

((AB=(hybrid OR hybridization OR hybridisation) OR AK=(hybrid OR hybridization OR hybridisation) OR TI=(hybrid OR hybridization OR hybridisation)) AND (ALL=(Odonata))) NOT ALL=(fluorescent OR fluorescence OR "in situ")

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HYBRIDIZATION BETWEEN 2 DAMSELFLY SPECIES (ODONATA, COENAGRIONIDAE) - MORPHOMETRIC AND GENITALIC DIFFERENTIATION

LEONG, JM; HAFERNIK, JE. 1992

In allopatry and in a **hybrid** zone, we analyzed the relationship between morphometric characters and the inferior abdominal appendage angle in two damselflies, *Ischnura gemina* (Kennedy) and *Ischnura denticollis* (Burmeister), to assess the taxonomic reliability and genetic basis of this genitalic trait. Changes in the morphometric-genitalic relationship in the **hybrid** zone strongly imply that introgression has occurred, although there is some evidence of **hybrid** unfitness and partial reproductive isolation. We conclude that the inferior appendage trait is not taxonomically reliable because it fails to convey the recombinant nature of individuals in the **hybrid** zone. A combination of field and lab data suggest that the genitalic angle trait is polygenic but controlled by only a few genes. We argue for retention of full species status for both *I. gemina* and *I. denticollis*, despite genetic exchange between the two.

Disturbed population genetics: Suspected introgressive hybridization between two *Mnais* damselfly species (Odonata)

Hayashi, F; Dobata, S; Futahashi, R. 2005

Mnais costalis and *M. pruinosa* are damselflies (Odonata: Calopterygidae) with low dispersal abilities, both during their aquatic stream-living immature stage and their flying adult stage. A previous nuclear DNA (nDNA) sequencing and morphology study showed that these two species are very closely related, and cohabit widely in western Japan. The two species, however, segregate microhabitats along a stream: *M. costalis* lives in the lower reaches, and *M. pruinosa* in the upper reaches. In this study, our analyses were based on mitochondrial DNA (mtDNA), which usually mutates faster and is more variable among individuals than nDNA, and which is inherited maternally. We found that most COI haplotypes were shared between the two species, and that for most study sites interspecific riverine genetic structures were not clarified by mtDNA analysis. Incongruent population genetic structures based on nDNA and mtDNA suggested **hybridization** and introgression of mtDNA between the two species.

Hybridization between *Ischnura graellsii* (Vander Linder) and *I. elegans* (Rambur) (Odonata : Coenagrionidae): are they different species?

Monetti, L; Sanchez-Guillen, RA; Rivera, AC. 2002

Two closely related damselflies, *Ischnura graellsii* and *I. elegans*, were analysed for morphological differences and reproductive isolation in the north coast of Galicia (NW Spain). We compared animals from sympatric and allopatric localities, including *I. elegans* from Belgium and *I. graellsii* from southern Spain as pure allopatric populations. A set of morphometric characters were studied by means of multivariate discriminant analysis to determine if these two species can be unambiguously distinguished. Discriminant analysis revealed that *I. graellsii* and *I. elegans* are well differentiated on the first two axis (86% and 11%, respectively). *I. graellsii* individuals are distinguished from *I. elegans* by their smaller size and, specifically, by their narrower and shorter wings and shorter tibiae. In addition, *I. elegans* has a narrower space between the branches of each cercus, and greater distance between the branches of each paraproct. Sympatric individuals are morphologically intermediate, suggesting **hybridization**. When the species were put together in the laboratory, they showed partial temporal separation in mating behaviour, but males of *I. elegans* readily mated with females of *I. graellsii*, and **hybrid** individuals were obtained. The opposite heterospecific cross was almost impossible, apparently because of mechanical problems with the tandem linkage. Laboratory-reared **hybrids** (from male *I. elegans* x female *I. graellsii*) are morphologically intermediate, mainly resembling the maternal phenotype. Although **hybridization** between both taxa is common, we suggest maintaining the specific status for both phenotypes because they show incipient reproductive isolation, as it is reported in the literature. (C) 2002 The Linnean Society of London, Biological Journal of the Linnean Society, 2002, 76, 225-235.

Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata : Coenagrionidae)

Sanchez-Guillen, RA; Van Gossum, H; Rivera, AC. 2005

Female-limited polychromatism is frequent in many species of Odonata. *Ischnura elegans* has three colour morphs: one male-like coloured (androchrome) and two additional gynochrome brown morphs (*infuscans* and *rufescens-obsolata* morphs). A total of 19 progenies obtained from once-mated females were reared in the laboratory in three generations. Results indicate that the colour morphs are controlled by the same genetic system as previously described for *I. graellsii*, i.e. an autosomal locus with female-limited expression and with three alleles with a hierarchy of dominance ($p(a) > p(i) > p(o)$). Five interspecific crossings between female *I. graellsii* and male *I. elegans*, five crossings between **hybrid** females and male *I. elegans* and one crossing between female *I. graellsii* and a **hybrid** male further confirmed that the genetic system is the same in both species. A survey of morph frequencies in north-west Spain revealed that *I. elegans* shows high variability in androchrome frequency (4-91%) between nearby populations, whereas in *I. graellsii* androchromes never are the majority morph (5-40%). The highest androchrome frequency in *I. graellsii* was found in populations closest to a locality where both species have **hybridized**, and that now has the highest androchrome frequency of *I. elegans*. We hypothesize that *I. elegans* genes have been incorporated into the genome of *I. graellsii* resulting in increased androchrome frequency in the latter species. Low androchrome frequency in *I. elegans* seems also related to the influence of *I. graellsii* genes. Therefore, we suggest that **hybridization** between both taxa is contributing to the temporal maintenance of contrasting androchrome frequencies in nearby populations. (c) 2005 The Linnean Society of London.

Taxonomic uncertainty of a highly endangered brook damselfly, *Copera tokyoensis* Asahina, 1948 (Odonata: Platycnemididae), revealed by the mitochondrial gene genealogy

Kiyoshi, T; Takahashi, J; Yamanaka, T; Tanaka, K; Hamasaki, K; Tsuchida, K; Tsubaki, Y. 2011

In the Japanese main islands, two brook damselfly species are sympatrically distributed. One is highly endangered damselfly, *Copera tokyoensis*, Asahina, 1948, and the other is a congeneric common species, *C. annulata* (Selys, 1863). Mitochondrial gene genealogy reconstructed by the maximum likelihood method showed that they are not reciprocally monophyletic. These two congeneric species might have experienced mitochondrial introgressions possibly through **hybridizations**. The effect of **hybridization** against endangered species is generally poorly understood. Taxonomic uncertainty might also explain this situation because extremely dispersed pattern of the haplotype network could not be appeared by once or twice **hybridization**. Three closely located populations of *C. tokyoensis* in the Kanto district showed significant population differentiation. It might suggest the low dispersal tendency of this endangered species.

Genetic diversity, population structure and taxonomy of *Calopteryx splendens* (Odonata: Calopterygidae): An AFLP analysis

Sadeghi, S; Kyndt, T; Dumont, HJ. 2010

Calopteryx splendens is a widely distributed palaearctic damselfly with a remarkably uniform morphology. Variation in the size and shape of the pigmented spot on the wing is the main diagnostic character used to discriminate subspecies across its huge geographic range. Here, AFLP analysis was used to assess the genetic structure and diversity of nine populations representing 3 putative subspecies and evaluate the pigment spot as a taxonomic marker. Genetic diversity was high, with the number of polymorphic loci per population ranging from 141 to 280 out of a total of 333 variable sites (42.3-84.1%) and Nei's gene diversity from 0.160 to 0.283 (overall 0.299). Overall population genetic differentiation ($F_{ST} = 0.2766$) suggests limited gene flow and adaptation to local environments. Restricted gene flow and genetic differentiation among populations are supported by significant F_{ST} estimates. High levels of gene flow ($N_m > 1$) were only recorded among three Asian populations (Russia - Kazakhstan - Turkey). The patterns of genotypic diversity suggest that a given wing spot size and shape may arise from the **hybridization** of a limited number, possibly not more than four, ancestral gene pools in different ways and at different times. Clearly, the sample analyzed was not sufficient to capture all of the complex history of *C. splendens*, but sufficient to indicate the taxa *ancilla*, *waterstoni*, and *orientalis* possibly represent three of the four ancestral gene pools, and originated in western Asia. The origin of the fourth, *xanthostoma*, is the western Mediterranean.

