

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

28/03/2022

### Widespread hybridization within mound-building wood ants in Southern Finland results in cytonuclear mismatches and potential for sex-specific hybrid breakdown

Beresford, J; Elias, M; Pluckrose, L; Sundstrom, L; Butlin, RK; Pamilo, P; Kulmuni, J. 2017

Hybridization and gene flow between diverging lineages are increasingly recognized as common evolutionary processes, and their consequences can vary from hybrid breakdown to adaptive introgression. We have previously found a population of wood ant hybrids between *Formica aquilonia* and *F. polyctena* that shows antagonistic effects of hybridization: females with introgressed alleles show hybrid vigour, whereas males with the same alleles show hybrid breakdown. Here, we investigate whether hybridization is a general phenomenon in this species pair and analyse 647 worker samples from 16 localities in Finland using microsatellite markers and a 1200-bp mitochondrial sequence. Our results show that 27 sampled nests contained parental-like gene pools (six putative *F. polyctena* and 21 putative *F. aquilonia*) and all remaining nests (69), from nine localities, contained hybrids of varying degrees. Patterns of genetic variation suggest these hybrids arise from several hybridization events or, instead, have backcrossed to the parental gene pools to varying extents. In contrast to expectations, the mitochondrial haplotypes of the parental species were not randomly distributed among the hybrids. Instead, nests that were closer to parental-like *F. aquilonia* for nuclear markers preferentially had *F. polyctena*'s mitochondria and vice versa. This systematic pattern suggests there may be underlying selection favouring cytonuclear mismatch and hybridization. We also found a new hybrid locality with strong genetic differences between the sexes similar to those predicted under antagonistic selection on male and female hybrids. Further studies are needed to determine the selective forces that act on male and female genomes in these newly discovered hybrids.

### Segregation distortion causes large-scale differences between male and female genomes in hybrid ants

Kulmuni, J; Seifert, B; Pamilo, P. 2010

Hybridization in isolated populations can lead either to hybrid breakdown and extinction or in some cases to speciation. The basis of hybrid breakdown lies in genetic incompatibilities between diverged genomes. In social Hymenoptera, the consequences of hybridization can differ from those in other animals because of haplodiploidy and sociality. Selection pressures differ between sexes because males are haploid and females are diploid. Furthermore, sociality and group living may allow survival of hybrid genotypes. We show that hybridization in *Formica* ants has resulted in a stable situation in which the males form two highly divergent gene pools whereas all the females are hybrids. This causes an exceptional situation with large-scale differences between male and female genomes. The genotype differences indicate strong transmission ratio distortion depending on offspring sex, whereby the mother transmits some alleles exclusively to her daughters and other alleles exclusively to her sons. The genetic differences between the sexes and the apparent lack of multilocus hybrid genotypes in males can be explained by recessive incompatibilities which cause the elimination of hybrid males because of their haploid genome. Alternatively, differentiation between sexes could be created by prezygotic segregation into male-forming and female-forming gametes in diploid females. Differentiation between sexes is stable and maintained throughout generations. The present study shows a unique outcome of hybridization and demonstrates that hybridization has the potential of generating evolutionary novelties in animals.

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

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

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

### Hybridization between introduced *Torymus sinensis* (Hymenoptera: Torymidae) and indigenous *T. beneficus* (late-spring strain), parasitoids of the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)

Yara, K; Sasawaki, T; Kunimi, Y. 2010

Hybridization between introduced biological control agents and native species is a nontarget effect of biological control. However, the genetic impacts related to introduced insects, especially from the viewpoint of post-release evaluation, remain largely unexplored. *Torymus sinensis* (Hymenoptera: Torymidae) is a parasitoid wasp introduced from China into Japan to control the invasive Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). After *T. sinensis* was first released in 1982, the damage caused by *D. kuriphilus* fell greatly. Previous studies showed that hybridization between *T. sinensis* and indigenous *Torymus beneficus* (the early-spring strain) was rare in the field, but individuals morphologically intermediate between *T. sinensis* and *T. beneficus* then appeared in the field. Here, we examined the frequency of hybridization between *T. sinensis* and the late-spring strain of *T. beneficus* by using the internal transcribed spacer 1 (ITS1) region of nuclear rDNA. Increasing numbers of F-1 hybrids were detected every year from 1993 to 1996, reaching 22%. It seems that *T. sinensis* can hybridize more easily with the late-spring strain of *T. beneficus*. Morphological indices of the F-1 hybrid type ranged between indices of the late-spring strain of *T. beneficus* and *T. sinensis*, with considerable overlap. Simultaneous with the occurrence of the F-1 hybrids, the indigenous late-spring strain of *T. beneficus* was rapidly displaced by *T. sinensis*. (C) 2010 Elsevier Inc. All rights reserved.

### Pollination patterns limit hybridization between two sympatric species of *Narcissus* (Amaryllidaceae)

Marques, J; Rossello-Graell, A; Draper, D; Iriando, JM. 2007

Natural hybrids between rare and common sympatric species are commonly eradicated to avoid the potential extinction of the rare species, although there is currently no clear predictive framework to quantify this risk. As hybrids can have intrinsic value as new evolutionary pathways, further knowledge on the factors controlling hybridization is needed. In this study we evaluated the role of pollination patterns in hybridization events in two sympatric populations of *Narcissus cavanillesii* and *N. serotinus* in Portugal. *Narcissus cavanillesii* is a rare species, while *N. serotinus* is widely distributed across the Mediterranean. The hybrid, *N. xperzlarae*, is quite frequent in southeastern Spain but is scarce in Portugal. Reciprocal manual crossings confirmed compatibility between the two species, although hybridization was more successful when *N. cavanillesii* participated as female. *Narcissus cavanillesii* and *N. serotinus* only shared one pollinator, *Megachile* sp. (Hymenoptera), which had low visitation rates and high flower constancy. No single isolation mechanism was fully effective in preventing hybridization. Temporal

displacement of flowering peaks, strong pollinator specificity, and high flower constancy in the shared pollinator all contributed to limiting hybridization in this site. In other sympatric occurrences, different phenological windows and pollination assemblages may allow greater frequency of the hybrid.

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#### **Mixed colonies and hybridisation of *Messor* harvester ant species (Hymenoptera: Formicidae)**

Steiner, FM; Seifert, B; Grasso, DA; Le Moli, F; Arthofer, W; Stauffer, C; Crozier, RH; Schlick-Steiner, BC. 2011

The Mediterranean harvester ant species *Messor minor*, *M. cf. wasmanni*, and *M. capitatus* can co-occur in the same habitat. In Italian populations, we encountered colonies that contained workers from more than one species as identified via standard morphology, as well as colonies with workers that appeared to be morphologically intermediate between species. This unusual finding required further analysis. We analysed such colonies using microsatellites, mitochondrial DNA and refined morphometrics, and a simple inference key for the colony-level interpretation of data from the three sources combined. We infer that *Messor minor* and *M. cf. wasmanni* engage in bidirectional interspecific gene flow. Hybrids between these two species are inferred to produce fertile offspring, which would indicate that barriers to hybridisation do not exist or can be completely overcome. This is unexpected, given that they are non-sister species and broadly sympatric in nature. Our findings also indicate the possible occurrence of hybrid-hybrid crosses, a phenomenon rarely observed in ants. We cautiously interpret the data at hand as in support of the interspecific gene flow considerably shaping the genetic makeup of populations, raising the question about a potential adaptive value of this hybridisation. *Messor capitatus* mixes with hybrids of the other two species, but we found no indication of hybridisation involving this species. We discuss various hypotheses on the causations of colony mixing and hybridisation in the three *Messor* species at the proximate and ultimate level.

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#### **Electrophoretic evidence for hybridization in the ant genus *Acanthomyops* (Hymenoptera : Formicidae)**

Umphrey, GJ; Danzmann, RG. 1998

Enzyme electrophoresis on a GPI locus was used to provide the first genetic tests of the hypotheses that hybridization occurs between the ant species *Acanthomyops latipes* and *A. claviger*, that the hybrids are F-1's, and that the hybrids result from a *latipes* queen mating with a *claviger* male. A difference in GPI allele frequencies (fixed in *latipes*, polymorphic but strongly skewed to an alternate allele in *claviger*) was used to test five predictions that arise from these hypotheses. Test results were consistent with all components of these hypotheses, with the possible exception of a reciprocal cross in one colony. No evidence of backcrossing was found. We believe that two alternative hypotheses to explain the existence of morphologically intermediate queens can be rejected: the hypothesis that *latipes* produces dimorphic queens, and the hypothesis that colonies producing the morphologically intermediate queens represent a separate species. The pattern of hybridization, with F-1 hybrids commonly occurring throughout the broad range of sympatry of the parent species, contrasts sharply with most "hybrid zone" phenomena. (C) 1998 Elsevier Science Ltd. All rights reserved.

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

Seifert, B. 2019

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

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#### **Sperm parasitism in ants: Selection for interspecific mating and hybridization**

Umphrey, GJ. 2006

Interspecific mating in eusocial Hymenoptera can be favored under certain conditions even if all hybrid offspring are completely infertile. This exploits two key features of the eusocial Hymenoptera: a haplodiploid genetic system and reproductive division of labor in females. Interspecifically mated queens can still produce viable sons that will mate intraspecifically. Apparent reduced fitness resulting from producing infertile daughter gynes can be also offset by advantages conferred by hybrid workers. An important advantage is likely to be superior ability at using marginal habitats. Interspecifically mated queens can nest in sites where intraspecific competition will be low. By mating interspecifically, a queen trades expected reproductive success through female offspring for a higher probability of achieving some reproductive success. Females that mate interspecifically can be considered "sperm parasites" on the males of the other species. I provide evidence that sperm parasitism is responsible for widespread hybridization in North America among two species of the ant subgenus *Acanthomyops* (genus *Lasius*), and review evidence for sperm parasitism in other hybridization phenomena in ants. Sperm parasitism in ants represents a novel form of social parasitism in ants and a dispersal polymorphism. It may also act as a precursor to the evolution of some other recently discovered phenomena, such as genetic caste determination.

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#### **Use of malic enzyme to detect hybrids between *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae) attacking *Dryocosmus kuriphilus* (Hymenoptera: cynipidae) and possibility of natural hybridization.**

Izawa, H; Osakabe, M; Moriya, S; Toda, S. 1996

Hybrids between the early-season strain of *Torymus beneficus* and *T. sinensis*, in which the malic enzyme (ME) is fixed at the F and S alleles, respectively, can be discriminated from their parents by the enzyme banding pattern using PAG electrophoresis. The ME locus of hybrids between the late-season strains of *T. beneficus* and *T. sinensis* is fixed at the S allele. Females of *Torymus* spp. collected in the field at Tsukuba had various ratios of ovipositor sheath length to thorax length and three ME genotypes: SS, FS and FF. Detection of FS in this population indicates that natural hybridization occurs between early-season strains of *T. beneficus* and *T. sinensis*.

