environment localities colonized by *O. virens*. The populations of *O. virens* were structured in three genetic groups associated to different landscapes, revealing the presence of a secondary contact zone, possibly arisen from isolation followed by genetic divergence among populations and subsequent gene flow of divergent individuals of *O. virens*. At last, we found positive isolation by distance (IBD; $r: 0.427$; $P: 0.025$) which is an important factor, since it may be adding to the emergence of reproductive barriers among individuals of *O. virens* that have been experiencing isolation.

Experimental hybridisation between X0 and XY chromosome races in the grasshopper *Podisma sapporensis* Shir. (Orthoptera, Acrididae). I. Cytological analysis of embryos and F1 hybrids

Bugrov, AG; Warchalowska-Sliwa, E; Sugano, Y; Akimoto, S. 2004

The results of experimental **hybridisation** between some chromosome subraces belonging to the X0 and XY chromosome races of the brachypterous grasshopper *P. sapporensis* are presented. Pre-zygotic reproductive isolation mechanisms in experimental pairs were not confirmed. In crossings of XY-standard x X0-standard and XY-standard x X0-Naganuma chromosome subraces, a zygotic barrier has been found. All embryos of XY-standard x X0-standard crosses and the vast majority of embryos of XY-standard x X0-Naganuma crosses were obtained from female diploid or haploid/diploid cells as a result of parthenogenesis. In very rare cases, when the zygotic barriers had been surmounted, normal embryo heterozygotes and a F1 **hybrid** generation were obtained in XY-standard x X0-Naganuma crosses. On the contrary, crosses between the XY-Tanno and X0-standard subraces gave viable offspring in spite of many chromosome differences such as a X-A translocation and fixed pericentric inversions in four pairs of autosomes. The results obtained do not support the hypothesis that chromosomal differences play a key role in restricting gene flow between X0 and XY races of *P. sapporensis*. The presence of crossing barriers explains the phenomena of the purity of the X0 and XY chromosomes races.

Development and characterization of microsatellite loci in Mormon crickets (*Anabrus simplex*, Orthoptera, Tettigoniidae)

Bailey, NW; Hockham, LR; Graves, JA; Ritchie, MG. 2005

Anabrus simplex is an economically significant crop pest in the western United States and is a model organism for studying the influence of sex role reversal on sperm allocation and utilization patterns and population genetics. We isolated seven polymorphic microsatellite loci in *Anabrus simplex*, and within population allele numbers ranged from 10 to 25. High polymorphism is not unusual for Orthopteran insects, although observed heterozygosities ranged from 0.24 to 0.91 and were lower than expected heterozygosities, suggesting null alleles. These microsatellites will greatly facilitate studies of postcopulatory reproductive isolation in nuptial gift-giving insects and historical phylogenetics in the Rocky Mountains.

PATTERNS OF REPRODUCTIVE ISOLATION BETWEEN THE SIBLING GRASSHOPPER SPECIES *DOCIOSTAURUS-CURVICERCUS* AND *D-JAGOI-JAGOI* (ORTHOPTERA, ACRIDIDAE, GOMPHOCERINAE)

BLONDHEIM, SA. 1990

Positive selection and comparative molecular evolution of reproductive proteins from New Zealand tree weta (Orthoptera, Hemideina)

Twort, VG; Dennis, AB; Park, D; Lomas, KF; Newcomb, RD; Buckley, TR. 2017

Animal reproductive proteins, especially those in the seminal fluid, have been shown to have higher levels of divergence than non-reproductive proteins and are often evolving adaptively. Seminal fluid proteins have been implicated in the formation of reproductive barriers between diverging lineages, and hence represent interesting candidates underlying speciation. RNA-seq was used to generate the first male reproductive transcriptome for the New Zealand tree weta species *Hemideina thoracica* and *H. crassidens*. We identified 865 putative reproductive associated proteins across both species, encompassing a diverse range of functional classes. Candidate gene sequencing of nine genes across three *Hemideina*, and two *Deinacrida* species suggests that *H. thoracica* has the highest levels of intra-specific genetic diversity. Non-monophyly was observed in the majority of sequenced genes indicating that either gene flow may be occurring between the species, or that reciprocal monophyly at these loci has yet to be attained. Evidence for positive selection was found for one lectin-related reproductive protein, with an overall omega of 7.65 and one site in particular being under strong positive selection. This candidate gene represents the first step in the identification of proteins underlying the evolutionary basis of weta reproduction and speciation.

Acoustic signals, ecological features, and reproductive isolation of grasshoppers of the genus *Dociostaurus* (Orthoptera, Acrididae) in semidesert

Savitskii, VY. 2000

Acoustic signals, acoustic behavior, and some ecological features were investigated in 5 species of the genus *Dociostaurus* from various localities of Daghestan, Volgograd, and Astrakhan oblasts. The calling songs of *D. brevicollis*, *D. tartarus*, *D. kraussi*, *D. albicornis*, and *D. anatolicus* are described as well as response songs of *D. brevicollis*, protest songs of *D. brevicollis* and *D. tartarus*, and courtship songs of *D. tartarus*. A comparative analysis of calling songs in 10 species of *Dociostaurus* was performed. The calling songs were categorized into 4 groups by amplitude-temporal characters. The reproductive isolation between *Dociostaurus* species is shown not to be specified exclusively by differences in the acoustic behavior, but to be provided by some ecological mechanisms (for instance, by differences in phenology and biotopic preference).

Divergence in Potential Contact Pheromones and Genital Morphology Among Sympatric Song Types of the Bush Cricket *Mecopoda elongata*

Dutta, R; Balakrishnan, R; Tregenza, T. 2018

A well-established route to speciation in animals is via the evolution of divergent male mating signals and female preferences within a species. However, an open question is how common it is for near complete isolation to be achieved through a single signal-receiver system as opposed to multiple aspects of the mate-recognition system diverging simultaneously. The five highly divergent mate-attraction song types of the bush cricket *Mecopoda elongata* exemplify reproductive isolation in sympatry through long-distance mating signals. Female preference for their own song type has been established as a strong pre-mating reproductive barrier, but the potential existence of additional isolating mechanisms has not been investigated. We quantify divergence in cuticular lipid profiles and external genital structures between song types. These traits show significant variation among species of Orthoptera and are known to be used in mate recognition following contact. We show that divergence among sympatric *Mecopoda* song types in both cuticular lipid profiles and two external genital structures is sufficiently extensive that either of them can be used to identify individual song type with 90% accuracy. Our findings suggest that multiple isolating mechanisms are likely to evolve simultaneously facilitating a more robust reproductive isolation. Our study indicates a role for sexual selection in the divergence and potential future speciation of these populations and suggests that reproductive isolation may frequently evolve through simultaneous divergence across different aspects of mate recognition systems.

Taxonomic status of the grasshopper *Podisma tyatiensis* and reproductive isolation between *P.tyatiensis* and *Podisma sapporensis*

Bugrov, AG; Warchalowska-Sliwa, E; Akimoto, S. 2005

The grasshopper *Podisma tyatiensis*, which is distributed only at the summit of Mount Tyatya on Kunashiri Island, the Kuril Islands, is closely related to *Podisma sapporensis*, which has a broad distribution range on the islands of northern Japan and the Russian Far East (Hokkaido, Sakhalin and Kunashiri). The present study examined the taxonomic status of *P. tyatiensis* by crossing *P. tyatiensis* males with *P. sapporensis* females from Sakhalin. More than 90% of eggs from intrapopulation crosses developed to at least the last embryonic stage, whereas only 64% of eggs from the interpopulation crosses developed into that embryonic stage. Cytogenetic observations of prediapause embryos showed that the interpopulation crosses always led to the production of unfertilized eggs, and that all of the developing embryos had the maternal genome only. A mixture of haploid and diploid cells of maternal origin was found in most of those embryos. This result shows that unfertilized eggs produced by *P. sapporensis* females from Sakhalin developed parthenogenetically to at least the embryonic stage before hatching. The present crossing experiments revealed a high level of incompatibility between the genomes of the Sakhalin population and the Tyatya population, and confirmed the full species status of *P. tyatiensis*.

Multiple mating in natural populations of ground crickets

Gregory, PG; Howard, DJ. 1996

Diel variation in signalling and signal transmission in the bladder grasshopper, *Bullacris unicolor* (Orthoptera; Pneumoridae)

Couldridge, VCK; Gordon, ML. 2015

Here we investigate intraspecific variation in diel patterns of acoustic signalling in the bladder grasshopper, *Bullacris unicolor*. We observed that *B. unicolor* calls at different times during the night in different parts of its distribution. Males further north typically call just before dawn, while those further south signal throughout the night. Sound transmission experiments were conducted in order to determine whether the observed discrepancy in signal timing can be explained by differences in signal propagation at different times in the night, which might vary geographically. We found significant differences in signal attenuation and fidelity at different broadcast times as well as between locations. However, there was only partial support for the hypothesis that males time their calls to coincide with the most ideal transmission conditions. We suggest that other factors, such as predation pressure, might also contribute to the observed discrepancy in signal timing between populations.

Molecular divergence between *Gryllus rubens* and *Gryllus texensis*, sister species of field crickets (Orthoptera : Gryllidae)

Gray, DA; Barnfield, P; Seifried, M; Richards, MH. 2006

We assess the degree of sequence divergence in the maternally inherited mitochondrial cytochrome c oxidase I (COI) and cytochrome b (CytB) genes between two sister species of field crickets, *Gryllus rubens* Scudder, 1902 and *Gryllus texensis* Cade and Otte, 2000. We analyzed 1460 base pairs from 10 individuals of each species; individuals were sampled from areas of both allopatry and sympatry. Overall average pairwise mitochondrial sequence divergence between species was

1.4% +/- 0.1 % (mean +/- SD); however, there was almost an order of magnitude more divergence in COI (2.59% +/- 2.25%) than in CytB (0.35% +/- 0.24%). *Gryllus texensis* appears to harbor a much greater level of genetic variation than does *G. rubens*. Phylogenetic trees constructed from these sequences show reasonable separation of species; however, sequences are not reciprocally monophyletic. Gene tree polyphyly may reflect recent species-level divergence and (or) interspecific gene flow. The pattern of sequence divergence and genetic variation in these taxa is consistent with allopatric or peripatric speciation in Pleistocene glacial refugia in the southeastern (*G. rubens* ancestral lineage) and southcentral United States (*G. texensis* ancestral lineage).

ACOUSTIC SIGNALS IN INSECTS: A REPRODUCTIVE BARRIER AND A TAXONOMIC CHARACTER

Tishechkin, DY; Vedenina, VY. 2016

In singing insects, the song is an important component of the specific mate recognition system (SMRS). In communities of sympatric singing species, there is a partitioning of communication channels, the so-called "acoustic niches". Within one community, the songs of different species always differ in temporal or frequency characters, i.e. occupy different acoustic niches. However, conspecific songs do not always act as an inter specific reproductive barrier, despite always being a SMRS component. The species that do not communicate acoustically due to either allopatry or a different timing of vocalization, or inhabiting different biotopes, or unmatched food specializations can sing similar songs, thus forming reproductively isolated communities. Individuals of the opposite sex not only need to recognize a conspecific mate, but also have to evaluate its "quality". A close-range signal (courtship song) provides more opportunities for a choice of the "best" male than does a distant signal (calling song). In many species of Orthoptera, courtship includes not only acoustic, but also vibration, visual, chemical and mechanical signals. An analysis of cricket songs showed that, on average, the courtship songs are more elaborate and more variable than the calling songs. At the same time, due to the difference in mating behaviour between grasshoppers and crickets, the acoustic component of courtship is used for an evaluation of mate quality to a greater extent in the fowler than it is in the latter group. Courtship songs of grasshoppers are generally more elaborate in temporal structure than cricket songs and, moreover, can be accompanied by visual displays such as movements of various parts of the body. Thus, song evolution in grasshoppers is more driven by sexual selection than that of crickets. According to the reinforcement hypothesis, a premating barrier between **hybridizing** species becomes stronger in response to reduced **hybrid** fitness. However, our behavioural experiments conducted on two groups of **hybridizing** grasshopper species did not confirm the reinforcement hypothesis. We explain this firstly, by a low level of genetic incompatibility between the **hybridizing** species and secondly, by high **hybrid** fitness when attracting a mate. A high competitive capability of **hybrids** might be accounted for by the attractiveness for females of new elements in **hybrid** courtship songs. When we divide similar forms on the basis of songs, we distinguish biological species using their reproductive isolation as a criterion. Acoustic differences between species are usually greater than morphological ones. Therefore, analyzing the song allows us to find out the real status of doubtful taxa of species rank, to distinguish species in a flock of sibling forms and to reveal cryptic species in the cases when morphological studies fail to provide a univocal result. At the same time, songs have intra-specific variability and its range can be different in various groups. Therefore, it is necessary to study which degree of difference corresponds to the species level before interpreting the status of some forms based on song comparisons. Besides this, song similarities cannot be evidence that acoustically isolated forms are conspecific. On the other hand, song differences between these forms prove that they are full-rank species.

Habitat Association and Seasonality in a Mosaic and Bimodal Hybrid Zone between *Chorthippus brunneus* and *C. jacobsi* (Orthoptera: Acrididae)

Bailey, RI; Saldamando-Benjumea, CI; Tatsuta, H; Butlin, RK. 2012

Understanding why some **hybrid** zones are bimodal and others unimodal can aid in identifying barriers to gene exchange following secondary contact. The **hybrid** zone between the grasshoppers *Chorthippus brunneus* and *C. jacobsi* contains a mix of allopatric parental populations and inter-mingled bimodal and unimodal sympatric populations, and provides an ideal system to examine the roles of local selection and gene flow between populations in maintaining bimodality. However, it is first necessary to confirm, over a larger spatial scale, previously identified associations between population composition and season and habitat. Here we use cline-fitting of one morphological and one song trait along two valley transects, and intervening mountains, to confirm previously identified habitat associations (mountain versus valley) and seasonal changes in population composition. As expected from previous findings of studies on a smaller spatial scale, *C. jacobsi* dominated mountain habitats and mixed populations dominated valleys, and *C. brunneus* became more prevalent in August. Controlling for habitat and incorporating into the analysis seasonal changes in cline parameters and the standard errors of parental trait values revealed wider clines than previous studies (best estimates of 6.4 to 24.5 km in our study versus 2.8 to 4.7 km in previous studies) and increased percentage of trait variance explained (52.7% and 61.5% for transects 1 and 2 respectively, versus 17.6%). Revealing such strong and consistent patterns within a complex **hybrid** zone will allow more focused examination of the causes of variation in bimodality in mixed populations, in particular the roles of local selection versus habitat heterogeneity and gene flow between differentiated populations.

Inheritance of song and stridulatory peg number divergence between *Chorthippus brunneus* and *C-jacobsi*, two naturally hybridizing grasshopper species (Orthoptera : Acrididae)

Saldamando, CI; Miyaguchi, S; Tatsuta, H; Kishino, H; Bridle, JR; Butlin, RK. 2005

Knowledge of the genetic basis of divergence in mating signal characters that contribute to reproductive isolation is critical to understanding speciation. Here, we describe a semi-automated system for characterizing grasshopper acoustic signals. We used this system to study the genetic basis of divergence in three male calling song components [echeme (EL), syllable (SL) and phrase (PL) lengths] between *Chorthippus brunneus* and *C. jacobsi*, two species of grasshoppers that **hybridize** in northern Spain. We also studied the number of pegs in the stridulatory file. For all characters, additive effects accounted for most of the genetic differentiation between species. However, the three song components also showed small but significant epistatic effects. No sex linkage was detected. Wright-Castle-Lande estimates of the minimum numbers of genetic factors underlying song and peg number divergence were low: peg number ($n(e) = 5.87 \pm 5.84$), SL ($n(e) = 2.37 \pm 4.79$) and PL ($n(e) = 0.87 \pm 0.86$). On the other hand, EL appeared to be controlled by many genes. These results suggest that divergence in SL and PL might be driven by sexual selection whereas EL might not be under selection. This is consistent with experimental results on female song preference in related species. However, the fact that few factors appear to underlie the differences in peg number is surprising. Peg number is not closely related to song characteristics. It often varies between closely related grasshopper species and it has been assumed to be a neutral character. The biometrical approaches used here tend to underestimate the number of factors influencing a trait but provide valuable background for subsequent quantitative trait loci analyses.