Asymmetric hybridization in *Cordulegaster* (Odonata: Cordulegastridae): Secondary postglacial contact and the possible role of mechanical constraints

Solano, E; Hardersen, S; Audisio, P; Amorosi, V; Senczuk, G; Antonini, G. 2018

Two *Cordulegaster* dragonflies present in Italy, the Palaearctic and northern distributed *Cordulegaster boltonii* and the endemic to the south of the peninsula *Cordulegaster trinacriae*, meet in central Italy and give rise to individuals of intermediate morphology. By means of mitochondrial and nuclear markers and of Geometric Morphometrics applied to sexual appendages, we defined i) the geographical boundaries between the two species in Italy and ii) we determined the presence, the extent, and the genetic characteristics of the **hybridization**. Genetic data evidenced asymmetric **hybridization** with the males of *C. trinacriae* able to mate both interspecifically and intraspecifically. The results contrast with expectations under neutral gene introgression and sexual selection. This data, along with the morphological evidence of significant differences in size and shape of sexual appendages between the males of the two species, seem indicative of the role of mechanical constraints in intraspecific matings. The origin of the two species is dated about to 1.32 Mya and the **hybridization** resulted related to range expansion of the two species after Last Glacial Maximum and this led to the secondary contact between the two taxa in central Italy. At last, our results indicate that the range of *C. trinacriae*, a threatened and protected species, has been moving northward probably driven by climate changes. As a result, the latter species is currently intruding into the range of *C. boltonii*. The **hybrid** area is quite extended and the **hybrids** seem well adapted to the environment. From a conservation point of view, even if *C. trinacriae* has a strong genetic identity, the discovery of **hybridization** between the two species should be considered in a future species management.

SEASONAL-VARIATION IN ALLOPATRIC POPULATIONS OF ISCHNURA-DENTICOLLIS (BURMEISTER) AND ISCHNURA-GEMINA (KENNEDY) (ODONATA, COENAGRIONIDAE)

LEONG, JM; HAFERNIK, JE. 1992

We morphometrically evaluate the seasonal variation within two species of damselflies, *Ischnura gemina* (Kennedy) and *Ischnura denticollis* (Burmeister) in allopatry, in order to determine whether the same morphometric characters will be useful species discriminators in an *I. denticollis* and *I. gemina* **hybrid** zone. Canonical analysis of discriminance reveals that for both species, early emerging individuals are larger with wider heads than those that emerge later. In addition, each species displays other individual patterns of seasonal variation. Both damselfly species are phenetically distinct despite pronounced seasonal variation; this indicates that the morphometric characters used in this study are potentially suitable for use in diagnosis of **hybrid** zone individuals.

Integrative species delimitation based on COI, ITS, and morphological evidence illustrates a unique evolutionary history of the genus *Paracercion* (Odonata: Coenagrionidae)

Zhang, HG; Ning, X; Yu, X; Bu, WJ. 2021

Paracercion are common 'blue and black' colored damselflies. We explore the species boundaries of *Paracercion* (Odonata: Coenagrionidae) using ABGD, bPTP, GMYC and Distance-based clustering. We finally got the molecular data of all nine species of *Paracercion*. *P. hieroglyphicum* and *P. melanotum* were combined into one putative species based on cytochrome c oxidase I (COI). However, they were separated into two putative species based on the nuclear segment including ITS1-5.8S-ITS2 (ITS). This suggests the introgression of mtDNA in *Paracercion*. *Paracercion barbatum* and *Paracercion melanotum* can be separated into two species based on COI, whereas they were combined into one putative species based on ITS, which suggests a **hybridization** event between them. The lower interspecific divergence (COI: 0.49%) between *P. barbatum* and *Paracercion v-nigrum* indicates a recent speciation event in *Paracercion*. *Paracercion sieboldii* and *P. v-nigrum* can be separated into two putative species based on COI, while they were frequently merged into the same putative species based on ITS. This can be explained by incomplete lineage sorting in nDNA. Besides, *P. pendulum* and *P. malayanum* were synonymized as junior synonyms of *P. melanotum*. *P. luzonicum* was confirmed not to belong to *Paracercion*. The possibility of introgression, **hybridization**, recent speciation and incomplete lineage sorting makes species delimitation, based on molecular data, difficult and complicates understanding of the evolutionary history of *Paracercion*. The discordance in COI and ITS also indicates the value of using markers from different sources in species delimitation studies.

Variation in the shape of the wings and taxonomy of Eurasian populations of the *Calopteryx splendens* complex (Odonata: Calopterygidae)

Sadeghi, S; Dumont, HJ. 2014

We used geometric morphometrics to determine variation in the morphology of the forewings of individuals in 20 populations of *Calopteryx splendens* s. l. in Eurasia and related these to the circum-specific taxonomy of this taxon. We found differences in shape, with the largest and smallest centroid size of the wings in adjacent northern (*orientalis*) and western (*intermedia*) populations in Iran, respectively, so isolation and relationship are not necessarily determined by distance, but often associated with the stream basin inhabited. The variation in wing shape, however, was much greater. with Populations at the eastern edge of the range (Tajikistan, Kyrgyzstan and East Kazakhstan) uniquely different. Oddly, no taxonomic name is associated with them, although they may be among the oldest representatives of the *splendens* complex. The European and Asian populations are in two separate clades. One of these includes insects with no to a medium-sized wing spot, which does not reach the tip of the wing (*waterstoni*-group), while the other includes insects with very broad wing spots, or, when short, it extends to the very tip of the wings and most females are androchrome (*ancilla* or *intermedia* group). Turkmenistan and northern Iranian population form a separate line inside this clade, which we equate with *Calopteryx orientalis*. South Albanian and Greek populations are in a separate branch corresponding to ssp. *balcanica*; two populations from Ireland and Italy form a branch that has no equivalent in traditional taxonomy, while *Calopteryx xanthostoma* was not identified by its wing shape. Understanding this multitude of phenotypes and the enormous amount of variation within certain populations but not in others becomes easy if one assumes there were two probable late Pleistocene waves of migration, the first of insects lacking spots that migrated east and westwards from the South Black Sea basin, the second, perhaps from the west Caspian, composed of heavily spotted insects with androchromic females. The **hybridization** between these two waves resulted in the current plethora of colour forms and accounts for why similar phenotypes may turn up independently in widely distant locations.

Interspecific crossing between blue-tailed damselflies *Ischnura elegans* and *I. senegalensis* in the laboratory

Ischnurasppecies (Odonata) are among the most common damselflies in the world, which often exhibit female color polymorphisms. One morph, called androchrome, is similar to males in its color pattern, whereas the other morphs, generally referred to as gynochromes, exhibit female-specific colors. In several ischnurasppecies, the female polymorphism is heritable, although molecular and genetic mechanisms remain largely unknown. The dominant-recessive patterns of the female color morphs may differ between species. For example, androchromic females are dominant to gynochromic females in *Ischnura elegans*, whereas androchromic females are recessive in *Ischnura senegalensis*. Here we report a case of interspecific **hybridization** between a gynochrome female of *I. elegans* and a male of *I. senegalensis* in the laboratory. We obtained 61 **hybrid** adult offspring, of which all 31 females were of gynochrome morph. DNA analyses of the **hybrids** confirmed that nuclear DNA sequences were derived from both parent species, whereas mitochondrial DNA sequences were maternally inherited. In the **hybrids**, the postocular spots of female heads, the shape of male appendages, and the color of female's cerci resembled those of *I. elegans*, whereas the size of abdominal blue spots was similar to that of *I. senegalensis*. The shape of prothorax and basal abdominal markings were intermediate in females. The larval developmental traits and the morphological changes in the final larval instar of the **hybrids** were similar to those of *I. senegalensis*. To our knowledge, this is the first report of **hybrids** between two damselfly species with different dominant-recessive patterns of female color morphs.

Phylogeny and classification of Odonata using targeted genomics

Bybee, SM; Kalkman, VJ; Erickson, RJ; Frandsen, PB; Breinholt, JW; Suvorov, A; Dijkstra, KDB; Cordero-Rivera, A; Skevington, JH; Abbott, JC; Herrera, MS; Lemmon, AR; Lemmon, EM; Ware, JL. 2021

Dragonflies and damselflies are a charismatic, medium-sized insect order (similar to 6300 species) with a unique potential to approach comparative research questions. Their taxonomy and many ecological traits for a large fraction of extant species are relatively well understood. However, until now, the lack of a large-scale phylogeny based on high throughput data with the potential to connect both perspectives has precluded comparative evolutionary questions for these insects. Here, we provide an ordinal hypothesis of classification based on anchored **hybrid** enrichment using a total of 136 species representing 46 of the 48 families or incertae sedis, and a total of 478 target loci. Our analyses recovered the monophyly for all three suborders: Anisoptera, Anisozygoptera and Zygoptera. Although the backbone of the topology was reinforced and showed the highest support values to date, our genomic data was unable to strongly resolve portions of the topology. In addition, a quartet sampling approach highlights the potential evolutionary scenarios that may have shaped evolutionary phylogeny (e.g., incomplete lineage sorting and introgression) of this taxon. Finally, in light of our phylogenomic reconstruction and previous morphological and molecular information we proposed an updated odonate classification and define five new families (Amanipodagrionidae fam. nov., Mesagrionidae fam. nov., Mesopodagrionidae fam. nov., Priscagrionidae fam. nov., Protolestidae fam. nov.) and reinstate another two (Rhipidolestidae stat. res., Tatocnemididae stat. res.). Additionally, we feature the problematic taxonomic groupings for examination in future studies to improve our current phylogenetic hypothesis.