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#### **Displacement of *Torymus beneficus* (Hymenoptera : Torymidae) by *T. sinensis*, an indigenous and introduced parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera : Cynipidae), in Japanese chestnut fields: Possible involvement in hybridization**

Yara, K; Sasawaki, T; Kunimi, Y. 2007

Although there is empirical knowledge to suggest the displacement of *Torymus beneficus* (Hymenoptera: Torymidae) by *Torymus sinensis*, an indigenous and introduced parasitoid of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), respectively, in Japanese chestnut fields, the underlying mechanisms are unclear. In this study, the displacement of the early-spring strain of *T. beneficus* by *T. sinensis* was surveyed in a chestnut field for nine successive years ( $n = 418$ ), using two molecular markers, the internal transcribed spacer 2 of nuclear rDNA (ITS2) and the mitochondrial cytochrome oxidase subunit I (COI). We also investigated whether or not hybridization between the parasitoids was involved as an important factor in the displacement. All individuals from 1993 to 1995 were of the early-spring strain of *T. beneficus* type for both ITS2 and COI. After 1996, individuals with the same types decreased and ones of the *T. sinensis* type for both ITS2 and COI increased. On the other hand, there was only one individual that had *T. sinensis* type for ITS2 and the early-spring strain of *T. beneficus* type for COI, suggesting that individuals are descendants of the F<sub>1</sub> hybrid between the early-spring strain of *T. beneficus* females and *T. sinensis* males. These results indicate that hybridization between them was not closely related to displacement in the field. We have also found hybridization between *T. sinensis* and another type of *T. beneficus*: the late-spring strain; this is the first report to show their hybridization in chestnut fields. (c) 2007 Elsevier Inc. All rights reserved.

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

Seifert, B. 1999

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

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#### **HYBRIDIZATION** AND VARIATION IN THE *LEPTOTHORAX TUBERUM* GROUP (HYMENOPTERA, FORMICIDAE)

DOUWES, P; STILLE, B. 1991

Morphological and allozymatic analyses show that there are four species - *L. tuberum* (mainly in the north) and *L. nigriceps*, *L. "tubero-interruptus"*, and *L. unifasciatus* (sympatrically in the south) - in the *Leptothorax tuberum* group in West Europe north of the Alps, and that these species hybridize. The two commonly co-occurring species, *L. nigriceps* and *L. unifasciatus*, rarely hybridize, suggesting that premating isolating mechanisms have evolved, whereas the rare species, *L. "tubero-interruptus"*, easily interbreeds possibly with all the other species of this group. There are distinct morphological gaps between the species, but hybridization tends to fill these gaps or even produces morphological copies (*L. nigriceps* x *L. "tubero-interruptus"*) of a third species (*L. tuberum*).

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

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

In the framework of biological control, the selection of effective natural enemies determines the final pest control. Thus, the genetic improvement of biocontrol agents could enhance the efficiency of biocontrol programs. Although promising, this approach has rarely been applied in this field. At the intraspecific level, hybridization between divergent populations of biocontrol agents is expected to promote hybrid vigor (heterosis), but it is not clear to what extent. An even more difficult task is the ability to predict the fitness of hybrids from the biological characteristics of their parents. We investigated these general questions by crossing seven populations of the parasitoid wasp *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Our results show different levels of mating compatibilities among populations, including asymmetric or almost complete reproductive isolation. Hybrids' performance (fitness of the F<sub>1</sub> generation) ranges from inbreeding depression to heterosis. It was possible, to some extent, to predict hybrid fitness from pairwise genetic and phenotypic distances among parents, in accordance with the dominance hypothesis. This may provide general guidelines for the genetic improvement of biological control agents.

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#### **Ecological niche overlap in sister species: how do oil-collecting bees *Macropis europaea* and *Macropis fulvipes* (Hymenoptera: Melittidae) avoid hybridization and competition?**

Bassin, L; Alvarez, N; Pellissier, L; Triponez, Y. 2011

Oil-collecting bees are found worldwide and always in association with particular oil-producing flowers. In the Western Palearctic, three oil-collecting bee species within the genus *Macropis* (Hymenoptera, Melittidae) interact in a tight pollination mutualism with species of the only European oil-producing plant genus *Lysimachia* L. (Myrsinaceae). Two of these oil-collecting bees (*Macropis europaea* and *Macropis fulvipes*) show overlapping geographic distributions, comparable morphologies, and similar ecological characteristics (e.g., habitat type, floral preferences). In view of these similarities, we presume that hybridization should occur between the two species unless potential variation among the species' ecological niches prevents it, simultaneously decreasing competition for resources. Using modern genetic analyses and ecological niche modeling on a large bee sampling throughout Europe, we discuss new perspectives on the ecology and evolutionary history of this mutualism.

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#### **Hybridization enables the fixation of selfish queen genotypes in eusocial colonies**

Weyna, A; Romiguier, J; Mullan, C. 2021

A eusocial colony typically consists of two main castes: queens that reproduce and sterile workers that help them. This division of labor, however, is vulnerable to genetic elements that favor the development of their carriers into queens. Several factors, such as intracolony relatedness, can modulate the spread of such caste-biasing genotypes. Here we investigate the effects of a notable yet understudied ecological setting: where larvae produced by hybridization develop into sterile workers. Using mathematical modeling, we show that the coevolution of hybridization with caste determination readily triggers an evolutionary arms race between nonhybrid larvae that increasingly develop into queens, and queens that increasingly hybridize to produce workers. Even where hybridization reduces worker function

and colony fitness, this race can lead to the loss of developmental plasticity and to genetically hard-wired caste determination. Overall, our results may help understand the repeated evolution toward remarkable reproductive systems (e.g., social hybridogenesis) observed in several ant species.

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

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

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

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#### **Populations composed entirely of hybrid colonies: bidirectional hybridization and polyandry in harvester ants**

Anderson, KE; Novak, SJ; Smith, JF. 2008

In eusocial Hymenoptera, haplodiploid life cycles, obligate sterile castes, and polyandry may facilitate selection for hybridization. We analyzed a broad hybrid zone between the ecologically distinct seed-harvester ants *Pogonomyrmex occidentalis* (Cresson) and *Pogonomyrmex maricopa* (Wheeler) using mitochondrial (mt)DNA sequence data, eight morphological markers, and 14 random amplified polymorphic DNA (RAPD) markers. Average mtDNA sequence divergence among parental species was 11.34%, indicating secondary contact. RAPD markers were significantly correlated with morphological variation, confirming the interspecific hybrid origin of all morphologically putative hybrid colonies. A morphological hybrid index indicates an abundance of both F<sub>1</sub> hybrids and parental morphotypes within colonies. Individual character frequencies plotted against distance show coincident and concordant clines, suggesting little to no introgression. The structure of the hybrid zone is two-fold. Within the western region, stark reversals in character frequencies coincide with overt soil differences, indicating a mosaic hybrid zone structure. The eastern region is a riparian habitat where four adjacent populations were composed entirely of hybrid colonies. These habitat associations suggest that hybrid worker genomes permit dispersal into intermediate environments that select against one or both parental species. The present study suggests that, in addition to retaining reproductive compatibility, ecologically distinct species of ants may generate hybrid colonies maintained by environmental selection. (C) 2008 The Linnean Society of London, Biological Journal of the Linnean Society, 2008, 95, 320-336.

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#### **Molecular characterization of *Psytalia lounsburyi*, a candidate biocontrol agent of the olive fruit fly, and its *Wolbachia* symbionts as a pre-requisite for future intraspecific hybridization**

Cheyppe-Buchmann, S; Bon, MC; Warot, S; Jones, W; Malausa, T; Fauvergue, X; Ris, N. 2011

Numerous arthropod species are genetically differentiated across their distribution area. Diversifying the geographical origins of a biocontrol agent species can be used to favour their perennial establishment by the sampling of pre-adapted genotypes and/or the production of new genotypes through hybridization. Hybridization can be nevertheless challenged by reproductive isolations induced by some common microbial endosymbionts. In this study, we aimed at characterizing (i) the genetic diversity of six populations of *Psytalia lounsburyi* (Hymenoptera: Braconidae), a candidate biocontrol agent of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) and (ii) the diversity of their *Wolbachia* endosymbionts. Both mitochondrial and microsatellite markers evidence clustering between the South African population and several Kenyan/Namibian populations. The survey of the *Wolbachia* also distinguished two main variants with a spatial heterogeneity in the infection status. All these results are discussed in the context of the use of these *P. lounsburyi* populations for hybridization and further field releases.

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#### **Genotypic Characterization of *Torymus sinensis* (Hymenoptera: Torymidae) After Its Introduction in Tuscany (Italy) for the Biological Control of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)**

Viviani, A; Bernardi, R; Cavallini, A; Rossi, E. 2019

*Torymus sinensis* Kamijo (Hymenoptera: Torymidae) is an alien parasitoid that is used in many areas of the world for biological control of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). In Italy, this parasitoid was imported from Japan in 2003 and subsequently multiplied and released throughout the country. In this study, a phylogenetic investigation was carried out on insects from three different sites in northern Tuscany (Italy). Moreover, the possible hybridization between *T. sinensis* and some native *Torymus* species was evaluated. The conserved region 18S rRNA gene and the hypervariable ITS2 (Internal Transcribed Spacer 2) region of the ribosomal cistron were selected as molecular markers. Sequencing the amplified products, after cloning, ruled out any hybridization between *T. sinensis* and the native *Torymus* species, and also confirmed the presence of two haplotypes for the Tuscan population of *T. sinensis* both for the region of the 18S rRNA gene as well as for the ITS2 region. These results confirm that the environmental impact of the alien parasitoid *T. sinensis* in the study site is acceptable, although an extensive and repeated monitoring would be desirable.

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#### **Multiple mating in the context of interspecific hybridization between two *Tetramorium* ant species**

Cordonnier, M; Escarguel, G; Dumet, A; Kaufmann, B. 2020

In eusocial Hymenoptera, haplodiploidy and polyandry may facilitate selection for hybridization. Interspecific hybridization is widespread in ants and can lead to hybrid inviability as well as the formation of new species through hybrid speciation. However, in ants, polyandry is uncommon. By analyzing microsatellite markers on 15 ant workers per colony, we show that the mating system of 28 pure colonies of *Tetramorium immigrans*, 15 pure colonies of *Tetramorium caespitum*, and 27 hybrid colonies is a monogynous/polyandrous mating system, with a higher mating rate in *T. caespitum* (mean = 2.4 males vs. 1.7 in *T. immigrans*). Hybrid queens, but

no hybrid fathers, were deduced from workers' genotypes, in accordance with Haldane's rule extended to haplodiploid organisms, which states that the haploid sex should more often be sterile or inviable. In five colonies, hybridization and multiple mating allowed the simultaneous production of both hybrid and nonhybrid offspring. Although rare, these situations hinted at asymmetrical, larger contributions of *T. immigrans* vs. *T. caespitum* males to offspring production. Together, these findings point toward a complex and dynamic mating system in *T. immigrans* and *T. caespitum*, and contribute to better understand interspecific hybridization mechanisms and their consequences on genetic and taxonomic diversity. The study of polyandry within a hybrid zone is unprecedented and opens new opportunities to better understand interspecific hybridization mechanisms and their short- to long-term consequences.

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#### ~~Distribution of 18S rDNA sites and absence of the canonical TTAGG insect telomeric repeat in parasitoid Hymenoptera~~

Gokhman, VE; Anokhin, BA; Kuznetsova, VG. 2014

Karyotypes of six species belonging to three main clades of parasitoid Hymenoptera, the superfamilies Ichneumonoidea (Ichneumonidae: *Ichneumon amphibolus*), Cynipoidea (Cynipidae: *Diplolepis rosae*) and Chalcidoidea (Eurytomidae: *Eurytoma robusta*, *Eu. serratulae* and *Eu. compressa*, and Torymidae: *Torymus bedeguaris*) were studied using FISH with 18S rDNA and telomeric (TTAGG)(n) probes. Haploid karyotypes of *D. rosae*, *Eu. robusta* and *Eu. serratulae* carried the only 18S rDNA hybridization signal, whereas those of *I. amphibolus* and *Eu. compressa* carried three and two rDNA clusters respectively. In addition, three rDNA sites were visualized in the aneuploid female of *T. bedeguaris*. The number of rDNA clusters in parasitoid Hymenoptera generally correlates to the chromosome number. Apart from the overwhelming majority of the studied species of aculeate Hymenoptera, no hybridization signals were obtained from FISH with the telomeric (TTAGG)(n) probe in the examined parasitoid species. These data suggest absence of the canonical (TTAGG)(n) insect telomeric motif in the Ichneumonoidea, Cynipoidea and Chalcidoidea, and perhaps in parasitoid Hymenoptera in general.