Visual communication behaviour as a mechanism behind reproductive interference in three pygmy grasshoppers (Genus *Tetrix*, tetrigidae, orthoptera)

Hochkirch, A; Deppermann, J; Groning, J. 2006

Specific mate recognition systems should enable species to recognize conspecific mates correctly. However, heterospecific matings have been observed in a variety of taxa. One of these cases is the pygmy grasshopper genus *Tetrix*, in which three species show sexual interactions. *T. ceperoi* males mount preferably females of *T. subulata*, but they are rejected as mates. *T. subulata* males prefer *T. undulata* females over conspecific females and heterospecific matings occur. Here, we study the underlying behavioural mechanisms of this pattern by analysing the visual courtship behaviour of the three species video-graphically. We test the hypothesis that the displays of *T. ceperoi* are highly differentiated from the other species, while the courtship of *T. subulata* and *T. undulata* is more similar. This is supported by our results: while *T. ceperoi* males perform a fast movement of high amplitude ("pronotal bobbing"), the other two species show only minor movements ("lateral swinging," "frontal swinging"). The first function of a discriminant analysis of the temporal dimensions of the displays explained 96% of the variance. 100% of the *T. ceperoi* displays were classified correctly, whereas only 50% of the *T. subulata* and 81% of the *T. undulata* displays were grouped accurately. A two-way ANOVA revealed no significant effects of the direction of the "swinging" movements (laterally or frontally) and no interactions between direction and species either, whereas each temporal parameter differed significantly between the three species. The highest degree of differentiation was found between *T. ceperoi* and *T. undulata*, while

T. subulata and T. undulata only differed significantly in two of the six temporal parameters. Our results suggest that the mismatings between T. undulata and T. subulata are caused by an insufficient specificity of the courtship behaviour. Apparently, ecological segregation of these two species could impede sexual interactions in the field.

Spatial and temporal variation in three call traits and preferences of the tree cricket *Oecanthus forbesi*

Symes, LB. NA

Multiple selective forces act on the evolution of mating preferences. While mating preferences are central to pre-zygotic isolation, certain preferences and traits may make greater contributions. For some traits, females may exhibit preferences, but accept heterospecifics trait values when preferred values are scarce. For other traits, females may fail to reproduce before accepting heterospecifics trait values. Understanding patterns of variation and divergence in this later class of traits is particularly relevant to understanding divergence and speciation. Here, I focus on three call traits of Forbes' Tree Cricket (*Oecanthus forbesi*) to quantify their capacity to produce reproductive isolation and to compare patterns of variation and divergence in these traits. By generating female preference functions and measuring male call parameters, I test two hypotheses: (1) traits and preferences vary in their capacity to contribute to reproductive isolation and (2) traits that are important to reproductive isolation have lower intrapopulation, interpopulation, and interannual variation and weaker correlation with male body size. I find that female response to one trait (pulse rate) decreased sharply when trait values fell within the heterospecific range. This trait had low variation and no correlation with male morphology. For two other traits (pulse duration and dominant frequency), females responded to many values, including values characteristics of co-occurring heterospecifics. Trait variation was higher and pulse duration correlated with male leg length. These results indicate that the evolutionary dynamics of a low-variation trait (pulse rate) may be more important to speciation than changes in more conspicuously variable sexually selected traits. Significance statement Animals often attract and assess mates using complex signals. This paper tests whether some signal components contribute more to preventing mating between species. The data show that changes in a single trait [pulse rate of cricket calls] can eliminate female response to males, while other traits [dominant frequency (pitch) and pulse duration] can be changed to match the values produced by other species without reducing female response. Consequently, some traits may diverge without contributing to reproductive isolation between species. The paper then tests for correlations between trait function and patterns of trait variation within and between populations and species. Pulse rate has low variation within and between populations, but differs substantially between species. Dominant frequency and pulse rate are more variable within and between populations. Pulse duration also correlates with male body size, indicating that pulse duration could reflect male condition even if it is relatively unimportant for reproductive isolation.

Convergent and divergent patterns of morphological differentiation provide more evidence for reproductive character displacement in a wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae)

Jang, Y; Won, YJ; Choe, JC. 2009

Background: In ecological character displacement, traits involved in reproductive isolation may not evolve in arbitrary directions when changes in these traits are by-products of adaptation to an ecological niche. In reproductive character displacement, however, selection acts directly on reproductive characters to enhance the degree of reproductive isolation between sympatric populations. Thus, the direction of change in reproductive characters may be arbitrary in relation to changes in other morphological characters. We characterized both tegminal characters and characters indicative of body size in sympatric and allopatric populations of *Gryllus fultoni*, a species displaying character displacement in its calling song characters in areas of sympatry with *G. vernalis* populations, to infer the nature and direction of selection acting on reproductive and morphological characters in sympatry. Results: Except for mirror area, the number of teeth in a file, and ovipositor length of *G. fultoni*, all male and female morphological characters in *G. fultoni* and *G. vernalis* exhibited a uniform tendency to decrease in size with increasing latitude. There was no significant variation in female morphological characters between sympatric and allopatric *G. fultoni* populations. However, males of sympatric and allopatric *G. fultoni* populations significantly differed in head width, hind femur length, and mirror area even after controlling for clinal factors. Head width and hind femur length of *G. fultoni* were more similar to those of *G. vernalis* in sympatric populations than in allopatric populations, resulting in morphological convergence of *G. fultoni* and *G. vernalis* in sympatry. However, the mirror area of *G. fultoni* displayed the divergent pattern in relation to the sympatric *G. vernalis* populations. Conclusion: Divergence-enhancing selection may be acting on mirror area as well as calling song characters, whereas local adaptation or clinal effects may explain variation in other morphological characters in sympatric populations of *G. fultoni*. This study also suggests that structures and behaviors that directly enhance reproductive isolation may evolve together, independently of other morphological traits.

Inferring species boundaries using acoustic and morphological data in the ground cricket genus *Gymnogryllus* (Orthoptera: Grylloidea: Gryllinae)

Tan, MK; Yong, CYH; Ingrisch, S; Sah, HHA; Wahab, RBA; Johns, PM. 2018

An important function of song production by male crickets is to attract conspecific females. These sound signals can be used to infer species boundaries as they can provide indirect evidence for reproductive isolation. However, many studies of orthopteran diversity in South-east Asia are based mainly on morphology and only occasionally acoustics. As such, there is a lack of information on how acoustic data can be congruent with morphological data when used to delineate species. Crickets of the genus *Gymnogryllus* (Grylloidea, Gryllidae), are such an example. *Gymnogryllus* are relatively speciose, but their calling songs have not been studied. We collected specimens and calling songs of five *Gymnogryllus* species from South-east Asia. The acoustic parameters of the calls, along with male tegminal venation and morphology genitalia, were compared. All data types showed congruency in distinguishing *G. sylvestris* and *G. leucostictus* from each other and from the other species. Inferring species boundaries for *G. angustus*, *G. malayanus*, and *G. unexpectus* using acoustics and tegminal morphometry proves to be more challenging. While acoustics, tegminal morphometry, and genital morphology are likely to be useful for inferring species of *Gymnogryllus* from different species groups, greater coverage of taxa is needed to resolve taxonomy of closely related *Gymnogryllus*.

Divergent host plant adaptation drives the evolution of sexual isolation in the grasshopper *Hesperotettix viridis* (Orthoptera: Acrididae) in the absence of reinforcement

Grace, T; Wisely, SM; Brown, SJ; Dowell, FE; Joern, A. 2010

Early stages of lineage divergence in insect herbivores are often related to shifts in host plant use and divergence in mating capabilities, which may lead to sexual isolation of populations of herbivorous insects. We examined host preferences, degree of differentiation in mate choice, and divergence in cuticular morphology using near-infrared spectroscopy in the grasshopper *Hesperotettix viridis* aiming to understand lineage divergence. In Kansas (USA), *H. viridis* is an oligophagous species feeding on *Gutierrezia* and *Solidago* host species. To identify incipient mechanisms of lineage divergence and isolation, we compared host choice, mate choice, and phenotypic divergence among natural grasshopper populations in zones of contact with populations encountering only one of the host species. A significant host-based preference from the two host groups was detected in host-paired feeding preference studies. No-choice mate selection experiments revealed a preference for individuals collected from the same host species independent of geographic location, and little mating was observed between individuals collected from different host species. Female mate choice tests between males from the two host species resulted in 100% fidelity with respect to host use. Significant differentiation in colour and cuticular composition of individuals from different host plants was observed, which correlated positively with host choice and mate choice. No evidence for reinforcement in the zone of contact was detected, suggesting that divergent selection for host plant use promotes sexual isolation in this species. (C) 2010 The Linnean Society of London, Biological Journal of the Linnean Society, 2010, 100, 866-878.

Conservation of the red-winged grasshopper, *Oedipoda germanica* (Latr.): the influence of reproductive behaviour

Reinhardt, K; Kohler, G. 2002

Reproductive isolation can function as a mechanism to maintain locally adapted gene complexes while decreasing the heterozygosity in distinct populations. As a result, reproductive behaviour should be considered it fundamental factor influencing reproductive isolation. This is of interest to conservation biology when one desires to regulate gene flow between two populations either by creating opportunities for increased dispersal, by relocation of individuals, or by re-introduction of a species to its natural habitat. Reproductive behaviour can also influence the effective population size and the actual population size through the Allee effect. We investigated the reproductive behaviour of individuals from two isolated populations of the red winged grasshopper, *Oedipoda germanica*, an endangered species in Central Europe. We detail several methods to show how several aspects of the reproductive behaviour of this species interact with the conservation of this species. Foreign males were not disfavoured in mate choice and male body size was also unimportant in mating success. Heterogamic matings were as productive as homogamic matings in terms of total number of eggs per female, egg hatching rate, or nymph survival. Therefore, we suggest that cross-matings of individuals from different populations do not positively influence population size by heterosis effects nor act they negatively in the form of outbreeding depression. We found that female *O. germanica* were able to store viable sperm for extended periods but egg pods showed a decrease in hatching rate when these females were deprived of additional mating opportunities. Multiple mated females laid more eggs than once-mated females. Hence, females are capable of founding new Populations even after only one mating but their reproductive output may be reduced. Present-day gene flow between populations of *O. germanica* probably does not occur. Consequently, there is an urgent need to pursue habitat management and release programmes that maintain current population sizes of this species. With regard to the reproductive behaviour of the red-winged grasshopper, we suggest that relocation programmes release males and female together and at an early adult stage. Also, because female reproductive output increases with mating activity, the initial release should involve excess females. Because males have it shorter lifespan than females a subsequent release of males at a later time may enhance the overall success of local conservation efforts by resulting in more offspring per female. (C) 2002 Elsevier Science Ltd. All rights reserved.

The origins of postmating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*

Tregenza, T; Pritchard, VL; Butlin, RK. 2002

Although there are several well-established hypotheses for the origins of postmating isolation during allopatric divergence, there have been very few attempts, to determine their relative importance in nature. We have developed an approach based on knowledge of the differing evolutionary histories of populations within species that allows systematic comparison of the predictions of these hypotheses. In previous work, we have applied this methodology to mating signal variation and premating reproductive isolation between populations of the meadow grasshopper *Chorthippus parallelus*. Here we review the principles behind our approach and report a study measuring postmating isolation in the same set of populations. The populations have known and differing evolutionary histories and relationships resulting from the colonization of northern Europe following the last glaciation. We use a maximum-likelihood analysis to compare the observed pattern of postmating isolation with the predictions of the hypotheses that isolation primarily evolves either as a result of gradual accumulation of mutations in allopatry, or through processes associated with colonization, such as founder events., We also quantify the extent to which degree of postmating isolation can be predicted by genetic distance. Our results suggest that although there is only a weak correlation between genetic distance and postmating isolation, long periods of allopatry do lead to postmating isolation. In contrast to the pattern of premating isolation described in our previous study, colonization does not seem to be associated with increased postmating isolation.

The origins of premating reproductive isolation: Testing hypotheses in the grasshopper *Chorthippus parallelus*

Tregenza, T; Pritchard, VL; Butlin, RK. 2000

There are many proposed routes for the origin of premating reproductive isolation, but few systematic studies aimed at testing their relative importance. Accumulated information about the biogeographical history of the European meadow grasshopper, *Chorthippus parallelus*, has allowed us to make a planned series of comparisons among populations aimed at distinguishing the contributions of some of these hypotheses. We have compared the effects on assortative mating of long-term isolation in glacial refugia, founder events during postglacial colonization, and sympatry with a closely related species. A likelihood-based analysis allowed us to separate effects of variation in male and female mating propensity among populations from variation in mate choice leading to assortative mating. All three effects contributed significantly to the overall variation in mating pattern in a set of 21 pairwise comparisons among seven populations. Male cuticular composition, but not other candidate signals, was significantly associated with the level of assortative mating. Of the hypotheses for the origin of reproductive isolation, only the predictions of the founder hypothesis explained a significant amount of the variation in assortative mating. This does not rule out the possibility that there may be some other explanation. Having established the pattern of divergence, it is possible to generate hypotheses that explain our results at least as well as the founder hypothesis. However, because many such post hoc hypotheses are possible, they cannot be tested with this dataset. On this basis, our results favor the hypothesis that some aspect of the colonization process tends to accelerate divergence in mating signals leading to premating reproductive isolation. This could be accomplished through any one of several mechanisms. Colonization involves many bottlenecks as new populations are established at the edge of the range by long-distance migrants. Genetic effects may be important, but these bottlenecks may also alter the conditions under as suggested by Kaneshiro. At the same time, the colonizing populations may encounter novel environmental challenges.

Assortative preferences and discrimination by females against hybrid male song in the grasshoppers *Chorthippus brunneus* and *Chorthippus jacobsi* (Orthoptera : Acrididae)

Bridle, JR; Saldamando, CI; Koning, W; Butlin, RK. 2006

The grasshoppers *Chorthippus brunneus* and *Chorthippus jacobsi* are highly differentiated for male mating signals, and form a mosaic **hybrid** zone in northern Spain. At some sites within this zone, many **hybrids** are observed. At others, few **hybrids** are observed. Such bimodal sites may reflect recent contacts between parental genotypes, or local variation in levels of assortative mating or selection against **hybrids**. Playback of 12 parental and F1 male songs to 296 parental and **hybrid** females revealed positive assortative preferences in *C. brunneus* and *C. jacobsi* females, supporting a direct role of male mating signals in female choice. However, all female genotypic classes showed reduced responsiveness to F1 male songs. Such sexual selection against **hybrids** is consistent with the narrow cline width observed in the field for song characters relative to morphology. These results have implications for the genetic structure of the **hybrid** zone and for models of speciation by reinforcement.

A discrete neuropeptide difference between two hybridizing grasshopper subspecies

Roth, S; Kohler, G; Reinhardt, K; Predel, R. 2007

Population divergence can be detected by the divergence of functional and neutral characters. Under some circumstances, it is desirable to have available a character that is discretely expressed in either of the diverging genomes, rather than the evaluation of qualitative variation of continuous characters. In the present study, we investigated mass peaks of peptide hormones in a model system of population divergence, the **hybrid** zone of two *Chorthippus parallelus* subspecies in the French-Spanish Pyrenees. Mass spectra from neuroendocrine tissues have previously been identified as species-specific and may have a sufficient resolution to vary at the subspecies level. For the first time, we succeeded in the detection of a subspecies-specific expression of neuropeptides collected from single individuals. Mass

spectra sampled from populations across the *C. parallelus* grasshopper **hybrid** zone indicated neuropeptide identity between the sexes and within sample sites. The distribution of a single distinct but variable peptide signal, however, very closely followed the cline of the **hybrid** zone as derived from the mean variation in several continuous characters. The identity of this peptide in populations from the northern Pyrenees and central Europe supports a neuropeptide differentiation of preglacial origin. The observed differentiation in the peptide profile of the two subspecies demonstrates that a peptidomic approach may be a promising perspective to reconstruct reproductive isolation in an insect **hybrid** zone. (C) 2007 The Linnean Society of London.

Paraphyletic species no more - genomic data resolve a Pleistocene radiation and validate morphological species of the *Melanoplus scudderi* complex (Insecta: Orthoptera)

Huang, JP; Hill, JG; Ortego, J; Knowles, LL. 2020

Rapid speciation events, with taxa generated over a short time period, are among the most investigated biological phenomena. However, molecular systematics often reveals contradictory results compared with morphological/phenotypical diagnoses of species under scenarios of recent and rapid diversification. In this study, we used molecular data from an average of over 29 000 loci per sample from RADseq to reconstruct the diversification history and delimit the species boundary in a short-winged grasshopper species complex (*Melanoplus scudderi* group), where Pleistocene diversification has been hypothesized to generate more than 20 putative species with distinct male genitalic shapes. We found that, based on a maximum likelihood molecular phylogeny, each morphological species indeed forms a monophyletic group, contrary to the result from a previous mitochondrial DNA sequence study. By dating the diversification events, the species complex is estimated to have diversified during the Late Pleistocene, supporting the recent radiation hypothesis. Furthermore, coalescent-based species delimitation analyses provide quantitative support for independent genetic lineages, which corresponds to the morphologically defined species. Our results also showed that male genitalic shape may not be predicted by evolutionary distance among species, not only indicating that this trait is labile, but also implying that selection may play a role in character divergence. Additionally, our findings suggest that the rapid speciation events in this flightless grasshopper complex might be primarily associated with the fragmentation of their grassland habitats during the Late Pleistocene. Collectively, our study highlights the importance of integrating multiple sources of information to delineate species, especially for a species complex that diversified rapidly, and whose divergence may be linked to ecological processes that create geographic isolation (i.e. fragmented habitats), as well as selection acting on characters with direct consequences for reproductive isolation (i.e. genitalic divergence).