Update of the Odonata fauna of Georgia, southern Caucasus ecoregion

Schroter, A; Seehausen, M; Kunz, B; Gunther, A; Schneider, T; Jodicke, R. 2015

A total of 63 odonate taxa were recorded in Georgia during nationwide surveys in June/July 2014, and June and July-August 2015, corresponding to at least 85% of the country's Odonata fauna. For the majority of species information from Georgia is provided in English language for the first time. *Selysiotemis nigra* is a new addition to the country's list. The occurrence of *Chalcolestes parvidens* is confirmed and previous records from Georgia listed as *Lestes viridis* are doubted and believed to pertain to *parvidens*. The second and third records only for *L. macrostigma* are presented. Individuals intermediate between *Enallagma cyathigerum* and *E. c. risi* are reported from Georgia for the first time. As to Georgian *Lestes virens*, the infraspecific taxonomy is critically discussed, with special reference to Central Asian forms described as ssp. *marikovskii*. It is recommended to avoid any further splitting into inadequately defined subspecies, as the variability of eastern forms from Central Europe towards Central Asia can be better described as clinal variation within the ssp. *vestalis*. In consequence, the name *marikovskii* is regarded as a junior synonym of *vestalis*: *Lestes virens vestalis* Rambur, 1842 = *L. virens marikovskii* Belyshev, 1961, syn. nov. The diversity of taxa within the *Calopteryx splendens* complex in the Caucasus region is considered to comprise in fact three subspecies in Georgia: ssp. *intermedia*, spp. *tschaldirica*, and ssp. *mingrelica*. Despite of transition zones and **hybridisation** each subspecies represents in toto a spatially clearly delimited unit. In ssp. *intermedia* androchrome females frequently occurred in the Kakheti region in the east of Georgia. The distinct female colour form *feminalis* of *Calopteryx virgo* is illustrated for the first time and the availability of the name *Calopteryx virgo* var. *feminalis* Bartenev, 1910 is critically discussed. For a number of species the first information from Georgia is provided since their discovery over a century ago, such as *Coenagrion armatum*, *Aeshna serrata*, and *Onychogomphus assimilis*; for *Coenagrion lunulatum* and *C. scitulum* the first data since over 75 years are presented. *Coenagrion ponticum* was recorded throughout the country and at least at two sites found to reproduce syntopically with *C. puella*. New information is provided for the little known *Coenagrion vanbrinkae*, including a formerly unknown pink colour morph of reproductive females. The infraspecific taxonomy of *Ischnura elegans* is critically discussed, with special reference to the taxa *pontica* Schmidt, 1938 and *ebneri* Schmidt, 1938. In addition, new records of *Pyrrosoma nymphula* and *Coenagrion pulchellum*, both being rare in the Caucasus region, are given. The presence of distinct *Gomphus schneiderii* in Georgia is confirmed as well as the continuous presence of *Gomphus ubadschii* at the Rioni River over 80 years after its description under the homonym "*Gomphus flavipes* var. *lineatus* var. n.". *Onychogomphus assimilis* and *O. flexuosus* were found to be abundant in the eastern half of the country suggesting that Georgia is an important global stronghold for both threatened species. Males of *Caliaeschna microstigma* exhibited a distinctive tendency for reduced ante-humeral stripes, leaving only a small bluish patch at the posterior part in some males. Vital populations of *Libellula pontica*, endemic to the East Mediterranean, were found and the species is assumed to be well established in the Kakheti region in the East of the country.

Incidence and Diversity of TorixRickettsia-Odonata Symbioses

Thongprem, P; Davison, HR; Thompson, DJ; Lorenzo-Carballa, MO; Hurst, GDD. 2021

Heritable microbes are an important component of invertebrate biology, acting both as beneficial symbionts and reproductive parasites. Whilst most previous research has focussed on the 'Wolbachia pandemic', recent work has emphasised the importance of other microbial symbionts. In this study, we present a survey of odonates (dragonflies and damselflies) for *Torix* group *Rickettsia*, following previous research indicating that this clade can be common in other aquatic insect groups. PCR assays were used to screen a broad range of odonates from two continents and revealed 8 of 76 species tested were infected with *Rickettsia*. We then conducted further deeper screening of UK representatives of the *Coenagrionidae* damselfly family, revealing 6 of 8 UK *coenagrionid* species to be positive for *TorixRickettsia*. Analysis of *Rickettsia* gene sequences supported multiple establishments of symbiosis in the group. Some strains were shared between UK *coenagrionid* species that shared mtDNA barcodes, indicating a likely route for mitochondrial introgression between sister species. There was also evidence of coinfecting *Rickettsia* strains in two species. FISH analysis indicated *Rickettsia* were observed in the ovarioles, consistent with heritable symbiosis. We conclude that *TorixRickettsia* represent an important associate of odonates, being found in a broad range of species from both Europe and South America. There is evidence that coinfection can occur, vertical transmission is likely, and that symbiont movement following **hybridisation** may underpin the lack of 'barcoding gap' between well-established species pairs in the genus. Future work should establish the biological significance of the symbioses observed.

Hybridization between Calopteryx splendens and C. haemorrhoidalis confirmed by morphological and genetic analyses

Lorenzo-Carballa, MO; Watts, PC; Cordero-Rivera, A. 2014

Hybridization between *Calopteryx haemorrhoidalis* and any of its congeners has not been reported until now. We observed spontaneous matings between male *C.*

splendens and female *C. haemorrhoidalis* at a locality in Central Italy, together with some putative **hybrid** individuals that had a mixed phenotype. Here, we report the morphological and molecular characterization of five suspected **hybrids** collected from this population during 2001 (n=1), 2012 (n=2) and 2013 (n=2). A discriminant analysis based on 13 morphological variables correctly separated both parental species (with 100% assignation success) and classified the **hybrid** from 2001 as splendens phenotype and those from 2012 and 2013 as haemorrhoidalis. Genotype data (microsatellite loci) was used to confirm the **hybrid** origin of these specimens, although there were differences between the individual from 2001 and those from 2012 and 2013; the 2001 individual had alleles that were present in both parent species, suggesting it is an F1 **hybrid**, but the individuals collected in 2012 and 2013 had private alleles at eight (out of 12) loci and only a small portion of the genome in common with *C. splendens*, which suggests that introgression is occurring in this population. Similarities in mitochondrial DNA sequences indicate that the 2001 **hybrid** and the 2012-2013 **hybrids** have splendens and haemorrhoidalis maternal origins respectively, which, in contrast with behavioural observations, indicates that interspecific matings in both directions are possible. This is the first demonstration that *C. haemorrhoidalis* can **hybridize** with other congeners to produce viable offspring.

Seasonal Migration of *Pantala flavescens* Across the Bohai Strait in Northern China

Cao, LZ; Fu, XW; Hu, CX; Wu, KM. 2018

Pantala flavescens (Fabricius 1798) (Odonata: Libellulidae) is one of the most common species of migratory dragonflies. *P. flavescens* adults were captured by a searchlight trap on Beihuang Island (BH Island; 38 degrees 24'N, 120 degrees 55'E) from 2003 to 2016, where there is no freshwater. This inspired our research to analyze the pattern of seasonal migration and population dynamics. Stable hydrogen isotope measurement and the **Hybrid** Single Particle Lagrangian Integrated Trajectory (HYSPLIT) were used to simulate the migration pathway of *P. flavescens* between different breeding habitats. The results showed that there was no significant difference among population numbers of this overseas migration across years (F-13,F- 2161 = 0.85, P = 0.604); however, the numbers were significantly different across months (F-5,F- 2161 = 3.91, P = 0.003). Our geospatial natal assignment model suggested that *P. flavescens* trapped on BH were originated in different geographical regions and might have three movement strategies: wandering around northern China and north-bound (positive) and south-bound (negative) movements. Among them, the majority were engaged in wandering around northern China. Model simulations suggested that *P. flavescens* toured around BH. The results contribute to the knowledge of *P. flavescens* population ecology in a large-scale geographic region and will aid in the prediction and interpretation of insect migration patterns in response to climate change.