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#### ~~Mitonuclear discordance is caused by rampant mitochondrial introgression in Neodiprion (Hymenoptera: diprionidae) sawflies~~

Linnen, CR; Farrell, BD. 2007

We investigate the pervasiveness of hybridization and mitochondrial introgression in *Neodiprion* Rohwer (Hymenoptera; Diprionidae), a Holarctic genus of conifer-feeding sawflies. A phylogenetic analysis of the *lecontei* species group revealed extensive discordance between a contiguous mitochondrial region spanning three genes (COI, tRNA-leucine, and COII) and three nuclear loci (EF1 alpha, CAD, and an anonymous nuclear locus). Bayesian tests of monophyly and Shimodaira-Hasegawa (SH) tests of topological congruence were consistent with mitochondrial introgression; however, these patterns could also be explained by lineage sorting (i.e., deep coalescence). Therefore, to explicitly test the mitochondrial introgression hypothesis, we used a novel application of coalescent-based isolation with migration (IM) models to measure interspecific gene flow at each locus. In support of our hypothesis, mitochondrial gene flow was consistently higher than nuclear gene flow across 120 pairwise species comparisons ( $P < 1 \times 10^{-12}$ ). We combine phylogenetic and coalescent evidence to identify likely cases of recent and ancient introgression in *Neodiprion*, and based on these observations, we hypothesize that shared hosts and/or pheromones facilitate hybridization, whereas disparate abundances between hybridizing species promote mitochondrial introgression. Our results carry implications for phylogenetic analysis, and we advocate the separation of high and low gene flow regions to inform analyses of hybridization and speciation history, respectively.

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#### ~~HYBRIDIZATION OF CHROMOSOME POLYMORPHIC POPULATIONS OF THE INQUILINE ANT, DORONOMYRMEX KUTTERI (HYM., FORMICIDAE)~~

BUSCHINGER, A; FISCHER, K. 1991

The workerless, inquiline ant, *Doronomyrmex kutteri* has isolated populations with a haploid chromosome number of  $n = 23$  both in the Alps (Swiss and South Tyrolean Alps) and in Sweden, and a population with  $n = 25$  in southern Germany. Crossbreeding of sexuals from all populations proved successful. Backcrosses of F1-females with males from the parental populations produced F2-females, and hybrid males with  $n = 23, 24$ , or 25 chromosomes. The chromosome polymorphism is not due to B-chromosomes. Probably the  $n = 25$  karyotype originated from the  $n = 23$  karyotype by two Robertsonian fissions (2 MBAR  $\rightarrow$  4 ABAR), since the  $n = 25$  karyotype was found in only one of the populations. Diploid males occurred frequently in colonies from four out of five sites investigated.

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#### ~~Identification of 21 polymorphic microsatellites in the African parasitoid wasp, Psytalia lounsburyi (Silvestri) (Hymenoptera - Braconidae)~~

Bon, MC; Jones, W; Hurard, C; Loiseau, A; Ris, N; Pickett, C; Estoup, A; Fauvergue, X. 2008

We have developed 21 dinucleotide repeat microsatellite loci from African populations of *Psytalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae), a parasitoid wasp of the olive fruit fly, as part of a study assessing the role of introgression/hybridization in the success of a biological control introduction. We proposed suitable conditions for polymerase chain reaction multiplexing. All 21 loci were polymorphic with two to 21 alleles per locus within the Kenyan and South African populations tested. Most of them were successfully amplified in two other *Psytalia* species.

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#### Mating, hybridisation and introgression in *Lasius* ants (Hymenoptera: Formicidae)

van der Have, TM; Pedersen, JS; Boomsma, JJ. 2011

Recent reviews have shown that hybridisation among ant species is likely to be more common than previously appreciated, but that documented cases of introgression remain rare. After molecular phylogenetic work had shown that European *Lasius niger* (LINNAEUS, 1758) and *L. psammophilus* SEIFERT, 1992 (formerly *L. alienus* (FOERSTER, 1850)) are unlikely to be very closely related, we decided to analyse an old data set confirming the conclusion by PEARSON (1983) that these two ants can indeed form viable hybrids. We show that signatures of introgression can be detected in a Danish site and that interspecific gene-flow is asymmetrical (only from *L. niger* into *L. psammophilus*) as inferred previously by Pearson for the southern England site that he studied and from which we also collected data. We compare the observed patterns of hybridisation and introgression in the Danish and British site and infer that overlap in nuptial flights in Denmark may have contributed to the higher frequency of introgressed genes relative to the southern England site where nuptial flights are clearly separated in time. We also report the first mating system data for *L. psammophilus*, showing that this species has facultative multiple mating of queens similar to *L. niger*. We suggest that *L. psammophilus-niger* introgression may be much more common than previously appreciated, which would explain that European myrmecologists have often found it difficult to distinguish between these species at sites where they occur sympatrically. This would imply that multiple accessible field sites are available to study the molecular details of hybridisation and introgression between two ant species that have variable degrees of sympatry throughout their distributional ranges.

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#### ~~HYBRIDIZATION IN SIDA OVATA COMPLEX .2. EVIDENCE FROM BREEDING STUDIES~~

DAWAR, R; ABI, T; QAISER, M. 1994

Breeding studies reconfirmed the hybridization between *Sida ovata* Forssk., and *Sida tiagii* Bhandari. All the three taxa i.e., *S. ovata* Forssk., *S. tiagii* Bhandari and the



hybrid are facultative autogamous as indicated by pollen - ovule ratios and breeding experiments. *Chrysis* sp., (Hymenoptera) seems to be responsible for pollen transfer between *S. ovata* and *S. tiagii* in natural populations. Certain insect pollinators were specific such as *Bembix* sp., and *Bombus* sp., for *S. ovata*, *Apis* sp., and *Vespa* sp., for *S. tiagii* and *Ponera* sp., for the hybrid. The limited number of hybrids may be due to less frequent visitation of common pollinator (*Chrysis* sp.) along with the incomplete reproductive barrier between both the parents. The restricted gene flow is also evident by least fruit and seed set in hand pollinated reciprocal crosses of the putative parents as compared to the self pollination. It seems that back crosses are impeded due to the absence of common pollinators between the parents and hybrid and meiotic irregularities as indicated by the failure of hand pollinated (back crosses) experiments.

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**Parental genetic traits in offspring from inter-specific crosses between introduced and indigenous *Diadegma* Foerster (Hymenoptera: Ichneumonidae): Possible effects on conservation genetics**

Davies, AP; Takashino, K; Watanabe, M; Miura, K. 2009

The effects of natural enemy releases on conservation genetics within ecosystems are rarely considered. *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) was introduced and continues to be released for biological control of diamondback moth in Japan. *Diadegma semiclausum* and indigenous *Diadegma fenestrale* (Holmgren) (Hymenoptera: Ichneumonidae) share geographic ranges and hosts, and produce offspring when mated under laboratory conditions. We used DNA to examine whether offspring from inter-specific one-way parental crosses (*D. semiclausum* female and *D. fenestrale* male) were hybrid, as some Hymenoptera (e.g. *Aphidius colemani* Viereck (Hymenoptera: Braconidae)) exhibit thelytokous reproduction by gynogenesis. Molecular analyses revealed offspring mtDNA (COI) is maternally inherited, as expected, but rRNA (ITS-2) originates from both parents. Should similar hybridization occur in the field beyond the F1 generation, genetic mixing is a possible consequence that may influence biological control efficacy or pollute native population genetics.

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**Interaction between *Torymus sinensis* (Hymenoptera: Torymidae) and *T. beneficus*, Introduced and Indigenous Parasitoids of the Chestnut Gall Wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)**

Yara, K. 2014

*Torymus sinensis* is a parasitoid wasp that was introduced from China to Japan to control the invasive chestnut gall wasp *Dryocosmus kuriphilus*. Interaction between *T. sinensis* and the indigenous parasitoid *T. beneficus* has been of interest since *T. sinensis* was first released in chestnut orchards, as hybridization between them might impede the success of biological control by the introduced parasitoid. Such apprehensions disappeared with the drastic decrease in damage caused by *D. kuriphilus*. However, the emergence of morphologically intermediate individuals between them after the introduction of the parasitoid has triggered renewed interest in hybridization, specifically on the non-target effects of biological control. In this review, the interaction between both species, specifically hybridization, species composition and species displacement, is surveyed. Molecular markers are used, not only because *T. sinensis* and *T. beneficus* are similar morphologically, but also because the latter has two emergence strains (early- and late-spring). Surveys show that 1) *T. sinensis* has displaced both emergence strains of *T. beneficus*; 2) hybridization between *T. sinensis* and early-spring *T. beneficus* occurs at a low frequency (= 1%); but that 3), hybrid F1s between *T. sinensis* and late-spring *T. beneficus* occur at a much higher frequency (= 20%). A study of phylogenetic relationships indicates that the late-spring *T. beneficus* is closer to the different species *T. sinensis* than the early-spring *T. beneficus*. This can partly explain why *T. sinensis* hybridizes more readily with the late-spring *T. beneficus* than with the early-spring *T. beneficus*.

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**~~ANALYSIS OF GENETIC DIVERSITY AND STRUCTURE OF TWO CLADES OF APHELINUS MALI (HYMENOPTERA: APHELINIDAE) IN CHINA~~**

Zhou, HX; Zhang, RM; Guo, D; Tao, YL; Wan, FH; Wu, Q; Chu, D. 2014

Our prior research revealed that there are 2 mitochondrial clades of *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) in China, which are known as SD clade and LN clade. To further reveal their genetic characteristics and to determine the degrees of hybridization and gene flow between the 2 clades of *A. mali* in China, we analyzed the genetic diversities and genetic structures of 16 populations from 6 provinces (Shandong, Liaoning, Hebei, Shanxi, Xinjiang, and Yunnan) using 8 microsatellite loci. Our results showed that among the pure populations in the SD and LN clades, the greatest genetic diversities were found in the Qingdao, Shandong (QD) population and in the Dalian, Liaoning (DL) population. QD was the first population of the SD clade to be established, and DL was the first population of the LN clade to be established. In addition, genetic diversity was not substantially lower - and in some cases it was greater - in mixed-clade populations than in QD and DL. Individuals within each mitochondrial clade could not be differentiated based on microsatellite loci. Our data confirmed that the QD and DL populations, which were the first to be established in China, have served as bridgeheads for the other SD and LN populations in China. The results demonstrated that the hybridization or gene flow has occurred between the 2 mitochondrial clades.

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**Occurrence and hybridization of two parasitoid wasps, *Torymus sinensis* Kamijo and *T. beneficus* Yasumatsu et Kamijo (Hymenoptera : Torymidae) in the Oki islands**

Toda, S; Miyazaki, M; Osakabe, M; Komazaki, S. 2000

*Torymus sinensis*, an introduced parasitoid of *Dryocosmus kuriphilus*, was found to be present in the Oki islands though it had never been released there. To investigate the seasonal occurrence of two parasitoid wasps, *T. sinensis* and *Torymus beneficus*, and their hybrids, we collected 611 withered galls formed by *D. kuriphilus* in March 1996. Ninety-three females and 182 males of *Torymus* emerged from the galls. We separated species by the morphology and genotype of the malic enzyme (ME). Both parasitoids were found and *T. sinensis* was dominant. Seven females showed 5 bands of ME migrating between the S and F bands and were hybrids of *T. sinensis* with early-emerging *T. beneficus*. Only 8% of females were hybrids. Hybridization seemed to occur at low frequency in the field. Oki's *T. sinensis* is considered to have been accidentally introduced long before our investigation and was classified into a Chinese ecotype judging from their eclosion period.