Geographic variation in cuticular hydrocarbon profiles in Pacific field crickets

Kota, MV; Heinen-Kay, JL; Zuk, M. 2021

1. Insect cuticular hydrocarbons (CHCs) serve a role in both desiccation resistance and sexual attractiveness. These two functions are thought to be maximised by different CHC compositions, which should lead to local adaptation between populations experiencing different natural and sexual selection pressures.
 2. The Pacific field cricket (*Teleogryllus oceanicus*) is distributed throughout Oceania, including the Hawaiian and Cook Islands. In Hawaii, these crickets face strong natural selection from a parasitoid fly that has promoted the evolution of a novel male reproductive morph, dramatically altering sexual selection in the Hawaiian populations. The parasitoid and male polymorphism do not occur in the Cook Islands.
 3. We captured male and female *T. oceanicus* on two Hawaiian Islands and three Cook Islands, and quantified their CHCs. We also measured temperature and RH at our collection sites to test for associations with cricket CHC profiles.
 4. We discovered significant sexual dimorphism in CHC profiles, as well as differences between both island chains and particular islands. The two male reproductive morphs in Hawaii showed distinct CHC expression. There were no associations between weather variables and CHC profiles.
 5. Observed differences in *T. oceanicus* CHC profiles appear to reflect geographic isolation rather than selection in response to local and environmental and social conditions.
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AN ANALYSIS OF THE BEHAVIORAL-EFFECTS OF CROWDING AND RE-ISOLATION ON SOLITARY-REARED ADULT DESERT LOCUSTS (*SCHISTOCERCA-GREGARIA*) AND THEIR OFFSPRING

BOUAICHI, A; ROESSINGH, P; SIMPSON, SJ. 1995

The time-course of behavioural change in response to crowding and re-isolation was investigated in adults of *Schistocerca gregaria* Forskal (Orthoptera, Acrididae) using logistic regression analysis. Crowding solitary-reared adults for a period of 4 h caused them to behave similarly to crowd-reared insects, with their becoming much more active and moving towards rather than away from a stimulus group of locusts. Responsiveness to crowding was greatest in young adults. The behaviour acquired after 48 h of crowding was lost within 1 day of re-isolation. Although experience by solitary-reared adults of crowding for 48 h had only transitory effects on their own behaviour, there was also a long-term influence on the behaviour of their offspring. The strength of this effect was dependent on the age at which adults experienced crowding, increasing in a graded manner with adult age, and hence the recency of crowding before oviposition. Parents crowded at a late stage in the reproductive cycle yielded hatchlings which behaved indistinguishably from those from crowd-reared adults. Such an effect is consistent with the idea that females, through their previous experience of crowding, are effectively predicting the probability that their offspring will emerge into a high-density population, and predisposing their hatchlings' behaviour accordingly.

Sequence differentiation among inversion rearrangement are revealed by random amplified polymorphic DNA markers in the grasshopper *Trimerotropis pallidipennis* (Orthoptera : Acrididae : Oedipodinae)

Confalonieri, VA; Scataglini, MA; Remis, MI. 2002

South American populations of *Trimerotropis pallidipennis* Burmeister are polymorphic for pericentric inversions. In this species, rearrangement frequencies follow repetitive patterns of distributions along different geographic areas, which are determined by altitude, minimum temperature, and humidity variables. The current article reports the analysis of random amplified polymorphic DNA (RAPDs) in five populations of *T. pallidipennis* and shows the possible relationships of this kind of molecular variability with inversion and enzyme polymorphisms. Both comparisons of RAPDs versus chromosome and RAPDs versus enzyme genetic distances yielded significant results, indicating that the degree of differentiation between pairs of populations is significantly similar when all three types of variability are compared with each other. Specifically, the differentiation for loci amplified by some of the primers is significantly correlated with the differentiation in humidity conditions. Therefore, RAPD loci frequencies tend to follow similar patterns of variations as chromosome and enzymatic variability. Inversions are of evolutionary significance because they can generate supergenes that preserve special genetic sequences. A population of *T. pallidipennis* at high altitudes, which is monomorphic for basic sequences at all chromosomes, will not have the same allele sequence as a population at low altitudes with fixed inverted chromosomes. Because all seven inversions involve a good portion of the genome, a variation between supergenes that differentiate populations is expected to be observed when many random fragments of DNA are amplified, because an eventual recognition sequence of the primers sited into the inversion may be altered. The significance of the congruence between enzymatic and RAPD variability is also discussed.

A comparative study on the song and morphology of *Isophya stysi* Cejchan, 1958 and *Isophya modestior* Brunner von Wattenwyl, 1882 (Orthoptera, Tettigoniidae)

Orci, KM; Nagy, B; Szovenyi, G; Racz, IA; Varga, Z. 2005

We studied the song and morphology of *Isophya stysi* Cejchan, 1958, and *Isophya modestior* Brunner von Wattenwyl, 1882, two closely related bush-cricket species, treated as endangered in Hungary. Our main goals were to find song and morphometric characters that can be used reliably for the identification of specimens and to present comparative results that help us to see the relationship of the two taxa more clearly. We have found that the syllables of *Isophya stysi* always begin with 1-5 slowly repeated, distinct pulses, while in *I. modestior*, pulse-repetition rate was evenly high throughout the whole main pulse series of the syllable. Discriminant analysis showed that on the basis of their morphology, all the examined male specimens can be classified correctly to their song-based identification; furthermore, the arrangement pattern of stridulatory pegs also differed for the two species. Our results confirm that the two taxa are best treated as specifically distinct as they are distinguishable and the observed song differences may well be able to maintain reproductive isolation between them. We provide classification functions (based on four morphometric characters) that can be used confidently for the identification of males; however, we could not find any reliable method for identifying females from their morphology. Our results suggest that within *I. stysi* the population of the central-Transylvanian mountain range differs from the others by producing a higher number of pulses per syllable and having more stridulatory pegs and less elongated left elytron. (C) 2005 Elsevier GmbH. All rights reserved.

A comparison of five hybrid zones of the weta *Hemideina thoracica* (Orthoptera : Anostomatidae): Degree of cytogenetic differentiation fails to predict zone width

Morgan-Richards, M; Wallis, GP. 2003

Tension zones are maintained by the interaction between selection against **hybrids** and dispersal of individuals. Investigating multiple **hybrid** zones within a single species provides the opportunity to examine differences in zone structure on a background of differences in extrinsic factors (e.g., age of the zone, ecology) or intrinsic factors (e.g., chromosomes). The New Zealand tree weta *Hemideina thoracica* comprises at least eight distinct chromosomal races with diploid numbers ranging from $2n = 11$ (XO) to $2n = 23$ (XO). Five independent **hybrid** zones were located that involve races differing from one another by a variety of chromosomal rearrangements. The predicted negative correlation between extent of karyotypic differentiation (measured in terms of both percent of genome and number of rearrangements) and zone width was not found. Conversely, the widest zones were those characterized by two chromosome rearrangements involving up to 35% of the genome. The narrowest zone occurred where the two races differ by a single chromosome rearrangement involving approximately 2% of the genome. The five estimates of chromosomal cline width ranged from 0.5 km to 47 km. A comparative investigation of cline width for both chromosomal and mitochondrial markers revealed a complex pattern of zone characteristics. Three of the five zones in this study showed cline concordance for the nuclear and cytoplasmic markers, and at two of the zones the clines were also coincident. Zones with the widest chromosomal clines had the widest mitochondrial DNA clines. It appears that, even within a single species, the extent of karyotypic differentiation between pairs of races is not a good predictor of the level of disadvantage suffered by **hybrids**.

Transitions in cuticular composition across a hybrid zone: historical accident or environmental adaptation?

Buckley, SH; Tregenza, T; Butlin, RK. 2003

Two subspecies of the grasshopper *Chorthippus parallelus* form a **hybrid** zone in the Pyrenees. Transitions across the zone, including changes in mating signals and reproductive isolation have been intensively studied. Cuticular pheromones have been identified as likely mate recognition signals. Since the major role of the cuticle is in waterproofing, environmental adaptation of cuticular composition has the interesting potential to generate assortative mating as an incidental by-product. We describe the pattern of variation in cuticular hydrocarbon blend in four transects through the **hybrid** zone. We find no evidence for a previously observed displaced cline in one blend component. There were differences between subspecies but these varied among transects and were small compared with variation between transects. We examined environmental variation within one transect and found a correlation between vegetation and cuticular composition, suggesting that environment influences the constitution of the cuticle, and hence natural selection may interact with mating signals in this species. (C) 2003 The Linnean Society of London.

Parental crowding influences life-history traits in *Locusta migratoria* females

Chapuis, MP; Crespin, L; Estoup, A; Auge-Sabatier, A; Foucart, A; Lecoq, M; Michalakakis, Y. 2010

Parental environments could play an important role in controlling insect Outbreaks, provided they influence changes in physiological, developmental or behavioural life-history traits related to fluctuations in population density. However, the potential implication of parental influence in density-related changes in life-history traits remains unclear in man), insects that exhibit fluctuating Population dynamics, particularly locusts. In this study, we report a laboratory experiment, which enabled us to characterize the life-history trait modifications induced by parental crowding of female individuals from a frequently outbreaking population of *Locusta migratoria* (Linnaeus) (Orthoptera: Acrididae). We found that a rearing history of crowding led to reduced female oviposition times and increased offspring size but did not affect the developmental time, survival, fecundity, and the sex-ratio and the number of offspring. Because all Studied females were raised in a common environment (isolation conditions), these observed reproductive differences are due to trans-generational effects induced by density. We discuss the ecological and evolutionary implications of the observed density-dependent parental effects on the life-history of *L. migratoria*.

Temperature effects on the temporal properties of calling songs in the crickets *Gryllus fultoni* and *G. Vernalis*: Implications for reproductive isolation in sympatric populations

Jang, Y; Gerhardt, HC. 2007

Two closely related wood-cricket species, *Gryllus fultoni* (Orthoptera: Gryllidae) and *G. vernalis*, produce similar calling songs, consisting of 3-pulse chirps. Analysis of field and laboratory recordings of calling songs showed that, after correction to a common temperature, there was a divergence in chirp and pulse rates between far allopatric populations of *G. fultoni* and populations sympatric with *G. vernalis*. To determine whether the divergence in calling songs potentially provides reproductive isolation between *G. fultoni* and *G. vernalis* throughout the temperature range over which these insects sing, we recorded calling songs of lab-reared *G. fultoni* and *G. vernalis* populations between 18 and 28 degrees C. Mean chirp rate significantly differed between sympatric and far allopatric *G. fultoni* populations as well as between sympatric *G. fultoni* and *G. vernalis* populations. Although there was a significant difference in mean pulse rate between sympatric *G. fultoni* and *G. vernalis* populations, pulse rate did not differ between sympatric and far allopatric *G. fultoni* populations in the laboratory study. Considering the magnitudes of differences in calling song characters discriminated by females of *G. fultoni* and the mean differences and the variability in calling song characters between the two species, the joint difference in chirp and pulse rates may be adequate for species discrimination over most of the range at which these crickets breed.

New data on acoustic communication and ecology of grasshoppers of the genera *Eremippus* and *Dociostaurus* (Orthoptera, Acrididae) and notes on the use of bioacoustic data in supraspecific taxonomy of the subfamily Gomphocerinae

Savitsky, VY. 2007

The calling songs of *Dociostaurus hauensteini*, *Eremippus simplex*, *E. miramae*, and *E. sobolevi* are described for the first time. The species composition and ecology of grasshoppers of the genera *Eremippus* from the Lower Volga River basin were studied. In this region, the reproductive isolation between *Eremippus* species is shown to be provided principally by the differences in the acoustic behavior of the species. The calling songs were compared in five *Eremippus* species. According to their amplitude-temporal patterns, the calling songs were divided into three groups. The importance of bioacoustic data for supraspecific taxonomy of the subfamily

Climatic effects on population declines of a rare wetland species and the role of spatial and temporal isolation as barriers to hybridization

Rohde, K; Hau, Y; Kranz, N; Weinberger, J; Elle, O; Hochkirch, A. 2017

Climate change and climatic extremes may affect species directly or indirectly. While direct climatic effects have been intensively studied, indirect effects, such as increasing **hybridization** risk, are poorly understood. The goal of our study was to analyse the impact of climate on population dynamics of a rare habitat specialist, *Chorthippus montanus*, as well as the fine-scale spatial overlap with a sympatric habitat generalist, *Chorthippus parallelus* and the dispersion of **hybrids**. We were particularly interested in the role of spatiotemporal overlap on heterospecific encounter frequencies. We conducted high-precision mark-recapture studies on two sites over 7 years and genotyped 702 individuals of two *C. montanus* generations to detect **hybrids**. We tested the performance of three programs (structure, newhybrids and adegenet) and accepted only **hybrids** detected by the two best performing programs. We then tested for correlations between yearly population trends and climatic variables. Furthermore, we analysed the spatial dispersion of both taxa and the **hybrids** to calculate variation in spatial and temporal overlap and infer heterospecific encounter probabilities. Our results revealed that droughts during the egg phase and rainy weather during nymphal development were strongly correlated with population declines in the habitat specialist. The highest **hybridization** rate (196%) was found in the population with lowest population size. The combined effects of spatial and temporal niche overlap decreased heterospecific encounter probabilities to 42-76% compared to 20-28% and 11-19% calculated alone from phenology or spatial overlap respectively. **Hybrids** were detected in areas of higher heterospecific encounter probability, mainly at the edge of the specialists' occupied habitat in areas with intermediate soil moisture conditions compared to the parental species. This illustrates that the combination of spatial and temporal segregation provides an effective barrier to **hybridization**. However, the high **hybridization** rate in one of the populations suggests that this function may decrease with decreasing population size. This supports the hypothesis that climatic extremes threaten rare species directly by reducing reproductive success and may indirectly increase **hybridization** risk. A is available for this article.

The roles of geography, climate and sexual selection in driving divergence among insect populations on mountaintops

Pato, J; Illera, JC; Obeso, JR; Laiolo, P. 2019

Aim Analysing the drivers of intraspecific variation and how reproductive barriers arise is an essential step to infer the mechanisms of biogeographic differentiation. In populations of a specialized alpine species, we explore the role of geography and climate in the divergence of genetic, morphological and acoustic characters, and analyse the functional consequences of variation on mate choice. Taxon *Chorthippus cazurroi* (Orthoptera: Caelifera, Acrididae, Gomphocerinae). Location The entire distribution of the species (23 populations from six massifs of the Cantabrian Mountains, NW Spain). Methods First, we analysed the extent of intraspecific spatial divergence and the covariation among climatic niche, genetic (mtDNA), acoustic (song structure) and morphological (body size) traits. Then, we analysed the consequences of phenotypic variation by means of a crossing experiment among populations from different elevations. This served to test for differences in sexual selection among body size-divergent populations and for the relationship between male traits, female preference and reproduction. Results Genetic, morphologic and acoustic divergence increased with geographic distance. Female morphology was also affected by climate variation, while male one tightly covaried with the song differentiation. Females more closely approached males investing more time in song activities, but weakly responded to the rest of acoustic features and morphological variation. They also distanced themselves slightly more from males from different populations, although this behaviour did not lead to clear differences in reproductive parameters. Main conclusions The process of colonization of mountain massifs has led to significant genetic and phenotypic changes in *C. cazurroi*. Phenotypic divergence does not constitute a strong intrinsic barrier to reproduction and is largely unpaired from female preference, overall suggesting that sexual selection is a minor actor in the process of differentiation as compared, for instance, to drift. This does not exclude that traits associated with individual condition are under strong selection and, therefore, do not vary so extensively. This study dismisses the idea that alpine specialists with narrow distributions lack genetic and phenotypic variability, and highlights the importance of synthesizing biogeographic and experimental approaches to obtain stronger and deeper inferences about the dynamics and mechanisms of biological differentiation.