Morphological variability of *Cordulegaster trinacriae* in Italy (Odonata: Cordulegastridae)

Corso, A. 2019

This paper describes the most helpful features for field identification of *C. trinacriae* and the relevant morphological variability encountered. In *C. trinacriae* the yellow frons was without a dark horizontal marking in about 70 % of the sample or showed a barely patterned frons, while the remaining 30 % showed a more or less defined dark smudge, though always narrower and less bold than in *C. boltonii*. The percentage of unmarked versus marked frons was similar throughout the distributional range of *C. trinacriae* except in Sicily, where the unmarked yellow frons was much more common (87 %) and in Campania, where individuals with a dark mark on the frons were slightly more common than unmarked ones. In *C. trinacriae* the occipital triangle was almost always yellow with no or almost no dark markings, cleaner, and purer yellow than in *C. boltonii* from central Italy. The appendages of *C. trinacriae* always showed the characteristic form except for a few individuals, which might be **hybrids**. In *C. boltonii* they were found to be rather variable, especially on individuals from the southern part of its distributional range in central Italy, where intermediates and **hybrids** occur. Here, the appendages were sometimes similar (but not identical) to *C. trinacriae*, and therefore, this character is considered of limited use in the field. Individuals of *C. trinacriae* can be distinguished in areas of sympatry from intermediate *C. boltonii* and from **hybrids** when they show the following characters: i) unmarked yellow frons; ii) bright yellow, almost unmarked occipital triangle; ii) typical deeply notched lower appendages and long, sinuous upper appendages.

Strong genetic divergence indicates that congeneric damselflies *Coenagrion puella* and *C-pulchellum* (Odonata : Zygoptera : Coenagrionidae) do not hybridise

Lowe, CD; Harvey, IF; Thompson, DJ; Watts, PC. 2008

Coenagrionid damselflies are in general decline in the British Isles. Numerous factors have been implicated in the loss of these species including recent speculation that **hybridisation** between congeners may result in species decline. Here we use a panel of 12 microsatellite loci to examine levels of genetic divergence and the likely occurrence of **hybridisation** in five populations of *Coenagrion puella* and *C. pulchellum* using samples from four sites in south-east England. *Coenagrion puella* and *C. pulchellum* were highly genetically divergent, and there was no evidence of **hybridisation** between any of the populations examined, even where *C. puella* and *C. pulchellum* were sympatric. There was some suggestion that *C. pulchellum* was less genetically diverse than *C. puella*, though this may have been a result of ascertainment bias associated with cross-species application of microsatellite markers. We conclude that there is no evidence that **hybridisation** between *C. puella/pulchellum* could be responsible for the on-going demographic decline in *C. pulchellum*. Nevertheless, further genetic studies such as this one are likely to provide estimates of diversity, population structure and dispersal capacity that will be invaluable in future conservation management strategies for coenagrionid damselflies.

The Oracle of Delphi-a molecular phylogenetic approach to Greek *Cordulegaster* Leach in Brewster, 1815 (Odonata: Anisoptera: Cordulegastridae)

Schneider, T; Vierstraete, A; Ikemeyer, D; Van Pelt, GJ; Caspers, M; Muller, O; Dumont, HJ. 2022

Taxonomy of the genus *Cordulegaster* Leach in Brewster, 1815 in Greece is not completely understood. The taxonomic status of the subspecies *C. helladica buchholzi* (Lohmann, 1993), *C. helladica kastalia* (Lohmann, 1993), and *C. heros pelionensis* Theischinger, 1979 was still unclear. We applied a molecular genetic approach using sequences of mitochondrial and nuclear DNA fragments-cytochrome c oxidase I (COI) and Internal Transcribed Spacer 1 (ITS1). This approach revealed that specimens presently assigned to *C. heros pelionensis* should be considered as conspecific to the nominate subspecific taxon making *C. heros* a monotypic species. Two major monophyletic lines were found within the Greek representatives of the species grouped around *C. bidentata* Selys, 1843: the clade of the European endemic *C. bidentata* and the clade composed of three species: *C. helladica* (Lohmann, 1993), *C. buchholzi* (stat. nov., raised to species level), and *C. insignis* Schneider, 1845. *Cordulegaster helladica* is restricted to the Peloponnese. *Cordulegaster buchholzi* is not restricted to the Cyclades as previously thought, but widespread from the Cyclades over the island Euboea to south-east mainland Greece reaching in the west near Mount Parnassos, where it **hybridize** with *C. bidentata*. **Hybridization** between *C. bidentata* and *C. buchholzi* was detected at the Castalian Spring, where in ancient times the Oracle of Delphi was located, and some kilometres east of the Castalian Spring. These **hybrids** had been formerly named *C. helladica kastalia*. In the case of *C. insignis montandoni* St. Quentin, 1971 we have investigated specimens some kilometres away from the type locality in Romania, which all revealed **hybrids** between *C. bidentata* and *C. insignis*. However, we do not know if specimens phenotypically looking like *C. insignis* from further west in the SE Balkans represent isolated population of *C. insignis* within the range of *C. bidentata* or belong to a broader **hybrid** zone between *C. bidentata* and *C. insignis*.

ENALLAGMA OPTIMOLOCUS, A NEW SPECIES OF DAMSELFLY FROM MONTANA (ODONATA, COENAGRIONIDAE)

MILLER, KB; IVIE, MA. 1995

Enallagma optimolocus n. sp. is described and diagnosed from streams in western Montana. Garrison's 1984 key is modified to distinguish this species from the related *E. carunculatum* Morse and *E. civile* (Hagen). The competing aspects of two views of these specimens, i.e. full species or **hybrid** individuals, is discussed. The occurrence of *E. carunculatum* and *E. anna* Williamson at the collecting sites is noted. The absence of *E. civile* from the areas surrounding the range of *E. optimolocus* is noted, with Roemhild's 1975 (and Garrison's 1984) record of *E. civile* for Flathead Co., Montana, corrected to *E. carunculatum*. Illustrations of diagnostic features for the species are given.

Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion* (Odonata : Coenagrionidae)

Jordan, S; Simon, C; Polhemus, D. 2003

Damselflies of the endemic Hawaiian genus *Megalagrion* have radiated into a wide variety of habitats and are an excellent model group for the study of adaptive radiation. Past phylogenetic analysis based on morphological characters has been problematic. Here, we examine relationships among 56 individuals from 20 of the 23 described species using maximum likelihood (ML) and Bayesian phylogenetic analysis of mitochondrial (1,287 bp) and nuclear (1,039 bp) DNA sequence data. Models of evolution were chosen using the Akaike information criterion. Problems with distant outgroups were accommodated by constraining the best ML ingroup topology but allowing the outgroups to attach to any ingroup branch in a bootstrap analysis. No strong contradictions were obtained between either data partition and the combined data set. Areas of disagreement are mainly confined to clades that are strongly supported by the mitochondrial DNA and weakly supported by the elongation factor 1alpha data because of lack of changes. However, the combined analysis resulted in a unique tree. Correlation between Bayesian posterior probabilities and bootstrap percentages decreased in concert with decreasing information in the data partitions. In cases where nodes were supported by single characters bootstrap proportions were dramatically reduced compared with posterior probabilities. Two speciation patterns were evident from the phylogenetic analysis. First, most speciation is interisland and occurred as members of established ecological guilds colonized new volcanoes after they emerged from the sea. Second, there are several instances of rapid radiation into a variety of specialized habitats, in one case entirely within the island of Kauai. Application of a local clock procedure to the mitochondrial DNA topology suggests that two of these radiations correspond to the development of habitat on the islands of Kauai and Oahu. About 4.0 million years ago, species simultaneously moved into fast streams and plant leaf axils on Kauai, and about 1.5 million years later another group moved simultaneously to seeps and terrestrial habitats on Oahu. Results from the local clock analysis also strongly suggest that *Megalagrion* arrived in Hawaii about 10 million years ago, well before the emergence of Kauai. Date estimates were more sensitive to the particular node that was fixed in time than to the model of local branch evolution used. We propose a general model for the development of endemic damselfly species on Hawaiian Islands and document five potential cases of **hybridization** (*M. xanthomelas* x *M. pacificum*, *M. eudytum* x *M. vagabundum*, *M. orobates* x *M. oresitrophum*, *M. nesiotus* x *M. oahuense*, and *M. mauka* x *M. paludicola*).

New data on the distribution of the Italian endemic *Cordulegaster trinacriae* (Odonata: Cordulegastridae)

Corso, A; Penna, V; Janni, O; De Lisio, L; Biscaccianti, A; Holusa, O; Mastropasqua, F. 2020

Data on the northern and eastern limits of the distribution of the Italian endemic *C. trinacriae* are reported, together with further details on the first records for Abruzzo and Puglia, thereby re-defining its known range. The species is common in suitable habitats in central-southern Italy, extending further north along the Adriatic. In contrast to previous reports, along the central Tyrrhenian area *C. boltonii* and its **hybrids** replace *C. trinacriae* with phenotypically pure specimens only in southern Lazio and northern Campania. The flight period extends from late May to late September. The known altitudinal distribution ranges from 9 to 1639 m a.s.l. We consider the possibility that former records of *C. boltonii* from Abruzzo, Molise and Campania resulted from confusion with *C. trinacriae*. In these regions *C. boltonii* either never occurred or has now been largely replaced by *C. trinacriae* and is extremely localised.