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**~~DNA hybridization probe for endoparasitism by *Microplitis croceipes* (Hymenoptera - Braconidae)~~**

Greenstone, MH; Edwards, MJ. 1998

We describe a DNA hybridization assay for an insect endoparasitoid. The probe is a digoxigenin-labeled 438 bp fragment from a genomic library of the braconid *Microplitis croceipes* (Cresson), a larval endoparasitoid of heliothine noctuids. The assay is run on nylon membranes and can detect 125 pg of target DNA. Estimates of parasitization rate in large samples of simultaneously parasitized larvae of the host *Helicoverpa zea* (Boddie), made by hybridization probe assay of unamplified host homogenates and by dissection, are not statistically distinguishable for 1st or 2nd instar parasitoids. It should be possible to perform the entire procedure, from host collection and DNA extraction to evaluation of assay results: in 1 d, making this prototype technology useful as a monitoring and management tool.

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**~~Ecological and morphological differentiation of the honeybees, *Apis mellifera* Linnaeus (Hymenoptera : Apidae), of West Africa~~**

Radloff, SE; Hepburn, HR; Fuchs, S. 1998

Morphometric characters of worker honeybees, *Apis mellifera* Linnaeus, were analysed by multivariate methods to characterize their populations in the sahelian, dry and wet tropical and equatorial regions of western and eastern Africa (mainly between 0 degrees and 15 degrees N latitude, 18 degrees W and 39 degrees E longitude). Two distinct morphoclusters and a zone of hybridization between them were identified. The bees are identified as *Apis mellifera adansonii* Latreille and *A. m. jemenitica* Rutner. The former subspecies is distributed in the equatorial and wet tropical regions, the latter in the dry tropical and sahelian eco-climatic zones. The hybrid zone extends into the two tropical and savanna biomes and it is suggested that the stability of the hybridization zone is largely the effect of extensive annual fire in the region.

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~~Effect of hybridization of the *Quercus crassifolia* x *Quercus crassipes* complex on the community structure of endophagous insects~~

Tovar-Sanchez, E; Oyama, K. 2006

In a previous study, we showed that the geographic proximity of hybrid plants to the allopatric areas of parental species increases their morphological and genetic similarity with them. In the present work, we explored whether the endophagous fauna of hybrid plants show the same pattern. We studied the canopy species richness, diversity and composition of leaf-mining moths (Lepidoptera: Tischeriidae, Citheraniidae) and gall-forming wasps (Hymenoptera: Cynipidae) associated with two species of red oaks (*Quercus crassifolia* and *Quercus crassipes*) and their interspecific hybrid (*Quercus x dysphylla* Benth pro sp.) in seven hybrid zones in central Mexico, during four seasons in 2 years. The study was conducted on 194 oak trees with known genetic status [identified by leaf morphology and molecular markers (random amplified polymorphic DNAs)], and the results indicate a bidirectional pattern of gene flow. Hybrid plants supported intermediate levels of infestation of gall-forming and leaf-mining insects compared to their putative parental species. The infestation level of leaf-mining insects varied significantly following the pattern: *Q. crassifolia* > hybrids > *Q. crassipes*, whereas the gall-forming insects showed an inverse pattern. A negative and significant relationship was found between these two types of insect guilds in each host taxa, when the infestation percentage was evaluated. It was found that 31.5% (n=11) of the endophagous insects were specific to *Q. crassipes*, 22.9% (n=8) to *Q. crassifolia*, and 8.6% (n=3) to hybrid individuals. The hybrid bridge hypothesis was supported in the case of 25.7% (n=9) of insects, which suggests that the presence of a hybrid intermediary plant may favor a host herbivore shift from one plant species to another. Greater genetic diversity in a hybrid zone is associated with greater diversity in the endophagous community. The geographic proximity of hybrid plants to the allopatric site of a parental species increases their similarity in terms of endophagous insects and the Eje Neovolcanico acts as a corridor favoring this pattern.

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**Independent hybrid populations of *Formica polyctena* X *rufa* wood ants (Hymenoptera: Formicidae) abound under conditions of forest fragmentation**

Seifert, B; Kulmuni, J; Pamilo, P. 2010

Combined genetic and morphological data indicate frequent hybridisation between the wood ants *Formica polyctena* Forster 1850 and *F. rufa* Linnaeus 1761 in Central Europe. The genetic and morphological traits give a concordant picture of hybridisation with a strong correlation between the genotypic admixture proportions at 19 microsatellite loci and the first vectors of a principal component analysis ( $P < 0.001$ ) and of a 3-class discriminant analysis ( $P < 0.001$ ) of 15 quantitative morphological characters. This integrative approach enabled a grouping into *F. polyctena*, the hybrid and *rufa*. Genetic differentiation between the hybrid and *F. rufa* is significantly larger than between the hybrid and *polyctena*, indicating gene flow mainly between the latter entities. A suggested gene flow bias towards *F. polyctena* agrees with differential queen acceptance and mating behaviour. Both genetic and phenotypic colony parameters indicate predominance of monogyny in *F. rufa* but of polygyny in *polyctena* and the hybrid. Hybrids are intermediate between the parental species in body size, diagnostic morphological characters, monogyny frequency, size of nest population, nest diameter and infestation rate with epizootic fungi. The three entities respond differently to woodland fragmentation. Hybrids are significantly more abundant in forests with a coherent area < 300 ha than in woodland above this size. Regions with high hybrid frequency in Germany-the Eastern Oberlausitz (23%) and the Baltic Sea islands Darss, Hiddensee and Rugen (28%)-are characterised by a fragmented woodland structure whereas regions with low hybrid frequency-Brandenburg and the lower Erzgebirge (3.4%)-have clearly larger and more coherent forest systems. Data from other European countries indicate habitat fragmentation to be a facilitating factor but no essential precondition for interspecific hybridisation in these ants. Hybrids are hypothesised to have selective advantage in fragmented systems because of combining the main reproductive and dispersal strategies of the parental species.

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~~DIFFERENTIAL GENE EXPRESSION PROFILES IN THE VENOM GLAND/SAC OF *Orancistrocerus drewseni* (HYMENOPTERA: EUMENIDAE)~~

Baek, JH; Woo, TH; Kim, CB; Park, JH; Kim, H; Lee, S; Lee, SH. 2009

To determine differential gene expression profiles in the venom gland and sac (gland/sac) of a solitary hunting wasp species, *Orancistrocerus drewseni* Saussure (1857), a subtractive cDNA library was constructed by suppression subtractive hybridization. A total of 498 expressed sequence tags (EST) were clustered and assembled into 205 contigs (94 multiple sequences and 111 singletons). About 65% (134) of the contigs had matched BLASTx hits ( $E \leq 10^{-4}$ ). Among these, 115 contigs had similarity to proteins with assigned molecular function in the Gene Ontology database, and most of them (112 contigs, 83%) were homologous to genes from Hymenoptera, particularly to *Apis mellifera* (98 contigs). The contigs encoding hyaluronidase and phospholipase A2, known to be main components of wasp venoms, were found in high frequencies (27 and 4% respectively, as judged by the number of ESTs) in the gene ontology category of catalytic activity. Full-length open reading frames of hyaluronidase and phospholipase A2 were characterized and their abundance in the venom gland/sac was confirmed by quantitative real-time PCR. Several contigs encoding enzymes, including zinc-metalloproteinases that are likely involved in the processing and activation of venomous proteins or peptides, were also identified from the library. Discovery of venom gland/sac-specific genes should promote further studies on biologically active components in the venom of *O. drewseni*. (C) 2009 Wiley Periodicals, Inc.

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**DORONOMYRMEX POCAHONTAS - NOT A WORKERLESS PARASITE BUT STILL AN ENIGMATIC TAXON (HYMENOPTERA, FORMICIDAE)**

BUSCHINGER, A; HEINZE, J. 1993

New data suggest that the North American ant *Doronomyrmex pocahontas* Buschinger 1979, originally described as a workerless inquiline, is not a social parasite. Breeding experiments show a remarkable phenotypic plasticity, which is probably due to hybridization or to intraspecific polymorphism.

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~~Lifecycle closure, lineage sorting, and hybridization revealed in a phylogenetic analysis of European oak gallwasps (Hymenoptera: Cynipidae: Cynipini) using mitochondrial sequence data~~

Rokas, A; Melika, G; Abe, Y; Nieves-Aldrey, JL; Cook, JM; Stone, GN. 2003

Oak gallwasps are cyclically parthenogenetic insects that induce a wide diversity of highly complex species- and generation-specific galls on oaks and other Fagaceae. Phylogenetic relationships within oak gallwasps remain to be established, while sexual and parthenogenetic generations of many species remain unpaired. Previous work on oak gallwasps has revealed substantial intra-specific variation, particularly between regions known to represent discrete Pleistocene glacial refuges. Here we use statistical phylogenetic inference methods on sequence data for a fragment of the mitochondrial cytochrome b gene to reconstruct the relationships among 62 oak gallwasp species. For 16 of these we also include 23 additional cytochrome b haplotype sequences from different Pleistocene refuge areas to test the

effect of intra-specific variation on inter-specific phylogeny reconstruction. The reconstructed phylogenies show good intra-generic resolution and identify several conserved clades, but fail to reconstruct either very recent or very ancient divergences. Nine of the 16 species represented by multiple haplotypes are not monophyletic. The apparent discordance between the recovered gene tree and the current taxonomic classification can be explained through: (a) collapsing of some species currently known only from either a sexual or a parthenogenetic generation into a single cyclically parthenogenetic entity; (b) sorting of ancestral polymorphism in diverging lineages, and (c) horizontal transfer of haplotypes, perhaps due to hybridization within glacial refuges. Our conclusions emphasise the need for careful intra-specific sampling when reconstructing phylogenies for radiations of closely related species and imply that for certain taxonomic groups full phylogenetic resolution (using molecular markers) may not be attainable. (C) 2002 Elsevier Science (USA). All rights reserved.

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#### ~~Stock composition of northern neotropical honey bees: mitotype and morphotype diversity in Mexico (Hymenoptera: Apidae)~~

Dominguez-Ayala, R; Moo-Valle, H; May-Itza, WD; Medina-Peralta, S; Quezada-Euan, JGG. 2016

The stock of honey bees in the northern neotropics is likely a composite of European and African lineages, but the genetic makeup of most populations in this region has remained unstudied. We analyzed the genetic composition of honey bees across temperate and tropical regions of Mexico using mitochondrial and morphometric analyses. The results showed that honey bees from Mexico are descendent almost in similar proportion from matriline of African and European origins. However, morphometrics indicate that most colonies are the result of extensive introgressive hybridization with Africanized bees. While large-scale displacement of European honey bees seems to have occurred in the tropical regions, higher frequencies of colonies with a mixed range of African-European markers were identified in the temperate areas. Our results suggest that the outcome of the hybridization between Africanized and European honey bees in Mexico has been significantly associated with climate.