Patterns of mating between two closely related ground crickets are not influenced by sympatry

Gregory, PG; Remmenga, MD; Howard, DJ. 1998

Allonemobius fasciatus (De Geer) and *A. socius* (Scudder) (Orthoptera: Gryllidae) are small ground-dwelling crickets that meet and **hybridize**, to a limited extent, in a mosaic contact zone stretching from New Jersey to at least as far west as Illinois. To determine whether short-range signals such as visual cues, tactile stimulation, or pheromones are important in limiting gene flow between *A. fasciatus* and *A. socius* we carried out a study in the laboratory. A male or a female was placed in a cage with an *A. fasciatus* and *A. socius* individual of the opposite sex and the outcome, a conspecific or a heterospecific mating, was recorded. Overall, conspecific matings occurred more frequently than heterospecific matings. However, individuals from areas of sympatry were no more likely to mate with a conspecific than were individuals from areas of allopatry. Thus, the challenge posed by a related species has not resulted in a strengthening of positive assortative mating in these crickets.

Genetic, morphological, and dietary changes associated with novel habitat colonisation in the Canary Island endemic grasshopper *Acrostira bellamyi*

Hernandez-Teixidor, D; Lopez, H; Nogales, M; Emerson, BC; Juan, C; Oromi, P. 2014

1. The large flightless grasshopper *Acrostira bellamyi* Uvarov, endemic to the island of La Gomera (Canary Islands), inhabits two different environments: the xeric euphorb shrubland, as is typical for congeneric Canarian species, and the humid laurel forest, a novel habitat for the genus. 2. We investigate genetic, morphological, and ecological variation among individuals of *A. bellamyi* from the two habitats. DNA sequence data were used to evaluate whether grasshoppers from the two environments represent distinct lineages. Morphological and trophic analyses were performed to assess phenotypic differentiation between the two different habitats. 3. Population genetic analyses support the hypothesis that the euphorb shrubland is the ancestral habitat for this species. Female laurel forest specimens are larger than those inhabiting the euphorb shrubland, and some external body parts exhibit significant morphometric differences between the two populations. Diet of shrubland individuals is completely different from that of laurel forest individuals. Although in each habitat they consume the most abundant plants, individuals are able to select food plants, which appear to be explained by their nutrient content. 4. Our results suggest that *A. bellamyi* has colonised laurel forest from shrubland, and that this habitat shift has resulted in genetic, morphological, and ecological changes, perhaps as an adaptation to this new habitat.

Patterns of trait divergence between populations of the meadow grasshopper, *Chorthippus parallelus*

Tregenza, T; Pritchard, VL; Butlin, RK. 2000

To understand the process of speciation, we need to identify the evolutionary phenomena associated with divergence between populations of the same species. A powerful approach is to compare patterns of trait differences between populations differing in their evolutionary histories. A recent study of genetic divergence between populations of the meadow grasshopper *Chorthippus parallelus*, from different locations around Europe has allowed us to use this species to investigate which aspects of evolutionary history are associated with divergence in morphology and mating signals. During the last glaciation *C. parallelus* was confined to a

number of refugia in southern Europe and has subsequently recolonized the northern part of the continent. This process of isolation followed by range expansion has created populations differing markedly in their evolutionary pasts—some have been isolated from one another for thousands of years, others have undergone repeated founder events, and others now live in sympatry with a closely related species. Using laboratory-reared grasshoppers from 12 different populations with a range of evolutionary histories, we quantify differences in morphology, chemical signals, and male calling-song. The observed pattern of divergence between these populations is then compared with the pattern predicted by hypotheses about what drives divergence. This comparison reveals that long periods in allopatry and processes associated with repeated founder events are both strongly associated with divergence.

Phenotypic disparity in Iberian short-horned grasshoppers (Acrididae): the role of ecology and phylogeny

Garcia-Navas, V; Nogueras, V; Cordero, P.J; Ortego, J. 2017

Background: The combination of model-based comparative techniques, disparity analyses and ecomorphological correlations constitutes a powerful method to gain insight into the evolutionary mechanisms that shape morphological variation and speciation processes. In this study, we used a time-calibrated phylogeny of 70 Iberian species of short-horned grasshoppers (Acrididae) to test for patterns of morphological disparity in relation to their ecology and phylogenetic history. Specifically, we examined the role of substrate type and level of ecological specialization in driving different aspects of morphological evolution (locomotory traits, chemosensitive organs and cranial morphology) in this recent radiation. Results: We found a bimodal distribution of locomotory attributes corresponding to the two main substrate type guilds (plant vs. ground); plant-perching species tend to exhibit larger wings and thicker femora than those that remain on the ground. This suggests that life form (i.e., substrate type) is an important driving force in the evolution of morphological traits in short-horned grasshoppers, irrespective of ancestry. Substrate type and ecological specialization had no significant influence on head shape, a trait that showed a strong phylogenetic conservatism. Finally, we also found a marginal significant association between the length of antennae and the level of ecological specialization, suggesting that the development of sensory organs may be favored in specialist species. Conclusions: Our results provide evidence that even in taxonomic groups showing limited morphological and ecological disparity, natural selection seems to play a more important role than genetic drift in driving the speciation process. Overall, this study suggests that morphostatic radiations should not necessarily be considered as "non-adaptive" and that the speciation process can bind both adaptive divergence mechanisms and neutral speciation processes related with allopatric and/or reproductive isolation.

Comparative genome scan detects host-related divergent selection in the grasshopper *Hesperotettix viridis*

Apple, J.L; Grace, T; Joern, A; Amand, P.S; Wisely, S.M. 2010

In this study, we used a comparative genome scan to examine patterns of population differentiation with respect to host plant use in *Hesperotettix viridis*, a Nearctic oligophagous grasshopper locally specialized on various Asteraceae including *Solidago*, *Gutierrezia*, and *Ericameria*. We identified amplified fragment length polymorphism (AFLP) loci with significantly elevated F_{ST} (outlier loci) in multiple different-host and same-host comparisons of populations while controlling for geographic distance. By comparing the number and identities of outlier loci in different-host vs. same-host comparisons, we found evidence of host plant-related divergent selection for some population comparisons (*Solidago*- vs. *Gutierrezia*-feeders), while other comparisons (*Ericameria*- vs. *Gutierrezia*-feeders) failed to demonstrate a strong role for host association in population differentiation. In comparisons of *Solidago*- vs. *Gutierrezia*-feeding populations, a relatively high number of outlier loci observed repeatedly in different-host comparisons (35% of all outliers and 2.7% of all 625 AFLP loci) indicated a significant role for host-related selection in contributing to overall genomic differentiation in this grasshopper. Mitochondrial DNA sequence data revealed a star-shaped phylogeny with no host- or geography-related structure, low nucleotide diversity, and high haplotype diversity, suggesting a recent population expansion. mtDNA data do not suggest a long period of isolation in separate glacial refugia but are instead more compatible with a single glacial refugium and more recent divergence in host use. Our study adds to research documenting heterogeneity in differentiation across the genome as a consequence of divergent natural selection, a phenomenon that may occur as part of the process of ecological speciation.

Components of reproductive isolation between the closely related grasshopper species *Chorthippus biguttulus* and *C. mollis*

Finck, J; Ronacher, B. 2017

The formation and maintenance of species in nature is accomplished by the evolution of reproductive isolating mechanisms. To understand patterns of speciation and coexistence between species, it is crucial to obtain a complete synopsis of reproductive isolating barriers. We identified multiple reproductive isolation barriers between two closely related species, *Chorthippus biguttulus* and *Chorthippus mollis*, and quantified their respective contributions to isolation. To this end, we produced **hybrids** and backcrosses in the laboratory to examine reproductive isolation at multiple stages in the life history. The prezygotic barriers, based on chemical and acoustic cues, are currently the strongest impediments to gene flow between *C. biguttulus* and *C. mollis*. This indicates an important role for sexual selection in the maintenance of species isolation. However, extrinsic and intrinsic postzygotic isolation barriers also contributed to reproductive isolation. The virtual sterility of F₁ **hybrid** males was based on both the dysfunctionality of their testes and intermediate behavioral mating traits (behavioral sterility). This study demonstrates that a cascade of reproductive isolating mechanisms reduces the gene flow between *C. biguttulus* and *C. mollis*. Our results further demonstrate that the courtship display in these species consists of multimodal signals and indicates a key role of chemical cues in reproductive isolation and speciation in grasshoppers.

Combining RNA-seq and proteomic profiling to identify seminal fluid proteins in the migratory grasshopper *Melanoplus sanguinipes* (F)

Bonilla, M.L; Todd, C; Erlandson, M; Andres, J. 2015

Background: Seminal fluid proteins control many aspects of fertilization and in turn, they play a key role in post-mating sexual selection and possibly reproductive isolation. Because effective proteome profiling relies on the availability of high-quality DNA reference databases, our knowledge of these proteins is still largely limited to model organisms with ample genetic resources. New advances in sequencing technology allow for the rapid characterization of transcriptomes at low cost. By combining high throughput RNA-seq and shotgun proteomic profiling, we have characterized the seminal fluid proteins secreted by the primary male accessory gland of the migratory grasshopper (*Melanoplus sanguinipes*), one of the main agricultural pests in central North America. Results: Using RNA sequencing, we characterized the transcripts of similar to 8,100 genes expressed in the long hyaline tubules (LHT) of the accessory glands. Proteomic profiling identified 353 proteins expressed in the long hyaline tubules (LHT). Of special interest are seminal fluid proteins (SFPs), such as EJAC-SP, ACE and prostaglandin synthetases, which are known to regulate female oviposition in insects. Conclusions: Our study provides new insights into the proteomic components of male ejaculate in Orthopterans, and highlights several important patterns. First, the presence of proteins that lack predicted classical secretory tags in accessory gland proteomes is common in male accessory glands. Second, the products of a few highly expressed genes dominate the accessory gland secretions. Third, accessory gland transcriptomes are enriched for novel transcripts. Fourth, there is conservation of SFPs' functional classes across distantly related taxonomic groups with very different life histories, mating systems and sperm transferring mechanisms. The identified SFPs may serve as targets of future efforts to develop species specific genetic control strategies.

Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America

Knowles, L.L. 2000

There has been a resurgence of debate on whether the Pleistocene glaciations inhibited speciation. This study tests a model of Pleistocene speciation, estimating the phylogenetic relationships and divergence times of 10 species of montane grasshoppers, genus *Melanoplus*, using 1300 bp of the mitochondrial gene cytochrome oxidase I (COI). Based on average pairwise distances (corrected for multiple substitutions using Kimura's two-parameter model), all species appear to have originated within the Pleistocene. Sequence divergences between species are less than 4%, corresponding to divergence times less than 1.7 million years ago. Branching patterns among the species suggest that speciation was associated with more than one glacial-interglacial cycle. A likelihood-ratio test rejected a model of simultaneous species origins, the predicted branching pattern if species arose from the fragmentation of a widespread ancestor. These grasshoppers live in an area that was previously glaciated and, as inhabitants of the northern Rocky Mountain sky islands, underwent latitudinal and probably altitudinal shifts in distribution in response to climatic fluctuations. Given the repeated distributional shifts and range overlap of the taxa, there most likely has been ample opportunity for population mixing. However, despite periodic glacial cycles, with more than 10 major glaciations over the past million years and climatic fluctuations over as short a time scale as 10(3) to 10(4) years, the dynamic history of the Pleistocene did not preclude speciation. Although relationships among some taxa remain unresolved, these grasshopper species, even with their recent origins, exhibit genetic coherence and monophyletic or paraphyletic gene trees. The frequency of glacial cycles suggests that the speciation process must have been extremely rapid. These species of grasshoppers are morphologically very similar, differing primarily in the shape of the male genitalia. These characters are posited to be under sexual selection, may play an important role in reproductive isolation, and are known to diverge rapidly. This suggests the rapidity of evolution of reproductive isolation may determine whether species divergences occurred during the Pleistocene glaciations.

Species divergence in field crickets: genetics, song, ecomorphology, and pre- and postzygotic isolation

Gray, DA; Gutierrez, NJ; Chen, TL; Gonzalez, C; Weissman, DB; Cole, JA. 2016

Studies that simultaneously estimate levels of species divergence in genetics, reproductive and ecological traits, and pre- and postzygotic isolation are relatively rare. Here we compare levels of divergence in three allopatric sister species of field crickets. We compare divergence in both nuclear and mitochondrial DNA, male song, female ovipositor length, levels of pre- and postzygotic isolation, and male versus female contributions to prezygotic isolation. Taken together, our data show the accumulation of a multitude of potential reproductive isolating barriers if secondary contact were to become established. Furthermore, ecological and behavioural prezygotic isolation appear significantly more advanced than postzygotic isolation, with prezygotic isolation due to female behaviour exceeding that due to male behaviour. (C) 2015 The Linnean Society of London

A comparative study of aggressiveness in eastern North American field cricket species (genus *Gryllus*)

Jang, Y; Gerhardt, HC; Choe, JC. 2008

To understand the variation in aggressiveness and factors important for contest outcome, we quantified and compared agonistic interactions of four field cricket species in eastern North America: *Gryllus fultoni* (Orthoptera; Gryllidae), *G. vernalis*, *G. pennsylvanicus*, and *G. rubens*. The most aggressive behavior that we observed, the grapple, was frequently displayed in agonistic trials of *G. pennsylvanicus* and *G. rubens*. By contrast, we never observed this behavior in trials involving *G. fultoni* and *G. vernalis*. Consequently, species was the only significant variable affecting the variation in aggressiveness, whereas size difference, age difference, and contest duration had no significant effect. In species with high levels of aggressiveness, *G. pennsylvanicus* and *G. rubens*, the factors that might be indicative of resource holding potential such as size or age difference seemed to be significant for contest outcome. In species with low levels of aggressiveness, *G. fultoni* and *G. vernalis*, however, there were indications that contest outcomes were determined by aggressiveness itself rather than the size and age differences between opponents. Markov chain analyses revealed that the difference in aggressiveness between species with high and low levels of aggressiveness lay in a sequence of escalating behaviors: antennal fencing, mandible flare, and grapple. The escalated state of aggressiveness characterized by this behavioral sequence in *G. pennsylvanicus* and *G. rubens* seems to be the ancestral state in the North American *Gryllus* phylogeny. We argue that the loss of a tendency to use burrows in *G. fultoni* and *G. vernalis* might be related to low levels of aggressiveness in these species.

A Genome Scan and Linkage Disequilibrium Analysis among Chromosomal Races of the Australian Grasshopper *Vandiemena viatica*

Jackson, B; Kawakami, T; Cooper, S; Galindo, J; Butlin, R. 2012

In the past decade the interest surrounding the role of recombination in speciation has been re-kindled by a new generation of chromosomal speciation models that invoke the recombination-suppression properties of some types of chromosomal rearrangement. A common prediction of recombination-suppression models is that gene exchange between diverging populations will be more restricted in regions of the genome that experience low recombination. We carried out a genome scan of three chromosomal races of the grasshopper *Vandiemena viatica* (Orthoptera: Morabinae), occurring on Kangaroo Island, South Australia, using 1517 AFLP loci, with a view to elucidating the roles that selection and chromosomal variation have played in the formation of these taxa. An analysis of molecular variance demonstrated that chromosomal race accounted for a significant proportion of the genetic variance in the total dataset, which concurred with the findings of an earlier study. Sampling across one previously-identified **hybrid** zone, and the identification of outlier loci between parental races allowed us to establish that, in admixed populations, outlier loci which potentially pre-date the isolation of populations of races on Kangaroo Island exhibit higher levels of linkage disequilibrium with each other than putatively neutral loci. In turn this suggests that they might reside within genomic regions of low recombination, or be closely linked with each other.

Versatile Aggressive Mimicry of Cicadas by an Australian Predatory Katydid

Marshall, DC; Hill, KBR. 2009

Background: In aggressive mimicry, a predator or parasite imitates a signal of another species in order to exploit the recipient of the signal. Some of the most remarkable examples of aggressive mimicry involve exploitation of a complex signal-response system by an unrelated predator species. Methodology/Principal Findings: We have found that predatory *Chlorobalius leucoviridis* katydids (Orthoptera: Tettigoniidae) can attract male cicadas (Hemiptera: Cicadidae) by imitating the species-specific wing-flick replies of sexually receptive female cicadas. This aggressive mimicry is accomplished both acoustically, with tegmental clicks, and visually, with synchronized body jerks. Remarkably, the katydids respond effectively to a variety of complex, species-specific Cicadettini songs, including songs of many cicada species that the predator has never encountered. Conclusions/Significance: We propose that the versatility of aggressive mimicry in *C. leucoviridis* is accomplished by exploiting general design elements common to the songs of many acoustically signaling insects that use duets in pair-formation. Consideration of the mechanism of versatile mimicry in *C. leucoviridis* may illuminate processes driving the evolution of insect acoustic signals, which play a central role in reproductive isolation of populations and the formation of species.