Combining DNA sequences and morphology in systematics: testing the validity of the dragonfly species *Cordulegaster bilineata*

Pilgrim, EM; Roush, SA; Krane, DE. 2002

Morphological and molecular techniques are rarely combined when answering questions of taxonomic validity. In this study, we combine morphological techniques with DNA sequences to determine the validity of the dragonfly species *Cordulegaster bilineata*. The two dragonfly species *C. bilineata* and *C. diastatops* are very similar in size, body color, and morphological characters, and due to these similarities, the status of *C. bilineata* as a valid species is in question. In this study we compare morphological measurements of males and internal transcribed spacer 1 (ITS-1) sequences of rDNA between the two taxa. The hamule measurements (where copulation occurs) of males show little difference between the taxa in question, but the anal appendage measurements (where the male first contacts the female) show marked divergence between the two taxa. Cluster analysis with these anal appendage measurements correctly assigns almost all individuals measured into their respective taxon. PCR amplification products of ITS-1 display a similar to 50 bp size difference between *C. bilineata* (n = 4) and *C. diastatops* (n = 5) regardless of collection site. Sequence data for these amplifications show 51 bp missing in one locus in the ITS-1 of *C. bilineata* relative to *C. diastatops*. A lone population of *C. diastatops* from Wisconsin has three individuals with ITS-1 products that match the size of both *C. bilineata* and *C. diastatops*. One individual from this population appears to yield two ITS-1 amplification products that match both *C. bilineata* and *C. diastatops*. Although this population may be evidence for **hybridization** between the two taxa, such **hybridization** is not necessarily sufficient to disqualify the validity of a separate species designation for *C. bilineata*. Morphology and ITS-1 sequences depict a high degree of divergence that is consistent with species-level differences.

Hybridization in *Calopteryx* damselflies: the role of males

Tynkynen, K; Grapputo, A; Kotiaho, JS; Rantala, MJ; Vaananen, S; Suhonen, J. 2008

Females are often considered responsible for **hybridization** between two species because usually they are the choosier sex and their cooperation is needed for successful copulation. However, males can also be responsible for **hybridization**, for example in species in which males are able to force copulation. We studied the pattern of **hybridization** in two congeneric damselfly species, *Calopteryx splendens* and *Calopteryx virgo*, and provide evidence that F-1 **hybrids** between the two damselfly species occur in the wild. According to mitochondrial DNA analysis, **hybridization** is reciprocal: five of seven **hybrids** were sired by *C. splendens* and two by *C. virgo* males. We conducted an experiment that revealed that males of both species have surprisingly poor premating reproductive isolation in that they accept heterospecific females, but *C. splendens* males were less discriminating against con- and heterospecific females than were *C. virgo* males. Moreover, our data on the number of **hybrids** sired by either species in the wild are congruent with the results of the discrimination experiment, supporting the conclusion that males may be responsible for the **hybridization**. Our results suggest that the males' role in **hybridization** studies should no longer be neglected. (C) 2007 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Hybridization rate and climate change: are endangered species at risk?

Sanchez-Guillen, RA; Munoz, J; Hafernik, J; Tierney, M; Rodriguez-Tapia, G; Cordoba-Aguilar, A. 2014

Many species are altering their geographic range due to climate change creating new sympatric populations of otherwise allopatric populations. We investigated whether climate change will affect the distribution and thus the pattern of **hybridization** between two pairs of closely related damselfly species [*Ischnura damula* and

I. demorsa, and *I. denticollis* and *I. gemina* (this, an endangered species)]. Thus, we estimated the strength of pre and postmating reproductive barriers between both pairs of species, and we predicted future potential distribution under four different Global Circulation Models and a realistic emissions scenario of climate change by using maximum entropy modelling technique. Our results showed that reproductive isolation (RI) is complete in *I. damula* x *I. demorsa* individuals: F-1 (first generation) **hybrids** are produced but do not reach sexual maturation. However, RI in *I. denticollis* x *I. gemina* **hybrids** is high but incomplete and unidirectional: only *I. gemina* females produced F-1 **hybrids** which mate with males and females of *I. denticollis* and between them producing BC1 (back-crosses) and F-2 (second generation) viable **hybrids**. Maximum entropy models revealed a northern and westward shift and a general reduction of the potential geographic ranges. Based on the pattern of **hybridization**, for *I. damula* and *I. demorsa* there is a current threat as well as a rapid displacement and/or extinction of *I. gemina* by *I. denticollis*. However, the current pattern of extinction may not continue due to the contraction in ranges of the four species.

ACTUAL DISTRIBUTION AND TAXONOMIC RANK OF ORTHETRUM-COERULESCENS (FABRICIUS) AND O-RAMBURI (SELYS) = O-ANCEPS (SCHNEIDER) (INSECTA, ODONATA)

MAUERSBERGER, R. 1994

Orthetrum coerulescens (FABRICIUS) and *O. anceps* (SCHNEIDER) are very closely related forms, the first of which is distributed in Europe farther northwest than the latter. For the distinction of these two forms, only one reliable character (structure of lamina anterior of the 2nd abdominal segment in males) can be utilized. A review of the localities of museum specimens revealed that the two taxa are allopatric with a belt of secondary intergradation along the line of contact. **Hybrid** populations exist in Sicily as well as in Hungary and former Yugoslavia. Whereas the influence of *Orthetrum coerulescens* on the phenotypes decreases gradually from west to east on the Balkan peninsula (but is still recognizable even on Crete), the parental forms persist, isolated by the "Strait of Bonifacio", on Sardinia and Corsica, respectively. Single **hybrids** have been found, however, far outside the zone of secondary intergradation. It is obvious, hence, that *Orthetrum coerulescens* and *O. anceps* are not full species but either members of a superspecies or even subspecies which would have to be united under the older name *Orthetrum coerulescens* (FABRICIUS).

Introgression and rapid species turnover in sympatric damselflies

Sanchez-Guillen, RA; Wellenreuther, M; Cordero-Rivera, A; Hansson, B. 2011

Background: Studying contemporary **hybridization** increases our understanding of introgression, adaptation and, ultimately, speciation. The sister species *Ischnura elegans* and *I. graellsii* (Odonata: Coenagrionidae) are ecologically, morphologically and genetically similar and **hybridize**. Recently, *I. elegans* has colonized northern Spain, creating a broad sympatric region with *I. graellsii*. Here, we review the distribution of both species in Iberia and evaluate the degree of introgression of *I. graellsii* into *I. elegans* using six microsatellite markers (442 individuals from 26 populations) and five mitochondrial genes in sympatric and allopatric localities. Furthermore, we quantify the effect of **hybridization** on the frequencies of the genetically controlled colour polymorphism in females of both species. Results: In a principal component analysis of the microsatellite data, the first two principal components summarised almost half (41%) of the total genetic variation. The first axis revealed a clear separation of *I. graellsii* and *I. elegans* populations, while the second axis separated *I. elegans* populations. Admixture analyses showed extensive **hybridization** and introgression in *I. elegans* populations, consistent with *I. elegans* backcrosses and occasional F-1-**hybrids**, suggesting **hybridization** is on-going. More specifically, approximately 58% of the 166 Spanish *I. elegans* individuals were assigned to the *I. elegans* backcross category, whereas not a single of those individuals was assigned to the backcross with *I. graellsii*. The mitochondrial genes held little genetic variation, and the most common haplotype was shared by the two species. Conclusions: The results suggest rapid species turnover in sympatric regions in favour of *I. elegans*, corroborating previous findings that *I. graellsii* suffers a mating disadvantage in sympatry with *I. elegans*. Examination of morph frequency dynamics indicates that **hybridization** is likely to have important implications for the maintenance of multiple female morphs, in particular during the initial period of **hybridization**.

Taxonomic Revision of Eastern Part of Western Palaearctic Cordulegaster Using Molecular Phylogeny and Morphology, with the Description of Two New Species (Odonata: Anisoptera: Cordulegastridae)

Schneider, T; Vierstraete, A; Mueller, O; van Pelt, GJ; Caspers, M; Ikemeyer, D; Snegovaya, N; Dumont, HJ. 2021

Taxonomy of the genus *Cordulegaster* Leach in Brewster, 1815 in the Eastern part of the Western Palaearctic is poorly resolved. A two-step approach was applied: sequences of mitochondrial and nuclear DNA fragments were used to sort specimens; poorly known or new taxa with their phenotypic variation were described. The existence of two traditional groups (boltonii- and bidentata-group) was confirmed. *Cordulegaster coronata* Morton, 1916, however, belongs to a different group. Molecular-analysis supported three known and one new species (*C. heros* Theischinger, 1979, *C. picta* Selys, 1854, *C. vanbrinkae* Lohmann, 1993, and *C. kalkmani* sp. nov.) in the boltonii-group. In the bidentata-group, all specimens from West-Turkey belonged to *C. insignis* Schneider, 1845, all specimens further east to a complex of four closely related species, which we name charpentieri-complex (*C. amasina* Morton, 1916, stat. rev., *C. mzymtae* Bartenev, 1929 *C. charpentieri* (Kolenati, 1846), stat. rev. and *C. cilicia* sp. nov.). The following taxa: *C. insignis nobilis* Morton, 1916, syn. nov., *C. nachitschevanica* Skvortsov and Snegovaya, 2015, syn. nov. *C. plagionyx* Skvortsov and Snegovaya, 2015, syn. nov. and the Caucasian subspecies *C. insignis lagodechica* Bartenev, 1930, syn. nov., were synonymized with *C. charpentieri*. Finally, we provide a key for all Western Palaearctic *Cordulegaster*.