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#### Come and Gone: Description of a New Species of Eucharitidae (Hymenoptera) Attacking Solenopsis (Hymenoptera: Formicidae) in Texas

Chien, I; Heraty, JM. 2018

Eucharitidae (Hymenoptera: Chalcidoidea) are the only family in which all members are specialized parasitoids of ant brood, with species of *Orasema* Cameron (*Oraseminae*) parasitizing several genera of Myrmicinae, including *Solenopsis* Westwood and *Pheidole* Westwood (*Formicidae*). In 1988, a new species of *Orasema* was discovered in southern Texas attacking the brood of what is recognized as a *Solenopsis geminata* (Fabricius) (Hymenoptera: Formicidae) x *Solenopsis xyloni* McCook (Hymenoptera: Formicidae) hybrid. Adults of *Orasema* oviposit onto the green stems and leaves of *Vachellia* sp. (Fabaceae). *Orasema taii* n. sp. was only found attacking the hybrid colonies and was not recovered from adjacent nests of *Solenopsis invicta* Buren (Hymenoptera: Formicidae), which at that time were equally abundant in the area. Wasps were abundant at the time of collection, but since these initial collections, the parasitoids have not been recovered. Hybrid populations have declined precipitously with the invasion of the imported fire ant, and both the hybrid and parasitoid may have been extirpated at least from the area of discovery. Based on morphological and molecular evidence, a *taii* n. sp. is placed into the *Orasema bakeri* species group. The adult and immature stages and their behavior in the field are described.

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#### Contribution of integrative taxonomy to tracking interspecific hybridizations between the biological control agent *Torymus sinensis* and its related taxa

Viciruc, IM; Thaon, M; Moriya, S; Warot, S; Zhang, JP; Aebi, A; Ris, N; Fusu, L; Borowiec, N. 2021

Native to China, the chestnut gall wasps *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae) has invaded several countries on three continents in about half a century. To reduce the negative impact on the chestnut industry, the hymenopteran parasitoid *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) was firstly translocated from China to Japan and then from Japan to other countries. From an agronomic point of view, this classical biological control programme is perceived as a great success story even if in Japan it was overshadowed by evidence of hybridization with the indigenous species *Torymus beneficus* Yasumatsu & Kamijo. Based on numerous specimens collected in France and Italy but also in Asia, as well as on published data, we detected molecular and phenotypic signatures of a possible hybridization. Our results evidenced for the first time that the European stock of *T. sinensis* has some rare molecular signatures of historical hybridization that took place in Japan (0.66% of the mitochondrial haplotypes). Our morphometric study also shows that European and Japanese *T. sinensis* are morphologically intermediate between the two species that hybridized. The identity of the early- and late-season strains of *T. beneficus* is discussed. *Torymus beneficus* late-season strain (TbL) is considered to be the same as *T. sinensis* and only the early-season strain (TbE) is the true *T. beneficus*. *Torymus beneficus* is morphologically very close to *T. sinensis*, and the interspecific distance of COI, though large, is comparable with the larger intraspecific variability found in other Chalcidoidea species. Though the two could be regarded as subspecies, for the stability of nomenclature it is better to continue treating them as distinct species.

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#### ~~Introgression in hybrid ants is favored in females but selected against in males~~

Kulmuni, J; Pamilo, P. 2014

Hybridization is not a mere reproductive dead end but has been suggested to play a central role in speciation, for example, by introducing adaptive genetic variation. Our previous study uncovered a unique consequence of hybridization in *Formica* ants. In a population including two isolated but partially introgressed genetic groups, the females have an apparent hybrid background, whereas the males do not. This situation results in large-scale differences between male and female genomes that are stable throughout generations. Here, we compare genotypes from different developmental stages to investigate how sex-specific introgression and genetic differences between sexes are maintained. We show that strong selection rather than sex-dependent transmission maintains the genetic differences between sexes. All genotype combinations are produced and observed in the eggs of both sexes, but the alleles acquired through hybridization disappear from the haploid males during development from egg to adult as their frequencies drop toward zero. However, the same introgressed alleles increase in frequency and are favored when heterozygous in the females. Genotypes eliminated from males most likely represent incompatibilities arising from hybridization. Our results show an unusual situation of opposite selection, where introgression is favored in diploid females but selected against in haploid males. This finding suggests that introgressed genomic regions harbor both fitness-enhancing and -reducing elements. Our work highlights the complex consequences of hybridization and provides a rare opportunity to observe natural selection in real time in nature.

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#### DNA-barcoding of the Italian members of the *Aphaenogaster testaceopilosa*-group (Hymenoptera: Formicidae): hybridization and biogeographic hypothesis

Centorame, M; Moschella, F; Russini, V; Fanfani, A. 2018

The thermophilic *Aphaenogaster testaceopilosa*-group is a taxon consisting of 14 species in Europe. Recently, this group was recently revisited using morphological characters suggesting different changes in the systematic of this group. For the first time, in this work we used a molecular approach using the DNA barcoding on the European members of the genus *Aphaenogaster* in order to: i) validate the current systematic of the *A. testaceopilosa* group in Italy, ii) test the efficiency of COI marker to delimit the species belonging to the genus *Aphaenogaster*. In the first phase, we morphologically identified 54 specimens of *A. testaceopilosa* group collected along the entire Italian range. In the second phase, fragments of COI and CAD markers were sequenced from phenotyped individuals. Phylogenetic reconstructions and genetic distances clearly showed six lineages (MOTUs) in agreement with morphological identification except for one specimen collected in a likely contact zone between *Aphaenogaster picena* and *Aphaenogaster semipolita*. Furthermore, this study confirms the powerful of DNA barcoding to support the



morphological analysis to delimit the species belonging to *Aphaenogaster testaceopilosa*-group. Therefore, genetic data could be a valid tool to investigate other more problematic groups of this little-known genus. (C) 2018 Elsevier GmbH. All rights reserved.

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#### Winter survival of *Solenopsis invicta* and the *Solenopsis* hybrid (Hymenoptera: Formicidae) in Georgia

Diffie, S; Bass, MH; Bondari, K. 1997

The hybrid imported fire ant (*Solenopsis invicta* Buren X *S. richteri* Forel) infests the northern half of Alabama and the Alabama border counties of northern Mississippi and northern Georgia in the United States. Its habitat in Georgia includes regions that were previously uninfested by imported fire ants. The winter temperatures in these regions were considered to be too cold for imported fire ant survival. The movement of the hybrid fire ant into previously uninfested northern counties of Georgia prompted this study to monitor the winter survival of the hybrid. Hybrid fire ant colonies were monitored for five consecutive winters, and their survival was compared with colonies of red imported fire ants (*S. invicta* Buren) in northern and southern Georgia during these five winters. Data showed no evidence that the hybrid can survive the cold winter temperatures of Georgia better than the red imported fire ant. Effect of location (southern or northern Georgia) on ant colonies was not significant, but the environmental conditions varying from one year to another numerically influenced colony survival of imported fire ants.

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#### Worker Size, Geographical Distribution, and Introgressive Hybridization of Invasive *Solenopsis invicta* and *Solenopsis richteri* (Hymenoptera: Formicidae) in Tennessee

Pandey, M; Addesso, KM; Archer, RS; Valles, SM; Baysal-Gurel, F; Ganter, PF; Youssef, NN; Oliver, JB. 2019

Worker size and geographical distribution of red imported fire ants (*Solenopsis invicta* Buren), black imported fire ants (*Solenopsis richteri* Forel), and their hybrid (*S. invicta* x *S. richteri*) (Hymenoptera: Formicidae) were evaluated from colonies sampled across Tennessee. The fire ant species and hybrid status were determined using cuticular hydrocarbon and venom alkaloid indices obtained from gas chromatography and mass spectrometry. Hybrids were the most common fire ant throughout Tennessee. With the exception of a few isolated *S. invicta* samples, only hybrids were found in east Tennessee, and hybrids predominated in middle Tennessee. In west Tennessee, mixed populations of *S. richteri* and hybrids were found. Hybrids were more common in west Tennessee than a survey performed a decade earlier. No statistical differences were detected in the average inter-colonial worker size of *S. richteri* and hybrids. Likewise, average worker size was not related to geographic location in Tennessee. The similarity in average worker size among hybrid colonies with a wide range of cuticular hydrocarbon and venom alkaloid values suggests introgression was not impacting ant size in colonies sampled throughout Tennessee.

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#### Genetic polymorphism in "mixed" colonies of wood ants (Hymenoptera: Formicidae) in southern Finland and its possible origin

Korczynska, J; Gajewska, M; Pilot, M; Czechowski, W; Radchenko, A. 2010

Wood ant colonies that appear to consist of individuals representing different species are described in several previous papers. The present study is the first to elucidate the genetic basis of the spectacular morphological variability observed within such colonies. Two seemingly mixed colonies (FM-1 and FM-2) from southern Finland were investigated. On the basis of the morphology of their workers these colonies were comprised of individuals with phenotypes typical of *Formica rufa* L., *F. polyctena* Forst., and *F. aquilonia* Yarr. The sequence of an mtDNA fragment (5' end of the cytochrome b gene) was used to examine the phylogenetic relationships among haplotypes of workers from homogeneous colonies of different wood ant species and the two supposedly mixed colonies, and to sort the individuals within the colonies into matriline. Six microsatellite loci were used to analyse the genetic differentiation between colonies and among workers within colonies, and to detect putative hybrids. The results show that, independently of their phenotype, workers from the "mixed" colonies were genetically more similar to other individuals in their colony than to those in the homogeneous *F. rufa*, *F. polyctena* or *F. aquilonia* colonies. However, while colony FM-1 consisted of offspring of the same queen or more likely several maternally related queens, colony FM-2 consisted of the offspring of at least four unrelated queens. The data suggest hybridisation between *F. polyctena* x *F. aquilonia* and *F. polyctena* x *F. rufa* (and possibly subsequent mating between these hybrids) as the most probable mechanism leading to the existence of these two colonies, which implies that the hybrids are fertile. This study shows that colonies of wood ant hybrids can arise spontaneously and persist under natural conditions. The results also revealed that even some morphologically homogeneous colonies are genetically heterogeneous. In the case of closely related, morphologically similar species that interbreed, morphology can be a bad predictor of genetic differences between individuals.

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#### Courtship displays of introgressed, interspecific hybrid *Nasonia* males: Further investigations into the 'grandfather effect'

Beukeboom, LW; van den Assem, J. 2002

Previously, we investigated courtship behaviour of bidirectional, interspecific hybrid males of two species of *Nasonia* (Beukeboom & van den Assem, 2001). Characteristics of the displays were intermediate between those of the parental species, but at the same time were biased towards the paternal side. Due to the haplodiploid sex determination system of the Hymenoptera, the nearest male parent of the haploid (hence, fatherless) *Nasonia* males are their grandfather. Therefore, we have called this bias the 'grandfather effect'. In the present paper, we investigate one of the possible causes of the 'grandfather effect': nucleocytoplasmic interaction. In interspecific hybrids, the paternally donated nuclear genes must operate in an 'alien' environment: the maternal (heterospecific) cytoplasm. Adverse effects may prevail in this situation, and result in a biased gene transmission (although to the maternal side). With introgression techniques and subsequent hybridisation of introgressed lines, we constructed male progeny in which paternally contributed nuclear genes are conspecific with the maternal cytoplasm. Courtship of these males provide a test of the interaction hypothesis. Because our results show a persistence of the 'grandfather effect', a simple nucleo-cytoplasmic interaction seems an unlikely explanation of the phenomenon.