Diverse reproductive barriers in hybridising crickets suggests extensive variation in the evolution and maintenance of isolation

Veen, T; Faulks, J; Tyler, F; Lloyd, J; Tregenza, T. 2013

Reproductive barriers reduce gene flow between populations and maintain species identities. A diversity of barriers exist, acting before, during and after mating. To understand speciation and coexistence, these barriers need to be quantified and their potential interactions revealed. We use the **hybridising** field crickets *Gryllus bimaculatus* and *G. campestris* as a model to understand the full complement and relative strength of reproductive barriers. We find that males of both species prefer

conspecific females, but the effect is probably too weak to represent a barrier. In contrast, prezygotic barriers caused by females being more attracted to conspecific male song and preferentially mounting and mating with conspecifics are strong and asymmetric. Postzygotic barriers vary in direction; reductions in fecundity and egg viability create selection against **hybridisation**, but **hybrids** live longer than pure-bred individuals. **Hybrid** females show a strong preference for *G. bimaculatus* songs, which together with a complete lack of **hybridisation** by *G. campestris* females, suggests that asymmetric gene flow is likely. For comparison, we review reproductive barriers that have been identified between other Gryllids and conclude that multiple barriers are common. Different species pairs are separated by qualitatively different combinations of barriers, suggesting that reproductive isolation and even the process of speciation itself may vary widely even within closely related groups.

Life-history adaptation and reproductive isolation in a grasshopper hybrid zone

Orr, MR. 1996

Patterns of life-history adaptation and reproductive isolation were investigated in the acridid grasshoppers *Melanoplus sanguinipes* and *M. devastator*, which **hybridize** along an altitudinal gradient in the Sierra Nevada of California. *Melanoplus sanguinipes* females crossed with *M. devastator* males produced eggs that were approximately half as viable as eggs from other crosses. Diminished viability was not attributable either to infection by *Wolbachia pipiensis* or to failure of sperm transfer. When offered an opportunity to choose a mate, females from all populations discriminated against males of the other species, whereas in no-choice tests measuring copulation duration only females from the tails of the dunes showed preferences. *Melanoplus sanguinipes*, found at high elevations where the growing season is short, exhibited faster egg hatch, faster larval development, smaller adult body sizes, and smaller clutch sizes than *M. devastator*. *Melanoplus devastator*, from California's Central Valley, endured a hot and dry summer in a reproductive diapause that was absent in *M. sanguinipes*. Clines in reproductive diapause and clutch size coincided with the region of reproductive incompatibility. Development time, body size, and hatch time also changed across the **hybrid** zone, but the regions of largest transitions in these traits were either difficult to locate using the limited populations studied here or were not coincident with the zone's center. A method is described for combining ecological and phylogenetic analyses to address the unknown issue of whether life-history divergence has contributed to reproductive isolation in this system.

Multiple barriers to gene exchange in a field cricket hybrid zone

Maroja, LS; Andres, JA; Walters, JR; Harrison, RG. 2009

Data on patterns of variation within **hybrid** zones, combined with studies of life history, mate choice, and **hybrid** performance, allow estimates of the contribution of different pre-zygotic and post-zygotic barriers to reproductive isolation. We examine the role of behavioural barriers to gene exchange in the maintenance of a **hybrid** zone between North American field crickets *Gryllus firmus* and *Gryllus pennsylvanicus*. We consider these barriers in the context of previous studies that documented temporal and ecological isolation and a one-way post-mating incompatibility (i.e. *G. firmus* females do not produce offspring when they mate only with heterospecific males). Based on no-choice mating experiments in the laboratory, we demonstrate strong behavioural pre-mating barriers between the two species, but no apparent fecundity or fertility costs for *G. firmus* females when they mate with both conspecific and heterospecific males. Furthermore, we show that *G. firmus* females do not discriminate between **hybrids** and conspecifics, whereas *G. pennsylvanicus* females do. This observation could explain the asymmetric allele introgression observed in the **hybrid** zone. We also document a failure of heterospecific males to induce normal oviposition in *G. firmus* females, which may be due to rapid evolution of accessory gland proteins and may serve as an additional barrier to gene exchange. (C) 2009 The Linnean Society of London, Biological Journal of the Linnean Society, 2009, 97, 390-402.

Premating Reproductive Barriers between Hybridising Cricket Species Differing in Their Degree of Polyandry

Veen, T; Faulks, J; Rodriguez-Munoz, R; Tregenza, T. 2011

Understanding speciation hinges on understanding how reproductive barriers arise between incompletely isolated populations. Despite their crucial role in speciation, prezygotic barriers are relatively poorly understood and hard to predict. We use two closely related cricket species, *Gryllus bimaculatus* and *G. campestris*, to experimentally investigate premating barriers during three sequential mate choice steps. Furthermore, we experimentally show a significant difference in polyandry levels between the two species and subsequently test the hypothesis that females of the more polyandrous species, *G. bimaculatus*, will be less discriminating against heterospecific males and hence **hybridise** more readily. During close-range mating behaviour experiments, males showed relatively weak species discrimination but females discriminated very strongly. In line with our predictions, this discrimination is asymmetric, with the more polyandrous *G. bimaculatus* mating heterospecifically and *G. campestris* females never mating heterospecifically. Our study shows clear differences in the strength of reproductive isolation during the mate choice process depending on sex and species, which may have important consequences for the evolution of reproductive barriers.

The genus *Drosophila* is characterized by a large number of sibling species showing evolutionary significance

Singh, BN. NA

Mayr (1942) defined sibling species as sympatric forms which are morphologically very similar or indistinguishable, but which possess specific biological characteristics and are reproductively isolated. Another term, cryptic species has also been used for such species. However, this concept changed later. Sibling species are as similar as twins. This category does not necessarily include phylogenetic siblings as members of a superspecies. Since the term sibling species was defined by Mayr, a large number of cases of sibling species pairs/groups have been reported and thus they are widespread in the animal kingdom. However, they seem to be more common in some groups such as insects. In insects, they have been reported in diptera, lepidoptera, coleoptera, orthoptera, hymenoptera and others. Sibling species are widespread among the dipteran insects and as such are well studied because some species are important medically (mosquitoes), genetically (*Drosophila*) and cytologically (*Sciara* and *Chironomus*). The well-studied classical pairs of sibling species in *Drosophila* are: *D. pseudoobscura* and *D. persimilis*, and *D. melanogaster* and *D. simulans*. Subsequently, a number of sibling species have been added to these pairs and a large number of other sibling species pairs/groups in different species groups of the genus *Drosophila* have been reported in literature. The present review briefly summarizes the cases of sibling species pairs/groups in the genus *Drosophila* with their evolutionary significance.

Genomic footprints of an old affair: Single nucleotide polymorphism data reveal historical hybridization and the subsequent evolution of reproductive barriers in two recently diverged grasshoppers with partly overlapping distributions

Tonzo, V; Papadopoulou, A; Ortego, J. 2020

Secondary contact in close relatives can result in **hybridization** and the admixture of previously isolated gene pools. However, after an initial period of **hybridization**, reproductive isolation can evolve through different processes and lead to the interruption of gene flow and the completion of the speciation process. *Omocestus minutissimus* and *O. uhagonii* are two closely related grasshoppers with partially overlapping distributions in the Central System mountains of the Iberian Peninsula. To analyse spatial patterns of historical and/or contemporary **hybridization** between these two taxa and understand how species boundaries are maintained in the region of secondary contact, we sampled sympatric and allopatric populations of the two species and obtained genome-wide single nucleotide polymorphism data using a restriction site-associated DNA sequencing approach. We used Bayesian clustering analyses to test the hypothesis of contemporary **hybridization** in sympatric

populations and employed a suite of phylogenomic approaches and a coalescent-based simulation framework to evaluate alternative hypothetical scenarios of interspecific gene flow. Our analyses rejected the hypothesis of contemporary **hybridization** but revealed past introgression in the area where the distributions of the two species overlap. Overall, these results point to a scenario of historical gene flow after secondary contact followed by the evolution of reproductive isolation that currently prevents **hybridization** among sympatric populations.

THE ROLE OF COURTSHIP SONGS IN REPRODUCTIVE ISOLATION AMONG POPULATIONS OF GREEN LACEWINGS OF THE GENUS *CHRYSOPELRA* (NEUROPTERA, CHRYSOPIDAE)

WELLS, MM; HENRY, CS. 1992

Male and female lacewings tremulate during courtship, establishing duets that always precede copulation. Three distinct courtship songs are found in populations of the green lacewing *Chrysoperla plorabunda* (P1, P2 and P3 song morphs). Analysis of five features of the songs for individuals collected from Connecticut, Idaho, Oregon and California showed few differences within song morphs, but sympatric song morphs differed significantly in temporal features of the songs and their mode of presentation. Playback experiments using recorded songs were performed on females with all possible sympatric and allopatric combinations of females and recorded songs. The results showed that females strongly prefer to duet with recordings of males of their own song type and usually showed no responses to songs of other types. Thus, song differences are effective barriers to reproduction between the sympatric morphs. Our results support the hypothesis that the three song morphs are true biological species.

Tegmina-size variation in a Neotropical cricket with implications on spectral song properties

Lima, RM; Schuchmann, KL; Tissiani, AS; Nunes, LA; Jahn, O; Ganchev, TD; Lhano, MG; Marques, MI. 2018

This study evaluates the relationship between shape and size of tegmen, harp, mirror, and spectral range of calling song frequency of a Neotropical cricket subpopulation (*Lerneca inalata* beripocone). In addition, we compare intraspecific morphological divergence and calling song properties between individuals from different sites of the Pantanal of Pocone, Mato Grosso, Brazil. Regression analysis showed that the dominant and maximum calling song frequencies were negatively correlated with tegmen size, i.e. frequencies are either lower or higher depending on the corresponding size variation in resonance structures of the forewings. Canonical variable analysis demonstrated marked intraspecific differences in morphometric characters between localities of a *L. inalata* subpopulation c. 35km apart (SESC-Pantanal Advanced Research Base and Pouso Alegre Farm, Mato Grosso, Brazil). *Lerneca inalata* beripocone at SESC had larger forewings than conspecifics from Pouso Alegre Farm. These morphological variations of wing properties related to reproductive behaviours were interpreted as fitness parameters, likely shaped by restricted gene flow during temporal habitat isolation episodes. Such isolation patterns occur in the Pantanal wetlands for several months during the annual hydrological cycle.

Spatio-temporal dynamics of the *Allonemobius fasciatus*-*A. socius* mosaic hybrid zone: a 14-year perspective

Britch, SC; Cain, ML; Howard, DJ. 2001

Long-term studies of **hybrid** zones can provide valuable insight into a number of questions that have long attracted the attention of evolutionists. These questions range from the stability and fate of **hybrid** zones to the relative fitness of **hybrids**. In this paper we report the results of a 14-year survey of the *Allonemobius fasciatus*-*Allonemobius socius* **hybrid** zone. Populations were collected intensively in 1986 and 1987 and then more sporadically through the end of the 1980s and throughout the 1990s. By documenting changes in the genetic composition of populations near and within the zone during this period of time we assessed: the strength of the reproductive isolation between the two species; the relative growth rates (which can be considered a surrogate of relative fitness) of genotype classes corresponding to **hybrids** and to pure species individuals; and, the power of single-year and multi-year measurements of relative growth rates to predict changes in the genetic composition of mixed populations through time. In brief, we found very large year-to-year variation in the relative growth rates of pure species and **hybrid** individuals. This variation may reflect the fact that both species are at the edge of their range and perhaps at the limits of their ability to deal with environmental perturbations. As a consequence of the variation, even multi-year estimates of relative growth rates often provided imprecise predictions regarding the future genotypic composition of mixed populations. Despite our limited ability to predict the dynamics of individual populations, some trends are apparent. *A. socius*, the southern species, has clearly increased in frequency along a transect through the Appalachian Mountains, indicating that the zone is moving north in this region. In contrast, the zone appeared to be more stable along the East Coast transect. Within mixed populations, character-index profiles are often bimodal and stable through time, indicating relatively strong reproductive isolation between the two species that is not being reinforced, nor is it breaking down.

Behavioral sterility of hybrid males in acoustically communicating grasshoppers (Acrididae, Gomphocerinae)

Gottsberger, B; Mayer, F. 2007

The effectiveness of **hybridization** barriers determines whether two species remain reproductively isolated when their populations come into contact. We investigated acoustic mating signals and associated leg movements responsible for song creation of **hybrids** between the grasshopper species *Chorthippus biguttulus* and *C. brunneus* to study whether and how songs of male **hybrids** contribute to reproductive isolation between these sympatrically occurring species. Songs of F1, F2, and backcross **hybrids** were intermediate between those of both parental species in terms phrase number and duration. In contrast, species-specific syllable structure within phrases was largely lost in **hybrids** and was produced, if at all, in an irregular and imperfect manner. These divergences in inheritance of different song parameters are likely the result of incompatibility of neuronal networks that control stridulatory leg movements in **hybrids**. It is highly probable that songs of **hybrid** males are unattractive to females of either parental species because they are intermediate in terms of phrase duration and lack a clear syllable structure. Males of various **hybrid** types (F1, F2, and backcrosses) are behaviorally sterile because their songs fail to attract mates.

Reproduction barrier between two lineages of bed bug (*Cimex lectularius*) (Heteroptera: Cimicidae)

Wawrocka, K; Balvin, O; Bartonicka, T. 2015

Populations of bed bugs, *Cimex lectularius*, have increased in recent years spreading into numerous urban areas across the Western world and making them an increasingly important pest of the twenty-first century. Research into **hybridization** within and between different lineages of bed bugs can help us to understand processes of micro- and macro-evolution in these ectoparasites and may inform the control of this pest species. **Hybridization** experiments between two host lineages of bed bug (*C. lectularius*) from Central Europe (Czech Republic), those associated with humans and those with bats, were conducted under laboratory conditions. Number of eggs and early instars were compared between crosses of mixed host lineages (interspecific mating) with pairs from the same host lineage, those from the same locality and same lineage from different localities (intraspecific mating). While crosses within host lineages resulted in egg production and later instars, crosses between different host lineages were unsuccessful, although of the mated females possessed sperm in their mesospermales and/or seminal conceptacles. These crosses did not even result in egg production. Moreover, in the mixed lineage crosses, mortality rates in adults were higher (51 and 50 % higher in bat and human lineage, respectively) than in those animals from the same lineage. Survival of adults was in pairs from the same locality slightly higher than in pairs from different localities and differed statistically. These results support the existence of post-mating barriers and show reproductive isolation between two lineages

of *C. lectularius*. Bat and human host adaptations can promote evolving of such barriers and can be product of allolexenic speciation.

Phylogeographic structure without pre-mating barriers: Do habitat fragmentation and low mobility preserve song and chorus diversity in a European bushcricket?

Mahamoud-Issa, M; Marin-Cudraz, T; Party, V; Greenfield, MD. 2017

When animal species have a strong phylogeographic structure questions arise on the origin, maintenance and future evolutionary trajectory of that structure. One prediction is that phenotypic differences among populations serve as pre-mating barriers should secondary contact occur. Post-mating barriers may also function and ensure further separation of the populations. We tested these predictions in an acoustic insect, the European bushcricket *Ephippiger diurnus*, that is geographically distributed in separated, genetically isolated populations distinguished by pronounced differences in male songs and chorusing and female preferences for song. We staged mixed-population choruses to examine how males sing when in the company of other populations, and we released females in these choruses to assess their preference for or aversion toward males of their own population versus a different one. We also paired males and females of the same and different populations to test mating success, spermatophore transfer, and oviposition in the various pairings. In most cases males sang as effectively when accompanied by males from another population as when in a single-population chorus, and females overall expressed little aversion toward males from a different population. Inter-population pairs did not mate less frequently, transfer smaller spermatophores, or deposit fewer or smaller eggs than pairs of males and females from the same population. We infer that pre-mating barriers play little role in maintaining phylogeographic structure despite the acoustic differences between populations. Rather, the structure probably reflects the fragmented distribution of suitable habitat and the low mobility of both juveniles and adults. Thus, if secondary contact does occur, and we predict that it will in several key locations owing to ongoing environmental change, coalescence of populations and reduction of genetic and acoustic diversity may follow.