Predicting hybridisation as a consequence of climate change in damselflies

Nava-Bolanos, A; Sanchez-Guillen, RA; Wellenreuther, M; Munoz, J; Torres-Pachon, M; Novelo-Gutierrez, R; Cordoba-Aguilar, A. 2019

Climate change is a key stressor for species. Two major consequences of climate-induced range shifts are the formation of new areas of geographic overlap (i.e. sympatry) and an increased probability of **hybridisation** in the de novo created contact zones. One method to effectively quantify the potential of **hybridisation** is to integrate ecological niche modelling and the propensity to **hybridisation** based on genetic divergence. In this paper, we have applied this methodology to predict **hybridisation** outcomes following different scenarios of climate change in 30 species of Argia damselflies. We (i) investigated how climate change may affect species' distributions; (ii) quantified if changed distributions generate new areas of sympatry between species; (iii) calculated the propensity to **hybridise** based on genetic divergence between species; and (iv) integrated these data to predict the future potential of species to **hybridise**. We found that the distribution of 29 of the 30 species was affected by a change in climate which led to a general increase in sympatric overlap among species. The degree of genetic divergence among the 108 species' combinations ranged from 0.06% to 0.36%. Based on the sympatric overlap and genetic divergence, it can be predicted that 97 of the species pairs are likely to **hybridise** in the future. Our results are useful to forecast how highly diverse and closely related groups, such as Argia damselflies, may respond to a change in climate and how this can impact the potential of species mixing under a scenario of increased global warming.

Regional divergence and mosaic spatial distribution of two closely related damselfly species (Enallagma hageni and Enallagma ebrium)

Bourret, A; McPeck, MA; Turgeon, J. 2012

North American *Enallagma* damselflies radiated during the Pleistocene, and species differ mainly by reproductive structures. Although morphologically very different, *Enallagma hageni* and *Enallagma ebrium* are genetically very similar. Partitioning of genetic variation (AFLP), isolation by distance and clustering analyses indicate

that these morphospecies are locally differentiated genetically. Spatial analyses show that they are rarely sympatric at local sites, and their distributions form a mosaic of patches where one is clearly dominant over hundreds of square kilometers. However, these morphospecies are also not genetically more similar when they are sympatric, indicating that **hybridization** is probably not occurring. Given that these morphospecies are ecologically equivalent, strong assortative mating, reproductive interference and fast post-glacial recolonization may explain the origin and maintenance of these distributional patches across eastern North America. By limiting opportunities for gene flow, reproductive interference may play an unsuspected role in accelerating genetic differentiation in the early phases of nonecological speciation.

Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*

Tynkynen, K; Rantala, MJ; Suhonen, J. 2004

Problems in species recognition are thought to affect the evolution of secondary sexual characters mainly through avoidance of maladaptive **hybridization**. Another, but much less studied avenue for the evolution of sexual characters due to species recognition problems is through interspecific aggression. In the damselfly, *Calopteryx splendens*, males have pigmented wing spots as a sexual character. Large-spotted males resemble males of another species, *Calopteryx virgo*, causing potential problems in species recognition. In this study, we investigate whether there is character displacement in wing spot size and whether interspecific aggression could cause this pattern. We found first that wing spot size of *C. splendens* in populations decreased with increasing relative abundance of *C. virgo*. Secondly, *C. virgo* males were more aggressive towards large- than small-spotted *C. splendens* males. Thirdly, in interspecific contests *C. virgo* males had better territory holding ability than *C. splendens* males. These results suggest that interspecific aggression may have caused character displacement in wing spot size of *C. splendens*, because the intensity of aggression towards large-spotted males is likely to increase with relative abundance of *C. virgo* males. Thus, interspecific aggression may be an evolutionarily significant force that is able to cause divergence in secondary sexual characters.

Mechanical and tactile incompatibilities cause reproductive isolation between two young damselfly species

Barnard, AA; Fincke, OM; McPeck, MA; Masly, JP. 2017

External male reproductive structures have received considerable attention as a cause of reproductive isolation (RI), because the morphology of these structures often evolves rapidly between populations. This rapid evolution presents the potential for mechanical incompatibilities with heterospecific female structures during mating and could thus prevent interbreeding between nascent species. Although such mechanical incompatibilities have received little empirical support as a common cause of RI, the potential for mismatch of reproductive structures to cause RI due to incompatible species-specific tactile cues has not been tested. We tested the importance of mechanical and tactile incompatibilities in RI between *Enallagma anna* and *E. carunculatum*, two damselfly species that diverged within the past similar to 250,000 years and currently **hybridize** in a sympatric region. We quantified 19 prezygotic and postzygotic RI barriers using both naturally occurring and laboratory-reared damselflies. We found incomplete mechanical isolation between the two pure species and between **hybrid** males and pure species females. Interestingly, in mating pairs for which mechanical isolation was incomplete, females showed greater resistance and refusal to mate with **hybrid** or heterospecific males compared to conspecific males. This observation suggests that tactile incompatibilities involving male reproductive structures can influence female mating decisions and form a strong barrier to gene flow in early stages of speciation.

STRONG ASYMMETRY IN THE RELATIVE STRENGTHS OF PREZYGOTIC AND POSTZYGOTIC BARRIERS BETWEEN TWO DAMSELFLY SISTER SPECIES

Sanchez-Guillen, RA; Wullenreuther, M; Rivera, AC. 2012

One of the longest debates in biology has been over the relative importance of different isolating barriers in speciation. However, for most species, there are few data evaluating their relative contributions and we can only speculate on the general roles of pre- and postzygotic isolation. Here, we quantify the absolute and cumulative contribution of 19 potential reproductive barriers between two sympatric damselfly sister species, *Ischnura elegans* and *I. graellsii*, including both premating (habitat, temporal, sexual and mechanical isolation) and postmating barriers (prezygotic: sperm insemination success and removal rate, oviposition success, fertility, fecundity; postzygotic: **hybrid** viability, **hybrid** sterility and **hybrid** breakdown). In sympatry, total reproductive isolation between *I. elegans* females and *I. graellsii* males was 95.2%, owing mostly to a premating mechanical incompatibility (93.4%), whereas other barriers were of little importance. Isolation between *I. graellsii* females and *I. elegans* males was also nearly complete (95.8%), which was caused by the cumulative action of multiple prezygotic (n= 4, 75.4%) and postzygotic postmating barriers (n= 5, 7.4%). Our results suggest that premating barriers are key factors in preventing gene flow between species, and that the relative strengths of premating barriers is highly asymmetrical between the reciprocal crosses.

Do allopatric male *Calopteryx virgo* damselflies learn species recognition?

Kuitunen, K; Haukilehto, E; Raatikainen, KJ; Hakkarainen, H; Miettinen, M; Hogmander, H; Kotiaho, JS. 2012

There is a growing amount of empirical evidence that premating reproductive isolation of two closely related species can be reinforced by natural selection arising from avoidance of maladaptive **hybridization**. However, as an alternative for this popular reinforcement theory, it has been suggested that learning to prefer conspecifics or to discriminate heterospecifics could cause a similar pattern of reinforced premating isolation, but this possibility is much less studied. Here, we report results of a field experiment in which we examined (i) whether allopatric *Calopteryx virgo* damselfly males that have not encountered heterospecific females of the congener *C. splendens* initially show discrimination, and (ii) whether *C. virgo* males learn to discriminate heterospecifics or learn to associate with conspecifics during repeated experimental presentation of females. Our experiment revealed that there was a statistically nonsignificant tendency for *C. virgo* males to show initial discrimination against heterospecific females but because we did not use sexually naive individuals in our experiment, we were not able to separate the effect of innate or associative learning. More importantly, however, our study revealed that species discrimination might be further strengthened by learning, especially so that *C. virgo* males increase their association with conspecific females during repeated presentation trials. The role of learning to discriminate *C. splendens* females was less clear. We conclude that learning might play a role in species recognition also when individuals are not naive but have already encountered potential conspecific mates.