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#### Resistance of *Salix caprea*, *S. phylicifolia*, and their F1 hybrids to herbivores and pathogens

Hjalten, J; Ericson, L; Roininen, H. 2000

Traits that make plants unpalatable to herbivores are often believed to have evolved as a response to herbivory. Thus, it has been suggested that the genetic re-combinations that occur when plants hybridize might result in a breakdown in plant resistance to herbivores and pathogens. In this study, we tested whether hybrid plants are less resistant to herbivores and pathogens than the parental species under controlled environmental conditions. Two common and widespread willow species, the tree-forming *Salix caprea* L. and the shrub-forming *S. phylicifolia* Sm. (L.) and their F1 hybrids were used in the study. To control the origin of plant material used in the experiments, we hand-pollinated plants in the field to create F1 hybrids and pure parental individuals, which were potted and kept outdoors in an experimental field. During the third growing season we measured plant survival, leaf numbers, and the densities of three species (gallers and leaf-folders of sub-genus *Pontania* and *Phyllocolpa*) of galling sawflies (Hymenoptera: Tenthredinidae) and the frequency of leaves infected by the parasitic fungus, *Rhizina salicinum*. The experimental design used should minimize the influence of environmental variation on the plant characters. Therefore, the results primarily reflect genetically based differences in quality between hybrids and parental species. Hybrids and *S. phylicifolia* had more leaves than *S. caprea*. In addition, hybrids and *S. caprea* had higher densities of the leaf-galler *Pontania pedunculi* and the leaf-folder *Phyllocolpa* sp. than *S. phylicifolia*, whereas no differences were found between *S. caprea* and hybrids. We found little evidence suggesting a resistance breakdown in hybrids. Instead, the results are consistent with prediction from the dominance hypothesis, i.e., similar herbivore load on hybrids and one of the parental species. The density of bran-galls (*Pontania bridgmanii*) and the frequency of leaves infected by the tar-spot

disease, *Rhytisma salicinum* did not differ significantly between the plant categories. We conclude that to understand the mechanisms responsible for the disease/attack patterns on hybrid plants, the life cycle/life history traits of the involved organisms must be considered.

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

Jeong, G; Stouthamer, R. 2006

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

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#### **An analysis of heterosis and outbreeding depression among lab-reared populations of the parasitoid *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae); potential implications for augmentative releases**

Vorsino, AE; Wicczorek, AM; Wright, MG; Messing, RH. 2012

Augmentative biological control is a process in which a biocontrol agent is mass-reared and released to either augment the existing population present in the field or inundate the target pest population. Although research into the utility of augmentative biocontrol has been conducted for similar to 50 years, little is known about the effect of inundative releases on the progeny of the released population and the wild population. We used the arrhenotokous haplo-diploid tephritid parasitoid *Diachasmimorpha tryoni* (Cameron) (Hymenoptera; Braconidae) to model a theoretical augmentative release interaction. By employing a combination of molecular techniques and a fitness proxy, our results were capable of documenting the hybridization of a "released" and "wild" population (as modeled in vitro). These analyses revealed significant hybridization interactions between two moderately differentiated populations ( $F_{st}/R_{host} = 0.1352/0.0641$ ), as would be the case during an augmentative release. Our analysis also revealed an outcrossing asymmetry of hybrid progeny relating to parental population origin. This interaction has the potential to either hinder or facilitate augmentative control and may prove useful to future augmentative releases. Published by Elsevier Inc.

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#### **BIOSYSTEMATICS OF THE COTESIA-FLAVIPES COMPLEX (HYMENOPTERA, BRACONIDAE) - INTERSPECIFIC HYBRIDIZATION, SEX-PHEROMONE AND MATING-BEHAVIOR STUDIES**

KIMANI, SW; OVERHOLT, WA. 1995

Mating behaviour, sex pheromone attraction and reciprocal breeding of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), *C. sesamiae* (Cameron) and *C. chilonis* (Matsumura) were studied. These three putative species comprise the *Cotesia flavipes* complex. Wing fanning and antennal vibration were the initial courtship signals from the males. Antennal stroking by the male was also an important contact signal and a prerequisite to successful mounting and copulation. Interspecific crosses revealed that males of *C. flavipes* exhibited courtship behaviour, and mounted and copulated with females of *C. chilonis* and *C. sesamiae*; the males transferred sperm but progeny from these crosses did not include females. Males of *C. sesamiae* copulated with females of *C. chilonis* and the progeny included viable females. The progeny backcrosses of the hybrid females to male parents also included viable females. Sex pheromone experiments were conducted in a Y-tube olfactometer and in large field cages. Males and females of *C. flavipes* perceived and responded to odours emitted by the opposite sex. There was no significant response to odours from conspecific individuals of the same sex in any of the three species. Pheromone bioassays in field cages using sticky traps baited with live virgin *C. flavipes* females attracted conspecific males.

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#### ~~Differential gene expression between alate and dealate queens in the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)~~

Tian, HS; Vinson, SB; Coates, CJ. 2004

The transition of fire ant queens from alates to dealates, following a mating flight, is associated with numerous important physiological changes. A molecular analysis of gene expression differences that occur between alates and dealates was performed using the suppression subtractive hybridization (SSH) method. 983 SSH clones were arrayed and screened by dot blot hybridization, followed by Northern blot analysis for selected clones. Gene expression profiles throughout fire ant development were determined using semi-quantitative reverse transcriptase polymerase chain reactions (RT-PCR). The cytochrome c oxidase subunit 11 and STARS (striated muscle activator of Rho signaling) transcripts were expressed at higher levels in dealates compared to alates and may be involved in the programmed cell death of the flight muscles. Three different vitellogenin genes and two unique yellow, g-like genes were identified that may be closely associated with the reproductive system and/or nutrient transport. Two putative antibacterial peptides, abaecin and hymenoptaecin precursors, were highly expressed in dealate queens, suggesting that they are present as an immune system component during this important stage of fire ant development. The genes identified in this study may be utilized as novel targets for fire ant control and will also provide molecular markers for studies of other social insects. (C) 2004 Elsevier Ltd. All rights reserved.

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#### ~~Ideal phenotypes and mismatching haplotypes - errors of mtDNA tracing in ants (Hymenoptera: Formicidae) detected by standardized morphometry~~

Seifert, B; Goropashnaya, AV. 2004

A total of 401 nest samples of *Formica lugubris* Zetterstedt, *F. pratensis* Retzius, *F. aquilonia* Yarrow, *F. rufa* Linnaeus, and *F. polyctena* Forster, covering the entire Palaearctic range of these species and including 2100 individual workers, was phenotypically investigated by a system of standardized morphometry, pairwise removal of allometric variance, and canonical discriminant functions. A mitochondrial DNA fragment including the cytochrome b gene was sequenced in 148 samples from basically the same range. In the more difficult *F. pratensis* vs. *F. lugubris* case, the phenotypic system correctly determined 99.6% of all nest samples, and 95.1% with  $p < 0.05$ . In all other pairwise species discriminations any nest sample was correctly determined with  $p < 0.01$ , and three samples with hybrids *F. rufa* x *lugubris* were identified. At four localities in the Pyrenees and the Urals, 9 samples with *F. pratensis* phenotypes (7 of them ideal) but *F. lugubris* mtDNA haplotypes could be identified, resulting in 14.5% of phenotype/haplotype mismatches. A local dominance of this mismatch combination was observed at one Pyrenean and one Ural locality. There was no indication of an *F. pratensis* haplotype associated with an *F. lugubris* phenotype. One ideal *F. polyctena* phenotype was associated with an *F. aquilonia* haplotype in a sample from the Urals, and one ideal *F. aquilonia* phenotype was combined with an *F. lugubris* haplotype in a sample from central Siberia, resulting in overall phenotype/haplotype mismatch frequencies of 12.5% and 11.1%, respectively. We conclude that all these samples cannot represent actual *F. lugubris* but are the result of hybridizations in the past followed by unidirectional purging of the nuclear genome. Whether this process of purging worked very fast or over longer periods of population history, and whether or not it was complete or incomplete, cannot be assessed from the available information. These facts of hybridizing in two thirds of the W Palaearctic wood ant species, of extreme regional hybrid frequencies (up to 26%), of unidirectional purging of mtDNA associated with mismatching mtDNA haplotypes, and of occasional achievement of local dominance of these mismatch combinations, may serve as urgent warning not to perform

isolated mtDNA phylogenetic studies without a geographically and locally wide sampling basis and without control by nDNA information or reliable phenotypic determination. The latter two systems definitely have superior significance when conflicts with mtDNA indications arise. (C) 2004 Elsevier GmbH. All rights reserved.

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#### **~~Apomictic parthenogenesis in a parasitoid wasp *Meteorus pulchricornis*, uncommon in the haplodiploid order Hymenoptera~~**

Tsutsui, Y; Maeto, K; Hamaguchi, K; Isaki, Y; Takami, Y; Naito, T; Miura, K. 2014

Although apomixis is the most common form of parthenogenesis in diploid arthropods, it is uncommon in the haplodiploid insect order Hymenoptera. We found a new type of spontaneous apomixis in the Hymenoptera, completely lacking meiosis and the expulsion of polar bodies in egg maturation division, on the thelytokous strain of a parasitoid wasp *Meteorus pulchricornis* (Wesmael) (Braconidae, Euphorinae) on pest lepidopteran larvae *Spodoptera litura* (Fabricius) (Noctuidae). The absence of the meiotic process was consistent with a non-segregation pattern in the offspring of heterozygous females, and no positive evidence was obtained for the induction of thelytoky by any bacterial symbionts. We discuss the conditions that enable the occurrence of such rare cases of apomictic thelytoky in the Hymenoptera, suggesting the significance of fixed heterosis caused by hybridization or polyploidization, symbiosis with bacterial agents, and occasional sex. Our finding will encourage further genetic studies on parasitoid wasps to use asexual lines more wisely for biological control.

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#### **~~MITOCHONDRIAL GENOME OF APHELINUS MALI (HALDEMAN) (HYMENOPTERA: APHELINIDAE) WITH A HIGHER AT PERCENT AND THE HIGHEST AT SKEW IN HYMENOPTERA~~**

Du, LJ; Wei, SJ; Tan, XM; Chu, XX; Yang, QM; Wang, JQ; Wan, FH; Zhou, HX. 2020

*Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) is an important specific endoparasitoid of *Eriosoma lanigerum* (Hausmann) (Hymenoptera: Aphididae) with high natural parasitism in the field. By studying the COI gene of the *A. mali* mitochondrion, two distinct clades, Shandong and Liaoning clades, were found in China. In this paper, the mitochondrial genome of *A. mali* populations from Shandong province was sequenced and analyzed. In total, 14415 bp were sequenced, representing 93.5% of the total length of the *A. mali* mitochondrial genome, with six tRNA, trnY, trnM, trnI, trnQ, trnW, trnS1, not sequenced. The unmeasured area was located in an A+T-rich region of approximately 1000 bp. The AT content in the A+T-rich region was as high as 91.3% and this may be why this region has not been detected until now. The A+T content was 84.4% in the measured region, which is higher than that of other Hymenoptera. The AT skew was 0.1291, the highest AT skew known among Hymenoptera, which might be related to the higher AT content of protein-coding genes, rRNA and RSCU AT content. Transport genes (tRNA) had a secondary clover shaped structure, compared with other Hymenoptera; there is a more incomplete secondary structure, and less base mismatch. The mitochondrial genome of *A. mali* showed considerable rearrangement when compared with the mitochondria' genome of the most primitive arrangement, mainly concentrated in six tRNA genes and the gene region from cob to tmE and from trnI, to nadl. The mitochondria' genome of *A. mali* is quite different from that of other Hymenoptera as can be demonstrated by constructing a phylogenetic tree. This paper provides a basis for further study of phylogeny, genetic structure, and hybridization of *A. mali* populations.

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#### **~~No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species~~**

Kohnen, A; Wissemann, V; Brandl, R. 2011

Differences in quality and quantity of secondary compounds, as well as in leaf traits of host plants, may influence the host choice of herbivores. Different host preferences could lead to host-associated differentiation, the first step of sympatric speciation. In the present study, we investigated whether the rose gall wasp *Diplolepis rosae* L. (Hymenoptera: Cynipidae) shows genetic differentiation related to its host plants (*Rosa canina* L., *Rosa corymbifera* Borkh., and *Rosa rubiginosa* L.). These three host species radiated recently and subsequently expanded their range. Therefore, we expected a diversification within the closely-associated phytophagous insects. The process of genetic differentiation should be intensified in *D. rosae* by its close relationship to the host plant, as well as by its parthenogenetic reproduction (infection rate by *Wolbachia* sp. of almost 100%). However, using 106 polymorphic amplified fragment length polymorphism markers, we found no genetic differentiation among the wasps from different host plants. The population structuring between geographical localities was also low, suggesting considerable gene flow between sites. In part, the low genetic differentiation between sites is explained by the wide distribution of host species and hybrids between host plants. Hybrids with intermediate traits may facilitate the gene flow between wasp populations exploiting different host species. (C) 2011 The Linnean Society of London, Biological Journal of the Linnean Society, 2011, 102, 369-377.