A statistical approach to understanding reproductive isolation in two sympatric species of tree crickets

Bhattacharya, M; Isvaran, K; Balakrishnan, R. 2017

In acoustically communicating animals, reproductive isolation between sympatric species is usually maintained through species-specific calls. This requires that the receiver be tuned to the conspecific signal. Mapping the response space of the receiver onto the signal space of the conspecific investigates this tuning. A combinatorial approach to investigating the response space is more informative as the influence on the receiver of the interactions between the features is also elucidated. However, most studies have examined individual preference functions rather than the multivariate response space. We studied the maintenance of reproductive isolation between two sympatric tree cricket species (*Oecanthus henryi* and *Oecanthus indicus*) through the temporal features of the calls. Individual response functions were determined experimentally for *O. henryi*, the results from which were combined in a statistical framework to generate a multivariate quantitative receiver response space. The predicted response was higher for the signals of the conspecific than for signals of the sympatric heterospecific, indicating maintenance of reproductive isolation through songs. The model allows prediction of response to untested combinations of temporal features as well as delineation of the evolutionary constraints on the signal space. The model can also be used to predict the response of *O. henryi* to other heterospecific signals, making it a useful tool for the study of the evolution and maintenance of reproductive isolation via long-range acoustic signals.

Sperm numbers vary between inter- and intra-population matings of the grasshopper *Chorthippus parallelus*

Reinhardt, K. 2006

Comparing the reproductive output of intra- and inter-population matings is the most common way to assess whether post-mating reproductive isolation is caused by genetic incompatibilities. Such genetic incompatibility can however, only assume that the quantity of the post-mating signals involved does not differ between intra- and inter-population matings. This assumption may not be true because sexual selection predicts reduced mating effort towards low-quality mates and in many circumstances, allopatric partners are low-quality mates. Post-mating efforts may, therefore, be reduced in inter-compared to intra-population matings. Here, I test this crucial assumption by studying variation in one post-mating trait, sperm number, in crosses of two parapatric grasshopper populations. In both populations, males transferred fewer sperm to allopatric than sympatric females. If such plasticity with respect to population is common in other post-mating traits, differences between inter- and intra-population crosses may be more frequently caused by differences in sperm number rather than gamete incompatibility. Additionally, I found that sperm numbers declined less rapidly in the female storage organ of allopatric than sympatric females but its rate differed markedly between populations. This is discussed with respect to female adaptations to male traits.

Dispersal differences predict population genetic structure in Mormon crickets

Bailey, NW; Gwynne, DT; Ritchie, MG. 2007

Research investigating the geographical context of speciation has primarily focused on abiotic factors such as the role of Pleistocene glacial cycles, or geotectonic events. Few study systems allow a direct comparison of how biological differences, such as dispersal behaviour, affect population genetic structure of organisms that were subdivided during the Pleistocene. Mormon crickets exist in solitary and gregarious 'phases', which broadly correspond with an east-west mtDNA division across the Rocky Mountains. Gregarious individuals form bands that can move up to 2 km daily. This study assessed whether population genetic structure results mainly from deep Pleistocene vicariance or if we can also detect more recent genetic patterns due to phase and dispersal differences superimposed on the older, deeper divisions. We found that separation in refugia was a more important influence on genetic divergence than phase, with the Rockies acting as a barrier that separated Mormon cricket populations into eastern and western refugia during Pleistocene glacial cycles. However, patterns of isolation by distance differ between eastern and western clades for both mitochondrial and nuclear DNA, with greater divergence within the eastern, solitary clade. An mtDNA haplotype mismatch distribution is compatible with historical population expansion in the western clade but not in the eastern clade. A persistent (and possibly sex-biased) difference in dispersal ability has most likely influenced the greater population genetic structure seen in the eastern clade, emphasizing the importance of the interaction of Quaternary climate fluctuations and geography with biotic factors in producing the patterns of genetic subdivision observed today.

Geographic Variation in Advertisement Calls in a Tree Frog Species: Gene Flow and Selection Hypotheses

Jang, Y; Hahm, EH; Lee, HJ; Park, S; Won, YJ; Choe, JC. 2011

Background: In a species with a large distribution relative to its dispersal capacity, geographic variation in traits may be explained by gene flow, selection, or the combined effects of both. Studies of genetic diversity using neutral molecular markers show that patterns of isolation by distance (IBD) or barrier effect may be evident for geographic variation at the molecular level in amphibian species. However, selective factors such as habitat, predator, or interspecific interactions may be critical for geographic variation in sexual traits. We studied geographic variation in advertisement calls in the tree frog *Hyla japonica* to understand patterns of variation in these traits across Korea and provide clues about the underlying forces for variation. Methodology: We recorded calls of *H. japonica* in three breeding seasons from 17 localities including localities in remote Jeju Island. Call characters analyzed were note repetition rate (NRR), note duration (ND), and dominant frequency (DF), along with snout-to-vent length. Results: The findings of a barrier effect on DF and a longitudinal variation in NRR seemed to suggest that an open sea between the mainland and Jeju Island and mountain ranges dominated by the north-south Taebaek Mountains were related to geographic variation in call characters.

Furthermore, there was a pattern of IBD in mitochondrial DNA sequences. However, no comparable pattern of IBD was found between geographic distance and call characters. We also failed to detect any effects of habitat or interspecific interaction on call characters. Conclusions: Geographic variations in call characters as well as mitochondrial DNA sequences were largely stratified by geographic factors such as distance and barriers in Korean populations of *H. japonica*. Although we did not detect effects of habitat or interspecific interaction, some other selective factors such as sexual selection might still be operating on call characters in conjunction with restricted gene flow.

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

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

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

Reinforcement and other consequences of sympatry

Noor, MAF. 1999

The pattern of greater species mating discrimination between sympatric taxa than between allopatric taxa has been attributed to the strengthening of mate discrimination to avoid maladaptive **hybridization**. This process, termed reinforcement, has been highly contentious, particularly with regard to its role in speciation. Here, I review some recent studies of reinforcement, discuss alternative explanations for the pattern of greater species discrimination in sympatric taxa, and point to some new directions that may help to clarify the evolutionary forces involved. In particular, we need more ecological work on putative cases of reinforcement? more theoretical models that give diagnostic predictions of reinforcement relative to other modes of divergence, and empirical studies to evaluate these diagnostic predictions.

SEXUALLY MONOMORPHIC MATING PREFERENCES CONTRIBUTE TO PREMATING ISOLATION BASED ON SONG IN EUROPEAN GREEN LACEWINGS

Noh, S; Henry, CS. NA

When species are in intermediate stages of divergence, complicated patterns of reproductive isolation can arise among males and females of the incipient species. The *Chrysoperla carnea* group of green lacewings (Neuroptera: Chrysopidae) has recently experienced rapid speciation. They possess sexually monomorphic mating signals that were assumed to be important in mate recognition. Our objective was to compare patterns of reproductive isolation among five European species within the species complex as well as sex differences in mating strategies that contribute to those patterns. The species were in fact reproductively isolated based on mating signals. In addition, different species showed different degrees of responsiveness to conspecific signals, and those species that responded more frequently to conspecific signals were less discriminating against heterospecific signals. This suggests differences in mating strategies among species that may be related to population densities and heterospecific encounter rates. Sexual dimorphism in mating preference was apparent only in *C. agilis*, whose males were more responsive to all signals and less discriminating against heterospecific signals compared to females. Thus high variance in male quality may be characteristic of *C. agilis*, but not the remaining four species, whose male versus female mating strategies were similar.

Historical isolation facilitates species radiation by sexual selection: Insights from *Chorthippus* grasshoppers

Nolen, ZJ; Yildirim, B; Irisarri, I; Liu, SL; Crego, CG; Amby, DB; Mayer, F; Gilbert, MTP; Pereira, RJ. 2020

Theoretical and empirical studies have shown that species radiations are facilitated when a trait under divergent natural selection is also involved in sexual selection. It is yet unclear how quick and effective radiations are where assortative mating is unrelated to the ecological environment and primarily results from sexual selection. We address this question using sympatric grasshopper species of the genus *Chorthippus*, which have evolved strong behavioural isolation while lacking noticeable ecomorphological divergence. Mitochondrial genomes suggest that the radiation is relatively recent, dating to the mid-Pleistocene, which leads to extensive incomplete lineage sorting throughout the mitochondrial and nuclear genomes. Nuclear data shows that **hybrids** are absent in sympatric localities but that all species have experienced gene flow, confirming that reproductive isolation is strong but remains incomplete. Demographic modelling is most consistent with a long period of geographic isolation, followed by secondary contact and extensive introgression. Such initial periods of geographic isolation might facilitate the association between male signaling and female preference, permitting the coexistence of sympatric species that are genetically, morphologically, and ecologically similar, but otherwise behave mostly as good biological species.

Does courtship behavior contribute to species-level reproductive isolation in field crickets?

Gray, DA. 2005

Reproductive behavior influences gene flow within and among species; thus, sexual selection may be a major contributor to the maintenance of species, and possibly their formation. Here I experimentally manipulate the courtship interactions of the field crickets *Gryllus rubens* and *G. texensis* to examine the potential of close-range courtship interactions to limit interspecific gene flow. Responses of males to females and of females to male courtship song and males per se were examined for four paired sympatric and allopatric populations. Male *G. rubens* were more likely to court conspecific females, but male *G. texensis* courted females of both species equally. If paired with conspecific males, female *G. rubens* and *G. texensis* both preferred conspecific courtship song. In none of these comparisons were the responses of males or females from allopatry different from those in sympatry. There was an asymmetry of courtship response across both sex and species: male *G. rubens* were more discriminating than male *G. texensis*, whereas female *G. texensis* were more discriminating than female *G. rubens*. Despite significant preferences for conspecifics, the net effect of courtship interactions would appear to limit interspecific gene flow only weakly. These results are consistent with courtship behavior evolving under the influence of sexual selection and only indirectly promoting species integrity.

Asymmetric mating in the brachypterous grasshopper *Podisma sapporensis*

Sugano, YC; Akimoto, S. 2007

Crossing of genetically differentiated populations often results in assortative mating within populations. However, asymmetric sexual isolation or negative assortative mating has occasionally been reported. Previous studies suggested that sexual selection or sexual conflicts would lead to asymmetric mating when local populations are crossed. In order to evaluate the extent of assortative or disassortative mating in population crosses, we conducted laboratory crosses using the flightless grasshopper *Podisma sapporensis*. Crossing was conducted for all pairwise combinations of three populations, 150-240 km from one another - Teine, Shimokawa, and Akan. We found evidence for asymmetric mating for all the pairs of the populations. In particular, when the Teine and Akan populations were crossed, mating in the Teine male-Akan female cross was significantly more frequent than mating in both within-population crosses, whereas mating in the Teine female-Akan male cross was significantly less frequent than mating in both within-population crosses. We examined whether these results can be explained by any of the three hypotheses: (1) Kaneshiro's hypothesis, (2) differentiation in attractiveness, or (3) coevolution between male vigor and female receptivity. All the results were consistent with male vigor differing between populations balanced by different female potential to reject males. The available evidence suggests that antagonistic coevolution between the sexes has led local populations to different equilibria and that crossing of populations at different equilibria has resulted in asymmetry in mating frequencies.

No reproductive character displacement in male advertisement signals of *Hyla japonica* in relation to the sympatric *H. suweonensis*

Park, S; Jeong, G; Jang, Y. 2013

Reproductive interaction between closely related taxa may leave a distinctive signature in which populations of interacting taxa are more dissimilar in sympatry than in allopatry. An ideal condition for such a pattern of reproductive character displacement (RCD) may occur when a population has limited gene flow and experiences strong selection pressure, exerted by an interacting taxon in areas of sympatry. In Korea, there are two closely related treefrog species: *Hyla japonica*, which is distributed widely throughout the country, and *Hyla suweonensis*, which occurs sympatrically in a narrow strip of western coastal plains. *H. suweonensis* is only found within the distribution of *H. japonica*. These two species have a similar single-note call structure. Here, we tested the possibility of RCD in *H. japonica* by examining geographic variation in advertisement calls. Although means of temporal and spectral characters were significantly different between the two species, sympatric populations of *H. japonica* and *H. suweonensis* overlapped in distributions of most characters. Furthermore, allopatric and sympatric *H. japonica* populations did not differ in all call characters. Weak genetic differentiation between sympatric and allopatric populations of *H. japonica* implied either substantial gene flow or recent genetic isolation. Possible explanations for no RCD in male advertisement calls of *H. japonica* include a difference in fine temporal characteristics between the two species, migration between sympatric and allopatric localities in *H. japonica*, RCD in female preferences in *H. japonica*, and weak selection pressure by *H. suweonensis*.

Patterns of male sterility in a grasshopper hybrid zone imply accumulation of hybrid incompatibilities without selection

Shuker, DM; Underwood, K; King, TM; Butlin, RK. 2005

It is now widely accepted that post-zygotic reproductive isolation is the result of negative epistatic interactions between derived alleles fixed independently at different loci in diverging populations (the Dobzhansky-Muller model). What is less clear is the nature of the loci involved and whether the derived alleles increase in frequency through genetic drift, or as a result of natural or sexual selection. If incompatible alleles are fixed by selection, transient polymorphisms will be rare and clines for these alleles will be steep where divergent populations meet. If they evolve by drift, populations are expected to harbour substantial genetic variation in compatibility and alleles will introgress across **hybrid** zones once they recombine onto a genetic background with which they are compatible. Here we show that variation in male sterility in a naturally occurring *Chorthippus parallelus* grasshopper **hybrid** zone conforms to the neutral expectations. Asymmetrical clines for male sterility have long tails of introgression and populations distant from the zone centre show significant genetic variation for compatibility. Our data contrast with recent observations on 'speciation genes' that have diverged as a result of strong natural selection.

Reproductive isolation in the acoustically divergent groups of tettigoniid, *Mecopoda elongata*

Dutta, R; Tregenza, T; Balakrishnan, R. 2017

Sympatric divergent populations of the same species provide an opportunity to study the evolution and maintenance of reproductive isolation. Male mating calls are important in sexual selection in acoustically communicating species, and they also have the potential to maintain isolation among species or incipient species. We studied divergent south Indian populations of the bush cricket *Mecopoda elongata* which are extremely difficult to distinguish morphologically, but which exhibit striking divergence in male acoustic signals. We performed phonotactic experiments investigating the relative preference of females of the "Chirper" song type for calls of all 5 of the song types found in the region (in varying degrees of sympatry). We found that Chirper females preferred their own song type and were completely unresponsive to three trilling song types. Chirper females were occasionally attracted to the call type "Double Chirper" (the call most similar to their own type), suggesting call preference alone cannot provide a complete isolating mechanism. To investigate the basis of call preference we investigated the response of chirper females to variation in chirp rate. Chirper females responded most frequently to a mean chirp rate characteristic of their own song type rather than a higher chirp rate which would be more characteristic of the Double-Chirper song type. This suggests females drive stabilising selection on male chirp rate, which may contribute to the maintenance of isolation. Finally, a no-choice mating experiment using Chirper females and Chirper and Double Chirper males revealed a significant preference of Chirper females to mate with their own song type, even without a requirement for phonotaxis. Overall, the strong specificity of Chirper females for their 'own' song type provides evidence for behavioural isolation among divergent sympatric *Mecopoda* song types being maintained by female preference for both male song type and subsequent mating probability driven by other cues.

Stable inversion clines in a grasshopper species group despite complex geographical history

Guzman, NV; Kempainen, P; Monti, D; Castillo, ERD; Rodriguez, MS; Sanchez-Restrepo, AF; Cigliano, MM; Confalonieri, VA. 2022

Chromosomal inversions are known to play roles in adaptation and differentiation in many species. They involve clusters of correlated genes (i.e., loci in linkage disequilibrium, LD) possibly associated with environmental variables. The grasshopper "species complex" *Trimerotropis pallidipennis* comprises several genetic lineages distributed from North to South America in arid and semi-arid high-altitude environments. The southernmost lineage, *Trimerotropis* sp., segregates for four to seven putative inversions that display clinal variation, possibly through adaptation to temperate environments. We analysed chromosomal, mitochondrial and genome-wide single nucleotide polymorphism data in 19 *Trimerotropis* sp. populations mainly distributed along two altitudinal gradients (MS and Ju). Populations across Argentina comprise two main chromosomally and genetically differentiated lineages: one distributed across the southernmost border of the "Andes Centrales," adding evidence for a differentiation hotspot in this area; and the other widely distributed in Argentina. Within the latter, network analytical approaches to LD found three clusters of correlated loci (LD-clusters), with inversion karyotypes explaining >79% of the genetic variation. Outlier loci associated with environmental variables mapped to two of these LD-clusters. Furthermore, despite the complex geographical history indicated by population genetic analyses, the clines in inversion karyotypes have remained stable for more than 20 generations, implicating their role in adaptation and differentiation within this lineage. We hypothesize that these clines could be the consequence of a coupling between extrinsic postzygotic barriers and spatially varying selection along environmental gradients resulting in a **hybrid** zone. These results provide a framework for future investigations about candidate genes implicated in rapid adaptation to new environments.