The movement responses of three libellulid dragonfly species to open and closed landscape cover

French, SK; McCauley, SJ. 2019

The land cover between habitats (i.e. matrix environment) can affect connectivity by impacting organismal movement. Many animals, however, have preferences for specific matrix environments, which can affect their movement through the landscape. We examined how different terrestrial matrix environments impacted the fine-scale movement of adult dragonflies. Based on previous studies of adult dragonfly dispersal and larval distributions, we hypothesised that dragonflies would prefer to enter fields rather than forests and that forests would be a barrier to dragonfly movement, due to forests' structural complexity, low understorey light availability, and lower air temperatures. To test how adult dragonflies responded to various terrestrial environments, we released 108 *Leucorrhinia intacta*, a mixture of 108 *Sympetrum rubicundulum* and *obtrusum/rubicundulum* **hybrids**, and 108 *Sympetrum vicinum*, at field-forest ecotones and assessed their preferences for fields or forests. Individual behavioural responses were recorded, including their probability of taking flight, their direction of movement with respect to the two matrix types,

and flight time. The likelihood of adult dragonflies taking flight was species-specific in response to release location. Adults moved more frequently towards fields than forests when released at a forest edge. Individuals released within forests had shorter flight times, but again this response was species-specific. The presence of an open matrix (field or meadow) is likely important for facilitating movement in dragonflies; however, forests are not movement barriers for all dragonfly species. Integrating assays of matrix and habitat preferences can provide insight into how landscape connectivity can be maintained for actively dispersing species.

Climate-Induced Range Shifts and Possible Hybridisation Consequences in Insects

Sanchez-Guillen, RA; Munoz, J; Rodriguez-Tapia, G; Arroyo, TPF; Cordoba-Aguilar, A. 2013

Many ectotherms have altered their geographic ranges in response to rising global temperatures. Current range shifts will likely increase the sympatry and **hybridisation** between recently diverged species. Here we predict future sympatric distributions and risk of **hybridisation** in seven Mediterranean ischnurid damselfly species (*I. elegans*, *I. fountaineae*, *I. genei*, *I. graellsii*, *I. pumilio*, *I. saharensis* and *I. senegalensis*). We used a maximum entropy modelling technique to predict future potential distribution under four different Global Circulation Models and a realistic emissions scenario of climate change. We carried out a comprehensive data compilation of reproductive isolation (habitat, temporal, sexual, mechanical and gametic) between the seven studied species. Combining the potential distribution and data of reproductive isolation at different instances (habitat, temporal, sexual, mechanical and gametic), we infer the risk of **hybridisation** in these insects. Our findings showed that all but *I. graellsii* will decrease in distributional extent and all species except *I. senegalensis* are predicted to have northern range shifts. Models of potential distribution predicted an increase of the likely overlapping ranges for 12 species combinations, out of a total of 42 combinations, 10 of which currently overlap. Moreover, the lack of complete reproductive isolation and the patterns of **hybridisation** detected between closely related ischnurids, could lead to local extinctions of native species if the **hybrids** or the introgressed colonising species become more successful.

Genetic similarity within and among populations of the Variable and Azure damselflies (*Coenagrion pulchellum* and *C. puella*)

Freeland, JR; Conrad, KF. 2002

In the first half of this century, seven species of the damselfly genus *Coenagrion* regularly bred in Britain. Since that time, two of these species have become extinct, and three currently have highly restricted distributions. Of the remaining two species, the Azure Damselfly (*C. puella*) is both common and abundant, but the Variable Damselfly (*C. pulchellum*), while more common than most *Coenagrion* species, is experiencing a national decline in Britain. The reasons for the decline of *C. pulchellum* are poorly understood, and therefore its future in Britain is difficult to predict. The aim of this study was to investigate genetic relationships among populations of *C. puella* and *C. pulchellum*. We obtained mitochondrial sequence data from 36 *C. puella* and *C. pulchellum* individuals collected from five different sites across central England. These revealed three haplotypes with high overall similarity. **Hybridisation** between *C. puella* and *C. pulchellum* was suggested by (1) The sharing of a haplotype between *C. puella* and *C. pulchellum*, and (2) The fact that morphological characters of sympatric *C. puella* and *C. pulchellum* populations are not always species-specific. More research is required before we can determine whether or not **hybridisation** is playing a role in the decline of *Coenagrion* species in the U. K.

Divergence in female damselfly sensory structures is consistent with a species recognition function but shows no evidence of reproductive character displacement

Barnard, AA; Masly, JP. 2018

Males and females transmit and receive signals prior to mating that convey information such as sex, species identity, or individual condition. In some animals, tactile signals relayed during physical contact between males and females before and during mating appear to be important for mate choice or reproductive isolation. This is common among odonates, when a male grasps a female's thorax with his terminal appendages prior to copulation, and the female subsequently controls whether copulation occurs by bending her abdomen to complete intromission. It has been hypothesized that mechanosensory sensilla on the female thoracic plates mediate mating decisions, but is has been difficult to test this idea. Here, we use North American damselflies in the genus *Enallagma* (Odonata: Coenagrionidae) to test the hypothesis that variation in female sensilla traits is important for species recognition. *Enallagma anna* and *E. carunculatum* **hybridize** in nature, but experience strong reproductive isolation as a consequence of divergence in male terminal appendage morphology. We quantified several mechanosensory sensilla phenotypes on the female thorax among multiple populations of both species and compared divergence in these traits in sympatry versus allopatry. Although these species differed in features of sensilla distribution within the thoracic plates, we found no strong evidence of reproductive character displacement among the sensilla traits we measured in regions of sympatry. Our results suggest that species-specific placement of female mechanoreceptors may be sufficient for species recognition, although other female sensory phenotypes might have diverged in sympatry to reduce interspecific **hybridization**.

Oviposition of *Culex tarsalis* (Diptera: Culicidae) Differs on Water Conditioned by Potential Fish and Insect Predators

Why, AM; Lara, JR; Walton, WE. 2016

The response of egg-laying *Culex tarsalis* Coquillett (Diptera: Culicidae) to water conditioned by three fish species used for mosquito control and three predatory aquatic insect species was examined in laboratory binary choice experiments. Oviposition by *Cx. tarsalis* was 72% less on water conditioned with the arroyo chub, *Gila orcutti* (Eigenmann & Eigenmann) (Cypriniformes: Cyprinidae) relative to control cups containing aged tap water, but no significant difference was found in the numbers of egg rafts laid on water conditioned with the fathead minnow (*Pimephales promelas* (Rafinesque), Cypriniformes: Cyprinidae) and the control treatment (water aged 24 h). Mosquito oviposition on water conditioned with the predominantly herbivorous/algivorous California Mozambique tilapia **hybrid** (*Oreochromis mossambicus* (Peters) x *Oreochromis urolepis hornorum* L. (Perciformes: Cichlidae)) or predatory insects (nymphs: *Sympetrum corruptum* (Hagen) (Odonata: Libellulidae); adults: *Thermonectus basillaris* (Harris) or *Cyrtobister fimbriolatus* (Say) (Coleoptera: Dytiscidae)) did not differ significantly relative to that onto water aged for 24 h. As compared with water aged 24 h and water conditioned with diving beetles, oviposition by *Cx. tarsalis* was significantly lower ($\geq 53\%$) when live predatory diving beetles were present in oviposition cups. Gravid *Cx. tarsalis* females do not respond equally to putative semiochemicals in water conditioned with the piscine or aquatic insect predators of immature mosquitoes tested here.

SIMULATING RANGE EXPANSION: MALE SPECIES RECOGNITION AND LOSS OF PREMATING ISOLATION IN DAMSELFLIES

Wellenreuther, M; Tynkkynen, K; Svensson, EI. 2010

Prolonged periods of allopatry might result in loss of the ability to discriminate against other formerly sympatric species, and can lead to heterospecific matings and **hybridization** upon secondary contact. Loss of premating isolation during prolonged allopatry can operate in the opposite direction of reinforcement, but has until now been little explored. We investigated how premating isolation between two closely related damselfly species, *Calopteryx splendens* and *C. virgo*, might be affected by the expected future northward range expansion of *C. splendens* into the allopatric zone of *C. virgo* in northern Scandinavia. We simulated the expected secondary contact by presenting *C. splendens* females to *C. virgo* males in the northern allopatric populations in Finland. Premating isolation toward *C. splendens* in northern allopatric populations was compared to sympatric populations in southern Finland and southern Sweden. Male courtship responses of *C. virgo* toward conspecific females showed limited geographic variation, however, courtship attempts toward heterospecific *C. splendens* females increased significantly from sympatry to allopatry. Our results suggest that allopatric *C. virgo* males have partly lost their ability to discriminate against heterospecific females. Reduced premating isolation in

allopatry might lead to increased heterospecific matings between taxa that are currently expanding and shifting their ranges in response to climate change.