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#### **~~CLONING OF CDNA FOR VITELLOGENIN OF ATHALIA (HYMENOPTERA) ROSAE AND CHARACTERIZATION OF THE VITELLOGENIN GENE-EXPRESSION~~**

KAGEYAMA, Y; KINOSHITA, T; UMESONO, Y; HATAKEYAMA, M; OISHI, K. 1994

*Athalia rosae* (Hymenoptera) was previously shown to have two vitellins (L-Vn and S-Vn) and the two corresponding vitellogenins (L-Vg and S-Vg). A cDNA expression library was constructed from poly(A)<sup>+</sup> RNA prepared from adult female fat body cells, and was screened for the vitellogenins by using antisera against the L- and S-Vn, respectively. Examinations of cloned cDNAs show that the vitellogenin gene is transcribed as a single unit, with the 5'-terminal site coding for the S-Vg and the 3'-terminal site for the L-Vg. Nucleotide sequence at the 5'-end suggests the presence of a 16 amino acid-long signal peptide. Deduced amino acid sequence following the signal peptide shows a complete match with up to the 28 N-terminal amino acid sequence determined on S-Vn. The S-L Vg boundary with deduced amino acid sequence matching with 6 N-terminal amino acid sequence determined on L-Vn is also detected. Northern blot hybridization analysis shows that the vitellogenin gene is expressed in the female fat body as a single 6.5 kb mRNA but not in the ovary, and not in the male fat body. Western blot analysis detects a large precursor polypeptide, reacting with the anti-L-Vn and S-Vn antisera, in the adult female fat body.

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#### **~~Are the TTAGG and TTAGGG telomeric repeats phylogenetically conserved in aculeate Hymenoptera?~~**

Menezes, RST; Bardella, VB; Cabral-de-Mello, DC; Lucena, DAA; Almeida, EAB. 2017

Despite the (TTAGG)(n) telomeric repeat supposed being the ancestral DNA motif of telomeres in insects, it was repeatedly lost within some insect orders. Notably, parasitoid hymenopterans and the social wasp *Metapolybia decorata* (Gribodo) lack the (TTAGG)(n) sequence, but in other representatives of Hymenoptera, this motif was noticed, such as different ant species and the honeybee. These findings raise the question of whether the insect telomeric repeat is or not phylogenetically predominant in Hymenoptera. Thus, we evaluated the occurrence of both the (TTAGG) n sequence and the vertebrate telomere sequence (TTAGGG)(n) using dot-blotting hybridization in 25 aculeate species of Hymenoptera. Our results revealed the absence of (TTAGG)(n) sequence in all tested species, elevating the number of hymenopteran families lacking this telomeric sequence to 13 out of the 15 tested families so far. The (TTAGGG)(n) was not observed in any tested species. Based on our data and compiled information, we suggest that the (TTAGG)(n) sequence was putatively lost in the ancestor of Apocrita with at least two subsequent independent regains (in Formicidae and Apidae).

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#### **Taxonomy and phylogeny of Leptopilina species (Hymenoptera: Cynipoidea: Figitidae) attacking frugivorous drosophilid flies in Japan, with description of three new species**

Novkovic, B; Mitsui, H; Suwito, A; Kimura, MT. 2011

Despite the intensive use of the *Leptopilina* genus and its drosophilid hosts as model systems in the study of host-parasitoid interactions, the diversity and distribution of the species occurring in the Asian region remain elusive. Here we report the phylogeny of Japanese *Leptopilina* species attacking frugivorous drosophilid flies, based on COI, ITS1 and ITS2 sequences. Consistent with molecular data, hybridization experiments and morphological examination, five species were recorded in Japan: *Leptopilina heterotoma*, *L. victorae* and three new species, two occurring in the Ryukyu archipelago, *L. ryukyuensis* and *L. pacifica*, and another species, *L. japonica*, distributed in Honshu and Hokkaido. *Leptopilina japonica* is further divided into two subspecies, *L. j. japonica* occurring in Japan, and *L. j. formosana* occurring in Taiwan. According to these results, we discuss the evolution, speciation and colonization history of Japanese *Leptopilina* species.

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

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

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

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

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

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

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#### **Evolution of hybridogenetic lineages in *Cataglyphis* ants**

Darras, H; Kuhn, A; Aron, S. 2019

In most social Hymenoptera, a diploid egg develops into either a queen or a worker depending on environmental conditions. Hybridogenetic *Cataglyphis* ants display a bizarre genetic system, where queen-worker caste determination is primarily determined by genetic factors. In hybridogenetic populations, all workers are F1 hybrids of two distinct lineages, whereas new queens are nearly always pure-lineage individuals produced by clonal reproduction. The distribution and evolutionary history of these hybridogenetic populations have not yet been thoroughly analysed. Here, we studied the phylogeographic distribution of hybridogenetic populations in two closely related Spanish species: *Cataglyphis humeya* and *Cataglyphis velox*. Hybridogenesis has been previously documented in a locality of *C. velox*, but whether this system occurs elsewhere within the range of the two species was yet unknown. Queens and workers from 66 localities sampled across the range of the species were genotyped at 18 microsatellite markers to determine whether queens were produced by parthenogenesis and whether workers were hybrids of divergent lineages. Populations with F1 hybrid workers were identified by combining genetic, geographical and mating assortments data. In most populations of *C. velox*, workers were found to be hybrids of two divergent lineages. Workers were however produced via random mating in two marginal populations of *C. velox*, and in all populations studied of its sister species *C. humeya*. High-throughput sequencing data were obtained to confirm inferences based on microsatellites and to characterize relationships between populations. Our results revealed a complicated history of reticulate evolution that may account for the origin of hybridogenetic lineages in *Cataglyphis*.

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#### **Detection of hybrids between introduced *Torymus sinensis* and native *T. beneficus* (Hymenoptera : Torymidae) in central Japan, using malic enzyme**

Yara, K; Yano, E; Sasawaki, T; Shiga, M. 2000

*Torymus sinensis* is an introduced parasitoid used to control chestnut gall wasp, *Dryocosmus kuriphilus*. After the release of *T. sinensis*, morphologically-intermediate individuals between *T. sinensis* and the native parasitoid, *T. beneficus* appeared in the field, which were suspected to be the result of interspecific hybridization. In order to confirm whether the intermediate individuals were hybrids of *T. sinensis* and *T. beneficus* (early-season strain), their genotype for malic enzyme was analyzed by polyacrylamide gel electrophoresis. Nine individuals of 821 analyzed *Torymus* females showed a banding pattern (FS genotype) of hybrids between *T. sinensis* and the early-season strain of *T. beneficus*. Of 388 individuals categorized as morphologically-intermediate, only 2 individuals showed the FS genotype.

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#### **Distribution of the fire ant (Hymenoptera : Formicidae) hybrid in Georgia**

Gardner, WA; Diffie, S; Meer, RKV; Brinkman, MA. 2008

## **Inter- and intraspecific effects of volatile and nonvolatile sex pheromones on males, mating behavior, and hybridization in *Eretmocerus mundus* and *E. eremicus* (Hymenoptera : Aphelinidae)**

Ardeh, MJ; de Jong, PW; Loomans, AJM; van Lenteren, JC. 2004

*Eretmocerus* species (Hym. Aphelinidae) are solitary parasitoids of *Bemisia tabaci* (Gennadius). Mate finding and mating behavior of two species, *E. mundus* and *E. eremicus*, were studied under laboratory conditions. We used three populations of *Eretmocerus*: typical arrhenotokous populations of *E. eremicus* ( from USA) and *E. mundus* ( from Spain), and an atypical thelytokous population of *E. mundus* ( from Australia). We studied the intra- and interspecific responses of males to volatile and nonvolatile components of the female sex pheromones, mating behavior, and hybridization between populations and species. In both arrhenotokous populations, males reacted to volatile pheromones by walking toward conspecific virgin females. Males also reacted to nonvolatile pheromones by spending more time on and around patches on leaves of poinsettia plants that had been exposed to virgin females. Males of *E. eremicus* showed the same reaction to the nonvolatile sex pheromone of *E. mundus* females, but *E. mundus* males did not show any reaction to the nonvolatile sex pheromone of *E. eremicus*. There was no response of males of both species to thelytokous females of *E. mundus*. In both species three phases were distinguished in the mating behavior: premating, mating, and postmating. The duration of the phases differed between the three populations. Successful copulation between the two *Eretmocerus* species did not occur. In contrast, we recorded some successful copulations between Australian males and Spanish females of *E. mundus*, but they did not produce any hybrid females.

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## **Differential gene expression in queen-worker caste determination in bumble-bees**

Pereboom, JJM; Jordan, WC; Sumner, S; Hammond, RL; Bourke, AFG. 2005

Investigating how differential gene expression underlies caste determination in the social Hymenoptera is central to understanding how variation in gene expression underlies adaptive phenotypic diversity. We investigated for the first time the association between differential gene expression and queen-worker caste determination in the bumble-bee *Bombus terrestris*. Using suppression subtractive hybridization we isolated 12 genes that were differentially expressed in queen- and worker-destined larvae. We found that the sets of genes underlying caste differences in larvae and adults failed to overlap greatly. We also found that *B. terrestris* shares some of the genes whose differential expression is associated with caste determination in the honeybee, *Apis mellifera*, but their expression patterns were not identical. Instead, we found *B. terrestris* to exhibit a novel pattern, whereby most genes upregulated (i.e. showing relatively higher levels of expression) in queen-destined larvae early in development were upregulated in worker-destined larvae late in development. Overall, our results suggest that caste determination in *B. terrestris* involves a difference not so much in the identity of genes expressed by queen- and worker-destined larvae, but primarily in the relative timing of their expression. This conclusion is of potential importance in the further study of phenotypic diversification via differential gene expression.

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## **Differential gene expression profiles in the venom gland/sac of *Eumenes pomiformis* (Hymenoptera: Eumenidae)**

Baek, JH; Lee, SH. 2010

To search for novel transcripts encoding biologically active venom components, a subtractive cDNA library specific to the venom gland and sac (gland/sac) of a solitary hunting wasp species, *Eumenes pomiformis* Fabricius (1781), was constructed by suppression subtractive hybridization. A total of 541 expressed sequence tags (ESTs) were clustered and assembled into 102 contigs (31 multiple sequences and 71 singletons). In total, 37 cDNAs were found in the library via BLASTx searching and manual annotation. Eight contigs (337 ESTs) encoding short venom peptides (10 to 16 amino acids) occupied 62% of the library. The deduced amino acid sequence (78 amino acids) of a novel venom peptide transcript shared sequence similarity with trypsin inhibitors and dendrotoxin-like venom peptides known to be K<sup>+</sup> channel blockers, implying that this novel peptide may play a role in the paralysis of prey. In addition to phospholipase A2 and hyaluronidase, which are known to be the main components of wasp venoms, several transcripts encoding enzymes, including three metalloproteinases and a decarboxylase likely involved in the processing and activation of venomous proteins, peptides, amines, and neurotransmitters, were also isolated from the library. The presence of a transcript encoding a putative insulin/insulin-like peptide binding protein suggests that solitary hunting wasps use their venom to control their prey, leading to larval growth cessation. The abundance of these venom components in the venom gland/sac and in the alimentary canal was confirmed by quantitative real-time PCR. Discovery of venom gland/sac-specific transcripts should promote further studies on biologically active components in the venom of solitary hunting wasps. (C) 2010 Elsevier Ltd. All rights reserved.