The genus *Drosophila* as a model for testing tree- and character-based methods of species identification using DNA barcoding

Yassin, A; Markow, TA; Narechania, A; O'Grady, PM; DeSalle, R. 2010

DNA barcoding has recently been proposed as a promising tool for the (1) rapid assignment of unknown samples to described species by non-expert workers and (2) a potential method of new species discovery based on degree of DNA sequence divergence. Two broad methods have been used, one based on degree of DNA sequence variation, within and between species and another requiring the recovery of species as discrete clades (monophyly) on a phylogenetic tree. An alternative method relies on the identification of a set of specific diagnostic nucleotides for a given species (characters). The genus *Drosophila* has long served as a model system in genetics, development, ecology and evolutionary biology. As a result of this work, species boundaries within this genus are quite well delimited, with most taxa being defined by morphological characters and also conforming to a biological species concept (e.g., partial or complete reproductive isolation has been used to erect and define species). In addition, some of the species in this group have also been subjected to phylogenetic analysis, yielding cases where taxa both conform and conflict with a phylogenetic species concept. Here, we analyzed 1058 COI sequences belonging to 68 species belonging to *Drosophila* and its allied genus *Zaprionus* and with more than a single representative to assess the performance of the three DNA barcoding methods. 26% of the species could not be defined using distance methods, i.e. had a barcoding gap of ≤ 0 , and 23% were not monophyletic. We focused then on four groups of closely-related species whose taxonomy is well-established on non-molecular basis (e.g., morphology, geography, reproductive isolation) and to which most of the problematic species belonged. We showed that characters performed better than other approaches in the case of paraphyletic species, but all methods failed in the case of polyphyletic species. For these polyphyletic species, other sources of evidence (e.g., morphology, geography, reproductive isolation) are more relevant than COI sequences, highlighting the limitation of DNA barcoding and the needs for integrative taxonomy approaches. In conclusion, DNA barcoding of *Drosophila* shows no reason to alter the 250 years old tradition of character-based taxonomy, and many reasons to shy away from the alternatives. (C) 2010 Elsevier Inc. All rights reserved.

Phylo-comparative analyses reveal the dual role of drift and selection in reproductive character displacement

Saglam, IK; Miller, MR; O'Rourke, S; Caglar, SS. 2019

When incipient species meet in secondary contact, natural selection can rapidly reduce costly reproductive interactions by directly targeting reproductive traits. This process, called reproductive character displacement (RCD), leaves a characteristic pattern of geographic variation where divergence of traits between species is greater in sympatry than allopatry. However, because other forces can also cause similar patterns, care must be given in separating pattern from process. Here we show how the phylo-comparative method together with genomic data can be used to evaluate evolutionary processes at the population level in closely related species. Using this framework, we test the role of RCD in speciation of two cricket species endemic to Anatolian mountains by quantifying patterns of character displacement, rates of evolution and adaptive divergence. Our results show differing patterns of character displacement between species for reproductive vs. non-reproductive characters and strong patterns of asymmetric divergence. We demonstrate diversification results from rapid divergence of reproductive traits towards multiple optima under the dual influence of strong drift and selection. These results present the first solid evidence for RCD in Anatolian mountains, quantify the amount of drift and selection necessary for RCD to lead to speciation, and demonstrate the utility of phylo-comparative methods for quantifying evolutionary parameters at the population level.

Assortative Mating and Lack of Temporality Between Corn and Rice Strains of *Spodoptera frugiperda* (Lepidoptera, Noctuidae) from Central Colombia

Saldamando-Benjumea, CI; Estrada-Piedrahita, K; Velasquez-Velez, MI; Bailey, RI. 2014

Spodoptera frugiperda is a Neotropical moth that has diverged into "corn" and "rice" strains. In the US, prezygotic isolation studies have shown that both populations mate assortatively. In addition, recent studies have demonstrated that mating by the same strain individuals is enhanced by an allochronic shift in mating activity and male pheromones are important during courtship. In Colombia, studies on mate choice have never been performed previously, although earlier analyses made in populations from Central Colombia showed no significant differences in time of first mating and copula duration between the strains. Here, we performed multiple choice experiments using a tetrad design composed of individuals of the corn and the rice strains. We found that corn strain females rarely mate with rice strain males, but rice strain females mate with both strains of males. In addition, no temporal isolation was found. A ML (maximum likelihood) approach was used to discriminate between mating propensity and mate choice behaviors in *S. frugiperda* strains. This approach showed that mating propensity of corn strain males is three times greater than rice strain males. In contrast, in females, the propensity of mating was slightly higher for the corn strain. Finally, the isolation index between the corn and the rice strains from Colombia produced a value of $I = 0.33$. Our results suggest that prezygotic isolation at the behavioral and temporal levels differ between the US and Colombia. Moreover, in the US temporal isolation appears to have an important role in behavioral isolation but in Colombia temporality is not necessary to reduce encounters between *S. frugiperda* strains.

Shifts in plant nutrient content in combined warming and drought scenarios may alter reproductive fitness across trophic levels

Rosenblatt, AE. 2018

Ecological effects of climate change are difficult to predict because climate change is a multi-variable phenomenon that can impact ecosystems through diverse pathways. Despite this fact, climate change ecology research has been dominated by relatively simplistic experiments and models. To test the importance of assessing more realistic climate and ecological scenarios an experiment was conducted to assess the interactive effects of multiple climate change variables (warming, drought) on survival and reproductive fitness across three trophic levels within a well-studied terrestrial food web. The effects of warming and drought on the nutrient content of plants and how such changes may affect consumers was also examined. Results showed that warming and drought in combination can significantly alter the nutrient profiles of plants relative to climate variables in isolation and that multi-variable climate change can severely impact plant reproductive fitness. Also, consumer nutrient profiles did not shift in accordance with their resources, but reproductive fitness of grasshoppers was nonetheless severely affected by warming and drought in combination. Predator survival rates decreased by more than 45% under all climate change scenarios, highlighting the variable responses of different trophic levels. The impacts across the entire food web were likely caused by a combination of metabolic changes due to warming, dehydration, altered nutrient availability and resource quality, and behavioral shifts. The results suggest that realistic climate change scenarios could dramatically affect long-term demographic patterns as well as food web dynamics, and that single variable studies may fail to reveal the true impacts of climate change.

HYBRIDIZATION STUDIES ON *BLATTELLA-GERMANICA* AND *B-ASAHINAI* (DICTYOPTERA, BLATTELLIDAE) - CHIASMA FREQUENCY AND DISTRIBUTION

ROSS, MH; LIU, H. 1995

Chiasma frequency and distribution were studied in *Blattella asahinai* Mizukubo and interspecific **hybrid** males from crosses of *B. asahinai* X *B. germanica* (L.). Observations were made on male meiocytes in late diakinesis. Chiasmata were localized at or near the ends of the chromosomes in both species. Chiasma frequency was lower and less variable in *B. asahinai* than in *B. germanica*. In the interspecific **hybrids**, the frequency was closer to that of *B. asahinai* than *B. germanica*. Chiasmata in **hybrids** were redistributed into regions where chiasmata are rare in the parent species. Chiasma frequency and the range of variation in chiasma frequency and in the frequency of internal chiasmata were affected by the species of the maternal parent. The maternal influence may be the result of a nonreciprocal translocation that involves the X chromosome and that separates the two species.

Female answer specificity to male drumming calls in three closely related species of the stonefly genus *Zwicknia* (Plecoptera: Capniidae)

Orci, KM; Muranyi, D. 2021

This study examines the rate of female answers to conspecific versus heterospecific male vibratory calls in three, closely related stonefly species: *Zwicknia bifrons*, *Z. acuta*, and *Z. rupprechti*. In a previous study those three species were recognized on the basis of their distinct male drumming calls along with differences in genital morphology and genetic divergence. During this study no-choice playback experiments using original male call samples from each species were performed, and the answer rate of females to conspecific and heterospecific signal variants was measured. Mixed effect logistic regression models were used to test if male call species identity had a statistically significant effect on female answer probability. Females answered conspecific male calls with significantly higher probability than heterospecific calls in all the three examined species, suggesting that the divergence of vibrational communication can be an important component of the prezygotic isolation between them. Low, but well detectable responsiveness to heterospecific calls was observable between *Z. bifrons* and *Z. acuta*, the two species closest to each other regarding mitochondrial genetic divergence and male call pattern similarity. Thus, our results are most congruent with a tight, gradual coevolution of male calls and female preferences.

Geographic variation in acoustic communication: reproductive character displacement and speciation

Gerhardt, HC. 2013

Background: Reproductive character displacement is a geographical pattern in which mate-attracting signals, preferences or both differ more in areas of sympatry than in areas of allopatry. Questions: What selective forces drive reproductive character displacement? What are its consequences? Methods: Reproductive character displacement in mate-attracting signals and/or female preferences are described for pairs of closely related field crickets (*Gryllus*), periodical cicadas (*Magicicada*), chorus and treefrogs (*Pseudacris* and *Hyla*), and tinkerbirds (*Pogoniulus*). These examples were characterized by data from multiple populations and evidence regarding the efficacy of sympatric divergence in signals or preferences. Conclusions: Whereas selection resulted in changes that reduce the production of genetically or behaviourally unfit **hybrids** in field crickets, chorus frogs, and green and grey treefrogs, reproductive character displacement in periodical cicadas and tinkerbirds was most likely driven by masking interference or mistakes in the identification of territorial rivals. Reproductive character displacement may not only accentuate or preserve divergence that arises in allopatry but can result in cascades of speciation if assortative mating arises between displaced and non-displaced populations.

The geography of behaviour: an evolutionary perspective

Foster, SA. 1999

Traditional approaches to the study of behaviour have typically assumed that behavioural patterns, especially elements of reproductive behaviour, are invariant within species. Recent research on a diversity of behavioural traits in a wide array of taxa provides evidence that genetically based geographic variation in behaviour is common. Comparisons of populations that display geographic variation in behaviour can offer substantial insight into mechanisms of adaptive divergence and constraining or generative roles of gene flow, initial stages of speciation, and the roles of phenotypic plasticity and ontogeny in determining patterns of behavioural evolution.

The evolution of insect mating structures through sexual selection

Sirot, LK. 2003

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

Wide prevalence of hybridization in two sympatric grasshopper species may be shaped by their relative abundances

Rohde, K; Hau, Y; Weyer, J; Hochkirch, A. 2015

Background: **Hybridization** between species is of conservation concern as it might threaten the genetic integrity of species. Anthropogenic factors can alter **hybridization** dynamics by introducing new potentially **hybridizing** species or by diminishing barriers to **hybridization**. This may even affect sympatric species pairs through environmental change, which so far has received little attention. We studied **hybridization** prevalence and the underlying behavioral mechanisms in two sympatric grasshopper species, a rare specialist (*Chorthippus montanus*) and a common generalist (*Chorthippus parallelus*). We conducted a mate choice experiment with constant intraspecific density and varying heterospecific density, i.e. varying relative frequency of both species. Results: Mate choice was frequency-dependent in both species with a higher risk of cross-mating with increasing heterospecific frequency, while conspecific mating increased linearly with increasing conspecific density. This illustrates that reproductive barriers could be altered by environmental change, if the relative frequency of species pairs is affected. Moreover, we performed a microsatellite analysis to detect **hybridization** in twelve syntopic populations (and four allotopic populations). **Hybrids** were detected in nearly all syntopic populations with **hybridization** rates reaching up to 8.9 %. Genetic diversity increased for both species when **hybrids** were included in the data set, but only in the common species a positive correlation between **hybridization** rate and genetic diversity was detected. Conclusion: Our study illustrates that the relative frequency of the two species strongly determines the effectiveness of reproductive barriers and that even the more choosy species (*Ch. montanus*) may face a higher risk of **hybridization** if population size decreases and its relative frequency becomes low compared to its sister species. The asymmetric mate preferences of both species may lead to quasi-unidirectional gene flow caused by unidirectional backcrossing. This might explain why genetic diversity increased only in the common species, but not in the rare one. Altogether, the **hybridization** rate was much higher than expected for a widely sympatric species pair.

MULTIVALENTS RESULTING FROM MONOBRACHIAL HOMOLOGIES WITHIN A HYBRID ZONE IN *DICHRPLUS-PRATENSIS* (ACRIDIDAE) - MEIOTIC ORIENTATION AND SEGREGATION

Natural populations of the South American grasshopper *Dichroplus pratensis* differ for seven polymorphic centric fusions involving the six longest telocentric autosomes of the karyotype (L1-L6). Hence, the same telocentric is involved in more than one fusion in different populations producing metacentrics with monobrachial homologies. The present study concerns the meiotic behaviour of quadrivalents and quinquivalents formed in natural **hybrids** that occur in two **hybrid** zones between chromosomal races with monobrachial homologies. The analysis revealed that: (a) non-alternate orientation of multivalents at Metaphase I (MI) was high, ranging from 18.5 to 64.2% of the cells in 18 **hybrid** males. Non-alternate Prometaphase (PMI) orientation was studied in six males (two of each type of **hybrid**) and in all cases values were higher than in MI, which suggests reorientation between PMI and MI. (b) All **hybrids** showed high frequencies of aneuploid and diploid second spermatocytes which are the result of abnormal segregation and lagging of chromosomes involved in multivalent formation. A highly significant correlation exists between the frequency of abnormal MI cells per male and abnormal second division spermatocytes. In most individuals, however, the frequency of abnormal second spermatocytes was lower than that of abnormal MI, which suggests further reorientation of multivalents towards alternate orientation at MI or spermatocyte selection between both meiotic divisions. (c) The **hybrids** have an increased production of macrospermatids. The behaviour of the multivalents suggests that the inter-racial **hybrids** have their fertility moderately to severely reduced which infers the existence of post-mating reproductive isolation between races. This is discussed in relation to the maintenance and adaptive role of the fusion polymorphisms in nature.

Molecular evidence of a peripatric origin for two sympatric species of field crickets (*Gryllus rubens* and *G. texensis*) revealed from coalescent simulations and population genetic tests

Gray, DA; Huang, HT; Knowles, LL. 2008

Species pairs that differ primarily in characters involved in mating interactions and are largely sympatric raise intriguing questions about the mode of speciation. When species divergence is relatively recent, the footprint of the demographic history during speciation might be preserved and used to reconstruct the biogeography of species divergence. In this study, patterns of genetic variation were examined throughout the geographical range of two cryptic sister taxa of field crickets, *Gryllus texensis* and *G. rubens*; mitochondrial cytochrome oxidase I (COI) was sequenced in 365 individuals sampled from 48 localities. Despite significant molecular divergence between the species, they were not reciprocally monophyletic. We devised several analyses to statistically explore what historical processes might have given rise to this genealogical structure. The analyses indicated that the biogeographical pattern of genetic variation does not support a model of recent gene flow between species. Instead, coalescent simulations suggested that the genealogical structure within *G. texensis*, namely a deep split between two geographically overlapping clades, reflects historical substructure within *G. texensis*. Additional tests that consider the concentration of *G. rubens* haplotypes in one of the two *G. texensis* genetic clusters suggest a model of speciation in which *G. rubens* was derived from one lineage of a geographically subdivided ancestor. These results indicate that, despite the contemporary sympatry of *G. texensis* and *G. rubens*, the data are indicative of an peripatric origin in which *G. rubens* was derived from one of the two historical partitions in the species currently recognized as *G. texensis*. This proposed model of species divergence suggests how the interplay of geography and selection may give rise to new species, although this requires testing with multilocus data. Specifically, the model highlights how that geographical partitioning of ancestral variation in the past may augment the selectively driven divergence of characters involved in the reproductive isolation of the species today.

Difference in calling song of three field crickets of the genus *Teleogryllus*: the role in premating isolation

Honda-Sumi, E. 2005

Crickets songs are species-specific signals that are important for species recognition. To prove the effectiveness of calling songs in premating isolation, studies need to take into account closely related species distributed sympatrically in nature. I compared the calling songs of three closely related cricket species that inhabit the Japanese archipelago: *Teleogryllus yezoemma*, *T. emma* and *T. taiwanemma*. *Teleogryllus emma* is widely distributed in the archipelago and overlaps the ranges of *T. taiwanemma* in the south and *T. yezoemma* in the north; *T. yezoemma* and *T. taiwanemma* are allopatric. I compared three temporal parameters (the long-chirp duration, the interphrase duration and the pulse cycle), a frequency parameter (the dominant frequency) and two structural parameters (the number of pulses in one long chirp and the number of short chirps following one long chirp). In addition, I conducted a two-speaker playback experiment using female *T. taiwanemma* to test whether they can accurately distinguish conspecific males based on their calling song. The species with partially overlapping ranges had divergent songs that females could distinguish accurately, whereas the allopatric species had similar songs that females could not accurately discriminate. These results suggest that the calling song has an important role as a premating isolation mechanism. (c) 2004 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Levels of genetic polymorphism: marker loci versus quantitative traits

Butlin, RK; Tregenza, T. 1998

Species are the units used to measure ecological diversity and alleles are the units of genetic diversity. Genetic variation within and among species has been documented most extensively using allozyme electrophoresis. This reveals wide differences in genetic variability within, and genetic distances among, species, demonstrating that species are not equivalent units of diversity. The extent to which the pattern observed for allozymes can be used to infer patterns of genetic variation in quantitative traits depends on the forces generating and maintaining variability. Allozyme variation is probably not strictly neutral but, nevertheless, heterozygosity is expected to be influenced by population size and genetic distance will be affected by time since divergence. The same is true for quantitative traits influenced by many genes and under weak stabilizing selection. However, the limited data available suggest that allozyme variability is a poor predictor of genetic variation in quantitative traits within populations. It is a better predictor of general phenotypic divergence and of postzygotic isolation between populations or species, but is only weakly correlated with prezygotic isolation. Studies of grasshopper and planthopper mating signal variation and assortative mating illustrate how these characters evolve independently of general genetic and morphological variation. The role of such traits in prezygotic isolation, and hence speciation, means that they will contribute significantly to the diversity of levels of genetic variation within and among species.