Inventing a Biologically Inspired, Energy Efficient Micro Aerial Vehicle

Ratti, J; Vachtsevanos, G. 2012

In recent years, research efforts have focused on the design, development and deployment of unmanned systems for a variety of applications ranging from intelligence and surveillance to border patrol, rescue operations, etc. Micro Aerial Vehicles are viewed as potential targets that can provide agility and accurate small area coverage while being cost-effective and can be easily launched by a single operator. The small size of MAVs allows such flight operations within confined space but the control effectors must provide sufficient maneuverability, while maintaining stability, with only limited sensing capability onboard the platform. To meet these challenges, researchers have long been attracted by the amazing attributes of biological systems, such as those exhibited by birds and insects. Birds can fly in dense flocks, executing rapid maneuvers with g-loads far in excess of modern fighter aircrafts, and yet never collide with each other, despite the absence of air traffic controllers. This paper introduces a novel framework for the design and control of a Micro Air Vehicle. The vehicle's conceptual design is based on biologically-inspired principles and emulates a dragonfly (Odonata-Anisoptera). A sophisticated multi-layered **Hybrid & Linear/Non-Linear** controller to achieve extended flight times and improved agility compared to other Rotary and Flapping Wing MAV designs. The paper addresses the design and control features of the proposed QV design and gives an overview on the developmental efforts towards the prototyping of the flyer. The potential applications for such a high endurance vehicle are numerous, including air-deployable mass surveillance in cluster and swarm formations. The disposable nature of the vehicle would help in battle-field deployment as well, where such a MAV would be made available to soldiers for proximity sensing and threat level assessment. Other applications would include search and rescue operations and civilian law-enforcement.

A Biologically-Inspired Micro Aerial Vehicle

Ratti, J; Vachtsevanos, G. 2010

This paper introduces a novel framework for the design, modeling and control of a Micro Aerial Vehicle (MAV). The vehicle's conceptual design is based on biologically-inspired principles and emulates a dragonfly (Odonata-Anisoptera). We have taken inspiration from the flight mechanism features of the dragonfly and have developed indigenous designs in creating a novel version of a Flapping Wing MAV (FWMAV). The MAV design incorporates a complex mechanical construction and a sophisticated multi-layered, **hybrid**, linear/non-linear controller to achieve extended flight times and improved agility compared to other rotary wing and FWMAV Vertical Take Off and Landing (VTOL) designs. The first MAV prototype will have a ballpark weight including sensor payload of around 30 g. The targeted lifting capability is about twice the weight. The MAV features state of the art sensing and instrumentation payload, which includes integrated high-power on-board processors, 6DoF inertial sensors, 3DoF compasses, GPS, embedded camera and long-range telemetry capability. A 3-layer control mechanism has been developed to harness the dynamics and attain complete navigational control of the MAV. The inner-layer is composed of a 'quad **hybrid**-energy controller' and two higher layers are at present, implementing a linear controller; the latter will be replaced eventually with a dynamic adaptive non-linear controller. The advantages of the proposed design compared to other similar ones include higher energy efficiency and extended flight endurance. The design features elastic storage and re-use of propulsion energy favoring energy conservation during flight. The design/modeling of the MAV and its kinematics & dynamics have been tested under simulation to achieve desired performance. The potential applications for such a high endurance vehicle are numerous, including air-deployable mass surveillance and reconnaissance in cluster and swarm formations. The efficacy of the design is demonstrated through a simulation environment. The dynamics are verified through simulations and a general linear controller coupled with an energy based non-linear controller is shown to operate the vehicle in a stable regime. In accordance with specified objectives a prototype is being developed for flight-testing and demonstration purposes.

Rapid evolution of sexual signals in sympatric *Calopteryx damselflies*: reinforcement or 'noisy-neighbour' ecological character displacement?

Mullen, SP; Andres, JA. 2007

Enhanced prezygotic isolation in sympatry is one of the most intriguing patterns in evolutionary biology and has frequently been interpreted as evidence for reinforcement. However, the frequency with which reinforcement actually completes speciation remains unclear. The Jewelwing damselflies (*Calopteryx acquadilis* and *C. maculata*) have served as one of the few classic examples of speciation via reinforcement outside of *Drosophila*. Although evidence for wing pattern displacement and increased mate discrimination in this system have been demonstrated, the degree of **hybridization** and gene flow in nature are unknown. Here, we show that sympatric populations of these two species are the result of recent secondary contact, as predicted under a model of speciation via reinforcement. However, we found no phenotypic evidence of **hybridization** in natural populations and a complete association between species-specific haplotypes at two different loci (mitochondrial CO I and nuclear EF1-alpha), suggesting little or no contemporary gene flow. Moreover, genealogical and coalescent-based estimates of divergence times and migration rates indicate that, speciation occurred in the distant past. The rapid evolution of wing colour in sympatry is recent, therefore, relative to speciation and seems to be better explained by selection against wasting mating effort and/or interspecific aggression resulting from a 'noisy neighbour' signalling environment.

Reproductive interference between *Nehalennia damselfly* species

Van Gossum, H; Beirincx, K; Forbes, MR; Sherratt, TN. 2007

We tested the hypotheses that reproductive interference between 2 congeneric damselfly species influences their local population densities and the female morph ratios in one of the species. *Nehalennia irene* has 2 female types (andromorph and gynomorph), whereas *N. gracilis* exhibits only one female type. Andromorphic *N. irene* females not only resemble conspecific males in body coloration, but also resemble heterospecific females of *N. gracilis*. We predicted male *N. irene* to be most attracted to gynomorphs of *N. irene* and male *N. gracilis* to be least attracted to them. Further, if *N. gracilis* males harass andromorphic *N. irene* females excessively, then they may reduce andromorph frequencies of *N. irene* locally. Our results indicate **hybridization** to be prevented by a "lock-and-key" mechanism, but male *N. irene* often attempt mating with female *N. gracilis*. Contrary to prediction, andromorph frequency in *N. irene* did not depend on whether *N. irene* populations were in sympatry or allopatry with *N. gracilis*. As predicted, *N. irene* males attempted tandem formation most frequently with conspecific gynomorphs, while *N. gracilis* males made most heterospecific tandem attempts on *N. irene* andromorphs. Collectively, our results suggest that *N. gracilis* females may be frequently harassed by *N. irene* males, and that this may help explain the relative rarity of *N. gracilis*.

Interspecific interactions and learning variability jointly drive geographic differences in mate preferences

Verzijden, MN; Svensson, EI. 2016

Co-occurrence of closely related species can cause behavioral interference in mating and increase **hybridization** risk. Theoretically, this could lead to the evolution of more species-specific mate preferences and sexual signaling traits. Alternatively, females can learn to reject heterospecific males, to avoid male sexual interference from closely related species. Such learned mate discrimination could also affect conspecific mate preferences if females generalize from between species differences to prefer more species-specific mating signals. Female damselflies of the banded demoiselle (*Calopteryx splendens*) learn to reject heterospecific males

of the beautiful demoiselle (*C. virgo*) through direct premating interactions. These two species co-occur in a geographic mosaic of sympatric and microallopatric populations. Whereas *C. virgo* males have fully melanized wings, male *C. splendens* wings are partly melanized. We show that *C. splendens* females in sympatry with *C. virgo* prefer smaller male wing patches in conspecific males after learning to reject heterospecific males. In contrast, allopatric *C. splendens* females with experimentally induced experience with *C. virgo* males did not discriminate against larger male wing patches. Wing patch size might indicate conspecific male quality in allopatry. Co-occurrence with *C. virgo* therefore causes females to prefer conspecific male traits that are more species specific, contributing to population divergence and geographic variation in female mate preferences.

An examination of competitive gametic isolation mechanisms between the damselflies *Ischnura graellsii* and *I. elegans*

Sanchez-Guillen, RA; Cordoba-Aguilar, A; Cordero-Rivera, A. 2013

Recent findings suggest that postmating prezygotic isolation (i.e. gametic barriers) could be an important factor preventing **hybrid** formation. Competitive gametic barriers emerge when a female is inseminated by a conspecific and a heterospecific male. We examined whether sperm proportions after double matings and copulation duration impede **hybrid** formation. For this, we used females of *Ischnura graellsii* that mated with one conspecific and one heterospecific (*I. elegans*) male and vice versa, and calculated paternity of the second male by using RFLPs. Values of paternity (although preliminary because of a small sample size) suggest no bias in paternity towards conspecific males. However, proportion of sperm stored in the bursa and spermatheca of the female was biased towards the conspecific male when the heterospecific male was the first male, while copulation duration did not differ between conspecific and heterospecific males. Our results suggest that the relative sperm volumes may play a role as a gametic barrier in this species. However, cryptic female choice mediated by the preferential use of the conspecific sperm, although not detected, could not be discarded owing to small sample sizes in some cases.