Mate recognition in a freshwater fish: geographical distance, genetic differentiation, and variation in female preference for local over foreign males

Wong, BBM; Keogh, JS; Jennions, MD. 2004

It often is assumed that more distant allopatry should reflect reduced rates of contemporary gene flow and/or greater divergence in mate recognition systems. This assumption, however, is rarely tested and may not always be appropriate. Here we investigated female preference for local and foreign males in a morphologically variable Australian freshwater fish, the Pacific blue-eye *Pseudomugil signifer*. Using a multidisciplinary approach that combined molecular phylogeography with conventional mate choice experiments, we found female blue-eyes spent more time in association with local males only when the alternative was a foreigner from a geographically and genetically more distant population. When offered the choice between two foreign males, females associated more with males from the population that was more closely adjacent to their own. Our results suggest that female preference for local over foreign males in blue-eyes may depend on how genetically and geographically separated populations are from one another.

The shape of female mating preferences

Ritchie, MG. 1996

The "shape" of a female mating preference is the relationship between a male trait and the probability of acceptance as a mating partner. The shape of preferences is important in many models of sexual selection, mate recognition, communication, and speciation, yet it has rarely been measured precisely. Here I examine preference shape for male calling song in a bushcricket (katydid). Preferences change dramatically between races of a species, from strongly directional to broadly stabilizing (but with a net directional effect). Preference shape generally matches the distribution of the male trait. This is compatible with a coevolutionary model of signal-preference evolution, although it does not rule out an alternative model, sensory exploitation. Preference shapes are shown to be genetic in origin.

Courtship and copulation, but not ejaculates, reduce the longevity of female field crickets (*Gryllus bimaculatus*)

Bateman, PW; Ferguson, JWH; Yetman, CA. 2006

Females of many taxa incur fitness costs from male sexual coercion and harassment leading to mating. Although male crickets cannot force copulations on females, female *Gryllus bimaculatus* in this study incurred significant reductions in longevity through being exposed to different levels of male courtship. Virgin females kept in isolation had the longest life spans. Reductions in longevity applied to females in sensory contact with males (without the opportunity to mate), females that courted and mated and females that mated but with fertilization being prevented. Females also incurred significant reductions in longevity when kept with other females, which may have been due to high levels of cannibalism. Consistent with previous studies, females appeared to incur no cost to longevity from receiving sperm or seminal fluid. It is known that female *G. bimaculatus* benefit genetically from multiple mating. However, this benefit could possibly be offset by the negative effect that male courtship and mating behaviour has on female longevity.

Divergent mechanisms of acoustic mate recognition between closely related field cricket species (*Teleogryllus* spp.)

Bailey, NW; Moran, PA; Hennig, RM. 2017

Effective recognition of conspecific mating signals reduces the risk of maladaptive **hybridization**. Dissecting the signal recognition algorithms that underlie preferences is a useful approach for testing whether closely related taxa evaluate the same or different signal features to achieve mate recognition. Such data provide information about potential constraints and targets of selection during evolutionary divergence. Using a series of mate choice trials, we tested whether closely related, but genetically and phenotypically divergent, field cricket species (*Teleogryllus oceanicus* and *Teleogryllus commodus*) use shared or distinct recognition algorithms when evaluating acoustic male calling songs. These species overlap in sympatry, show premating isolation based on female discrimination of male calling songs, yet are capable of producing **hybrid** offspring. Unexpectedly, female selectivity for features of male song differed between the two species. We found that the two species use a combination of shared and unique signal filtering mechanisms, and we characterized how information about male carrier frequency, pulse rate and temporal patterning is integrated to achieve song recognition in each species. These results illustrate how comparatively few, simple modifications in key components of signal recognition algorithms can lead to striking interspecific discrimination between closely related taxa, despite apparent signal complexity. The finding that some steps during signal recognition and filtering are shared between the species, while others differ, can help to identify behavioural traits targeted by selection during evolutionary divergence. (C) 2017 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Induced expression of a vestigial sexual signal

Gray, DA; Hormozi, S; Libby, FR; Cohen, RW. 2018

Vestigial morphological traits are common and well known in a variety of taxa. Identification of vestigial genes has illustrated the potential for evolutionary reversals and the re-expression of atavistic traits. Here we induce expression of a behavioural sexual signal, male calling song, in a cricket species, *Gryllus ovisopis*, which lacks a functional calling song. We successfully used acetylcholine injections in the frontal space of the head of male crickets to activate cerebral command neurons for cricket calling, and we recorded calling songs with a temporal chirp pattern similar to that of *G. ovisopis*' close evolutionary relatives, *G. firmus* and *G. pennsylvanicus*, implying that the neural pattern generators that underlie cricket calling behaviour persist in a vestigial state in *G. ovisopis*. To our knowledge, this is the first demonstration of the induced expression of a vestigial behaviour in any organism. The retention of latent neural capacity to express sexual behaviours could have important implications for rapid evolution, trait re-emergence and reproductive isolation.

Multiple differences in calling songs and other traits between solitary and gregarious Mormon crickets from allopatric mtDNA clades

Bailey, NW; Gwynne, DT; Bailey, WV; Ritchie, MG. 2007

Background: In acoustic species, traits such as male calling song are likely to diverge quickly between allopatric populations due to sexual selection, and divergence in parameters such as carrier frequency, chirp structure, and other important song characters can influence sexual isolation. Here we make use of two forms of Mormon crickets to examine differences in a broad suite of traits that have the potential to influence speciation via sexual isolation. Mormon crickets in "gregarious" populations aggregate into dense migratory bands, and females are the sexually competitive Fsex (sex-role reversal). There is also a non-outbreak "solitary" form. These two forms are largely but not perfectly correlated with a significant mtDNA subdivision within the species that is thought to have arisen in allopatry. Combined information about multiple, independently evolving traits, such as morphology and structural and behavioural differences in calling song, provides greater resolution of the overall differences between these allopatric populations, and allows us to assess their stage of divergence. We test two predictions, first that the forms differ in song and second that gregarious males are more reluctant to sing than solitary males due to sex role reversal. We also tested for a difference in the relationship between the size of the forewing resonator, the mirror, and carrier frequency, as most models of sound production in crickets indicate that mirror size should predict carrier frequency. Results: Multivariate analyses showed that solitary and gregarious individuals from different populations representing the two mtDNA clades had almost non-overlapping distributions based on multiple song and morphological measurements. Carrier frequency differed between the two, and gregarious males were more reluctant to sing overall. Mirror size predicted carrier frequency; however, the relationship between mirror size and surface area varied between solitary and gregarious forms, suggesting that factors above and beyond mirror size contribute to carrier frequency. Conclusion: The two clades of Mormon crickets differ in a broad suite of independent traits that probably justify subspecies status (the two can successfully mate so may not be reproductively isolated). However, our results emphasize the importance of carefully distinguishing the ultimate causation of differences between traits used to delineate species or subspecies boundaries.

Divergence of cuticular hydrocarbons in two sympatric grasshopper species and the evolution of fatty acid synthases and elongases across insects

Finck, J; Berdan, EL; Mayer, F; Ronacher, B; Geiselhardt, S. 2016

Cuticular hydrocarbons (CHCs) play a major role in the evolution of reproductive isolation between insect species. The CHC profiles of two closely related sympatric grasshopper species, *Chorthippus biguttulus* and *C. mollis*, differ mainly in the position of the first methyl group in major methyl-branched CHCs. The position of methyl branches is determined either by a fatty acid synthase (FAS) or by elongases. Both protein families showed an expansion in insects. Interestingly, the FAS family showed several lineage-specific expansions, especially in insect orders with highly diverse methyl-branched CHC profiles. We found five putative FASs and 12 putative elongases in the reference transcriptomes for both species. A dN/dS test showed no evidence for positive selection acting on FASs and elongases in these

grasshoppers. However, one candidate FAS showed species-specific transcriptional differences and may contribute to the shift of the methyl-branch position between the species. In addition, transcript levels of four elongases were expressed differentially between the sexes. Our study indicates that complex methyl-branched CHC profiles are linked to an expansion of FASs genes, but that species differences can also be mediated at the transcriptional level.

THE USE OF BIOACOUSTIC CHARACTERS FOR DISTINGUISHING BETWEEN CRYPTIC SPECIES IN INSECTS: POTENTIALS, RESTRICTIONS, AND PROSPECTS

Tishechkin, DY. 2013

In insects, differences in acoustic signal patterns between closely related species often form the main precopulatory reproductive barrier. For this reason, discrimination between similar forms, based on signal patterns, allows for reproductively isolated species to be recognized. A calling signal produced by a mature male for attracting a conspecific female is a "species passport" testifying that it belongs to the same species. This is the reason why the use of calling signal analysis for discriminating between the cryptic species or elucidating the taxonomic rank of dubious forms is highly efficient. In certain species, courtship signals also show species-specific patterns. In insect taxonomy, the study of acoustic signals is most promising in morphologically or/and ecologically heterogeneous groups. Chances that any morphologically homogeneous form will actually appear to be a complex of cryptic species are low. The degree of signal variability differs in different orders, families and even congeneric species. For this reason, in every taxon investigated for the first time, it is necessary to evaluate the limits of signal intraspecific variability prior to using the acoustic characters in taxonomy. Species not involved into acoustic interactions due to allopatry, differences in host specialization, etc. can produce calling signals with identical patterns. Consequently, the similarity of signal structure in such species is by no means evidence of their synonymy.

HYBRID BREAKDOWN IN PEROMYSCUS-LEUCOPUS AND EXAMINATION OF THE RECOMBINATIONAL-BREAKDOWN MODEL

ADKINS, RM; MCBEE, K; PORTER, CA; BAKER, RJ. 1991

The recombinational-breakdown model is based on disruption of normal recombinational patterns and increased rates of mutation in **hybrids** between chromosomally distinct morphs of a species. A **hybrid** zone between northeastern and southwestern cytotypes of the white-footed mouse, *Peromyscus leucopus*, was used to examine how taxonomically widespread the implications of this model are. Aberration frequencies and the proportion of breaks in biallelic and acrocentric chromosomes were determined for mice from both pure-cytotypic populations, from a pure-**hybrid** locality, and from a site containing a mixture of pure-southwestern cytotypes and non-F1 **hybrids**. The pure-**hybrid** population had aberration frequencies significantly elevated above those of the southwestern population and the mixed parental-**hybrid** locality. Non-F1 **hybrids** within the pure-**hybrid** population had an elevated frequency of breaks in biallelic chromosomes. Breakdown, as measured by chromosomal aberrations, was maximal in the center of the **hybrid** zone. Such chromosomal disruption is a prediction of the recombinational-breakdown model. Therefore, the model appears to have applicability across broadly divergent taxonomic groups.

Conservation of multivariate female preference functions and preference mechanisms in three species of trilling field crickets

Blankers, T; Hennig, RM; Gray, DA. 2015

Divergence in mate recognition systems among closely related species is an important contributor to assortative mating and reproductive isolation. Here, we examine divergence in male song traits and female preference functions in three cricket species with songs consisting of long trills. The shape of female preference functions appears to be mostly conserved across species and follows the predictions from a recent model for song recognition. Multivariate preference profiles, combining the pulse and trill parameters, demonstrate selectivity for conspecific pulse rates and high trill duty cycles. The rules for integration across pulse and trill timescales were identical for all three species. Generally, we find greater divergence in male song traits than in associated female preferences. For pulse rate, we find a strong match between divergent male traits and female peak preferences. Preference functions for trill parameters and carrier frequency are similar between species and show less congruence between signal and preference. Differences among traits in the degree of trait-preference (mis)match may reflect the strength of preferences and the potential for linkage disequilibrium, selective constraints and alternative selective pressures, but appear unrelated to selection for mate recognition per se.

Variation in complex mating signals in an "island" hybrid zone between *Stenobothrus* grasshopper species

Sradnick, J; Klopfel, A; Elsner, N; Vedenina, V. 2016

Two grasshopper species *Stenobothrus rubicundus* and *S. clavatus* were previously shown to meet in a narrow **hybrid** zone on Mount Tomaros in northern Greece. The species are remarkable for their complex courtship songs accompanied by conspicuous movements of antennae and wings. We analyzed variations in forewing morphology, antenna shape, and courtship song across the **hybrid** zone using a geographic information system, and we documented three contact zones on Mount Tomaros. All male traits and female wings show abrupt transitions across the contact zones, suggesting that these traits are driven by selection rather than by drift. Male clines in antennae are displaced toward *S. clavatus*, whereas all clines in wings are displaced toward *S. rubicundus*. We explain cline discordance as depending on sexual selection via female choice. The high covariance between wings and antennae found in the centers of all contact zones results from high levels of linkage disequilibria among the underlying loci, which in turn more likely results from assortative mating than from selection against **hybrids**. The covariance is found to be higher in *clavatus*-like than *rubicundus*-like populations, which implies asymmetric assortative mating in parental-like sites of the **hybrid** zone and a movement of the **hybrid** zone in favor of *S. clavatus*.

Heightened perception of competition hastens courtship

Santori, C; Bussiere, LF; Housley, TM. 2020

When animals use costly labile display or signal traits to display to the opposite sex, they face complex decisions regarding the degree and timing of their investment in separate instances of trait expression. Such decisions may be informed by not only the focal individual's condition (or pool of available resources) but also aspects of the social environment, such as perceptions of same-sex competition or the quality of available mates. However, the relative importance of these factors to investment decisions remains unclear. Here, we use manipulations of condition (through dietary nutrition), recent social environment (exposure to a silenced male, nonsilenced male, female, or isolation), and female mating history (single or multiple male) to test how quickly male decorated crickets (*Gryllodes sigillatus*) decide to begin courting an available female. We find that males that were previously housed with nonsilenced males started courting the female earlier than other males. Females only mounted males after courtship began. Our results suggest a strong effect of the perception of competition on the decision to invest resources in sexual signaling behavior and that females might exert directional selection on its timing.

Genetic variation in cricket calling song across a hybrid zone between two sibling species

Mousseau, TA; Howard, DJ. 1998

The sibling ground crickets *Allonemobius fasciatus* and *A. socius* meet along a mosaic **hybrid** zone at approximate to 40 degrees N latitude in eastern North America. In this paper we report the findings of a genetic analysis of calling-song variation within and among six cricket populations sampled along a transect through the **hybrid** zone in southern New Jersey. We compared aspects of the calling song of both wild-caught and laboratory-reared crickets to test the hypothesis that population differences in song observed in the wild were genetically based. We found significant, species-level differences in all aspects of the calling song, and these differences persisted even after a generation of common-garden rearing in the laboratory, supporting the hypothesis that interspecific variation observed in the wild largely reflects genetic differentiation between the two taxa. A discriminant function analysis indicated that individual crickets could be assigned to the proper taxon with less than 10% error, supporting the premise that calling song could be used by female crickets as a mechanism for species recognition. One population, collected from within the **hybrid** zone and containing significant numbers of **hybrid** individuals, was intermediate in its calling song, presumably reflecting this population's mixed genetic makeup. In this **hybrid** zone population, song phenotype was highly correlated to a **hybrid** index score generated using species-specific alleles at four diagnostic allozyme markers, suggesting a multigenic basis to calling-song variation in these crickets as well as linkage disequilibrium between markers and song. Based on an analysis of laboratory-reared full-sib families, broad-sense heritabilities for calling-song characteristics were generally significant in the two *A. socius* populations, whereas many components of song showed no significant family effects in the three *A. fasciatus* populations. The genotypically mixed, **hybrid** zone population showed very high heritabilities for most calling-song components, which likely reflect the influence of interspecific gene flow on genetic variation for quantitative traits.

High background noise shapes selective auditory filters in a tropical cricket

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

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

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

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

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