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## **Maternal microinjection of the endoparasitoid *Cardiochiles diaphaniae* (Hymenoptera: Braconidae)**

Presnail, JK; Hoy, MA. 1996

Maternal microinjection is the injection of DNA into an adult female to deliver DNA to the eggs. Here we report the adaptation of this method to the parasitoid *Cardiochiles diaphaniae* (Hymenoptera: Braconidae). Results of preliminary dissections and injections with the plasmid pJKP2, followed by slot blot analysis, suggested that this method could be used to deliver injected DNA to the ovaries of this wasp. Subsequently, 16 adult females were injected and allowed to produce offspring (the Go generation). Polymerase chain reaction analysis of the next (G(1)) generation revealed that several individuals contained the plasmid sequences. In a 2nd experiment, the plasmid phsopd, containing the parathion hydrolase gene (opd) of *Pseudomonas diminuta*, was injected. The plasmid probe hybridized to high molecular weight DNA from 3 of 38 G(1) adults. A 4th adult produced a hybridization pattern consistent with integration of plasmid into the nuclear genome. The results indicate maternal microinjection can result in transformation of this parasitoid.

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## **Development of genetic markers distinguishing two invasive fire ant species (Hymenoptera: Formicidae) and their hybrids**

Shoemaker, D. 2016

Three single nucleotide polymorphism (SNP) markers were developed that were completely diagnostic in distinguishing the 2 fire ant species *Solenopsis invicta* Buren and *Solenopsis richteri* Forel (Hymenoptera: Formicidae). Although a 4th marker was not fully diagnostic, it was still useful given that one of the variants was confined to *S. richteri*. Joint use of these markers was shown to consistently distinguish hybrids from the 2 parental species. The development of these SNP markers distinguishing the 2 introduced fire ant species and their hybrids represents a significant advance over existing methods because this method is robust, easily transferable, and cost effective.

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## **Breakdown of species boundaries in *Mandevilla*: floral morphological intermediacy, novel fragrances and asymmetric pollen flow**

Pisano, AR; More, M; Cisternas, MA; Raguso, RA; Benitez-Vieyra, S. 2019

Phenotypic intermediacy is an indicator of putative hybrid origin and has provided the main clues to discovering hybrid plants in nature. *Mandevilla pentlandiana* and *M. laxa* (Apocynaceae) are sister species with clear differences in floral phenotype and associated pollinator guilds: diurnal Hymenoptera and nocturnal hawkmoths, respectively. The presence of individuals with intermediate phenotypes in a wild population raises questions about the roles of visual and olfactory signals (i.e. corolla morphology and floral fragrances) as barriers to interbreeding, and how the breakdown of floral isolation occurs. We examined phenotypic variation in a mixed



Mandevilla population, analysing the chemical composition of floral fragrances, characterising floral shape through geometric morphometrics and assessing individual grouping through taxonomically relevant traits and an unsupervised learning algorithm. We quantified the visitation frequencies of floral visitors and tracked their foraging movements using pollen analogues. The presence of morphologically intermediate individuals and pollen analogue movement suggested extensive hybridisation between *M. laxa* and *M. pentlandiana*, along with asymmetrical rates of backcrossing between these putative hybrids and *M. laxa*. Floral volatiles from putative hybrid individuals showed a transgressive phenotype, with additional compounds not emitted by either parental species. Our results suggest the presence of a hybrid swarm between sympatric *M. pentlandiana* and *M. laxa* and indicate that initial hybridisation events between these parental species are rare, but once they occur, visits between putative hybrids and *M. laxa* are common and facilitate continued introgression.

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#### **Mitochondrial DNA diversity and geographical distribution of sexual and asexual strains of the braconid parasitoid *Meteorus pulchricornis***

Fujie, S; Wachi, N; Umemoto, H; Maeto, K. 2019

Asexuality is an important tool with regard to the use of parasitoid wasps as biocontrol agents. Asexual (apomictic thelytokous) strains of *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae), a polyphagous endoparasitoid of lepidopteran larvae, are sympatric with sexual (arrhenotokous) strains in Japan. The results of phylogenetic analyses of mitochondrial cytochrome c oxidase subunit I (COI) sequences indicated two major haplotype groups on the Japanese islands. The northern group I predominantly contained sexual strains, whereas the southern group II contained both sexual and asexual strains. Most asexual strains were likely derived within group II. An asexual strain recently established in New Zealand has the identical haplotype to a strain in Japan and was proven to have originated from East Asia. Three hypotheses on the evolution of asexuality are discussed for this parasitoid wasp: recessive gene, hybridization, and cytoplasmic element.

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#### **Statewide Survey of Imported Fire Ant (Hymenoptera: Formicidae) Populations in Tennessee**

Oliver, JB; Vander Meer, RK; Ochieng, SA; Youssef, NN; Pantaleoni, E; Mrema, FA; Vail, KM; Parkman, JP; Valles, SM; Haun, WG; Powell, S. 2009

Imported fire ants (*Solenopsis* spp.; Hymenoptera: Formicidae) occupy 54 counties (similar to 5.4 million ha) in Tennessee. To better understand the fire ant species distribution in Tennessee, the state was divided into 16.1 x 16.1 km grids, and a single colony was sampled for cuticular hydrocarbon and venom alkaloid analyses within each grid. A total of 387 samples was processed from which 9 (2.3%), 167 (43.2%), and 211 (54.5%) were identified as red (*Solenopsis invicta* Buren), black (*Solenopsis richteri* Forel), or hybrid (*S. invicta* x *S. richteri*) imported fire ants, respectively. The *S. invicta* was only found near metropolitan Nashville in Davidson and Williamson counties and at one site in Decatur Co. All samples east of Franklin Co. were identified as hybrids. Tennessee counties west of Lincoln were predominantly *S. richteri* (86.5%) as opposed to hybrid (13.0%) and *S. invicta* (0.5%). The exception was Hardin Co., which was predominantly hybrid. Counties containing both hybrid and *S. richteri* (all in the middle and western part of the state) included Bedford, Decatur, Franklin, Giles, Hardeman, Hardin, Haywood, Lawrence, Lincoln, Marshall, Maury, McNairy, Perry, and Wayne. The *S. invicta* samples collected from one Williamson Co. site were determined to be polygynous and infected with the *Solenopsis invicta* virus (genotype SINV-1A). This was the first detection of polygynous imported fire ant in Tennessee. The SINV-1A virus was also a new find at the time of detection, but has been previously reported. The survey results are being used to direct current and future biological control efforts against imported fire ants in Tennessee.

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#### **Cuticular hydrocarbons of tetramorium ants from central Europe: Analysis of GC-MS data with self-organizing maps (SOM) and implications for systematics**

Steiner, FM; Schlick-Steiner, BC; Nikoiforov, A; Kalb, R; Mistrik, R. 2002

Cuticular hydrocarbons were extracted from workers of 63 different nests of five species of *Tetramorium* ants (Hymenoptera: Formicidae) from Austria, Hungary, and Spain. The GC-MS data were classified (data mining) by self-organizing maps (SOM). SOM neurons derived from primary neuron separation were subjected to hierarchical SOM (HSOM) and were grouped to neuron areas on the basis of vicinity in the hexagonal output grid. While primary neuron separation and HSOM resulted in classifications on a level more sensitive than species differences, neuron areas resulted in chemical phenotypes apparently of the order of species. These chemical phenotypes have implications for systematics: while the chemical phenotypes for *T. ferox* and *T. moravicum* correspond to morphological determination, in *T. caespitum* and *T. impurum* a total of six chemical phenotypes is found. Three hypotheses are discussed to explain this disparity between morphological and chemical classifications, including in particular the possibility of hybridization and the existence of cryptic species. Overall, the GC-MS profiles classified by SOM prove to be a practical alternative to morphological determination (*T. ferox*, *T. moravicum*) and indicate the need to revisit systematics (*T. caespitum*, *T. impurum*).

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#### **Non-reciprocal cross-incompatibility in *Trichogramma deion***

Stouthamer, R; Luck, RF; Pinto, JD; Platner, GR; Stephens, B. 1996

In non-reciprocal cross-incompatibility (NRCI), the crossing of a female of a strain A with a male of a strain B results in hybrid offspring, whereas the reciprocal cross produces few or no hybrids. Only females are of hybrid origin in Hymenoptera because they arise from fertilized eggs; males arise from unfertilized (haploid) eggs. Crosses between many strains of *Trichogramma deion* showed some degree of NRCI. Crosses between a *T. deion* culture collected in Seven Pines, California (SVP) with one from Marysville, California (MRY) showed an extreme form of NRCI in which practically no female offspring was produced when MRY females were crossed with SVP males. The reciprocal cross produced a close to normal proportion of female and male offspring. Detailed studies of this cross indicated that 1) the female offspring produced in the compatible interstrain cross were not the result of parthenogenesis but were true hybrids, 2) the incompatible interstrain cross did not produce female offspring because fertilized eggs died during development, 3) the death of these eggs could not be prevented by either antibiotic or temperature treatment, 4) cytoplasmically inherited factors causing NRCI could be discounted because backcrossed females with the genome of MRY and the cytoplasm of SVP, exhibit the NRCI relationship characteristic of their genome. Therefore the NRCI between these strains appears to be caused by a modification coded for by the nuclear genes of MRY that results in incompatibility when SVP sperm fertilizes MRY eggs. In addition the level of incompatibility in crosses between the SVP females and MRY males is temperature sensitive, the higher the rearing temperature the lower the level of compatibility.

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#### **The structure of a single unit of ribosomal RNA gene (rDNA) including intergenic subrepeats in the Australian bulldog ant *Myrmecia croslandi* (Hymenoptera : Formicidae)**

Ohnishi, H; Yamamoto, MT. 2004

A complete single unit of a ribosomal RNA gene (rDNA) of *M. croslandi* was sequenced. The ends of the 18S, 5.8S and 28S rRNA genes were determined by using the sequences of *D. melanogaster* rDNAs as references. Each of the tandemly repeated rDNA units consists of coding and non-coding regions whose arrangement is the same as that of *D. melanogaster* rDNA. The intergenic spacer (IGS) contains, as in other species, a region with subrepeats, of which the sequences are different from those previously reported in other insect species. The length of IGSs was estimated to be 7-12 kb by genomic Southern hybridization, showing that an rDNA repeating unit of *M. croslandi* is 14-19 kb-long. The sequences of the coding regions are highly conserved, whereas IGS and ITS (internal transcribed spacer) sequences are not. We obtained clones with insertions of various sizes of R2 elements, the target sequence of which was found in the 28S rRNA coding region. A short segment in the IGS that follows the 3' end of the 28S rRNA gene was predicted to form a secondary structure with long stems.

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#### **Temnothorax crasecundus sp n. - a cryptic Eurocaucasian ant species (Hymenoptera, Formicidae) discovered by Nest Centroid Clustering**

Seifert, B; Csoz, S. 2015

The paper integrates two independent studies of numeric morphology-based alpha-taxonomy of the cryptic ant species *Temnothorax crassispinus* (Karavajev, 1926) and *T. crasecundus* sp. n. conducted by different investigators, using different equipment, considering different character combinations and evaluating different samples. Samples investigated included 603 individual workers from 203 nests -thereof 104 nest samples measured by Seifert and 99 by Csoz. The material originated from Europe, Asia Minor and Caucasia. There was a very strong interspecific overlap in any of the 29 shape characters recorded and subjective expert determination failed in many cases. Primary classification hypotheses were formed by the exploratory data analysis Nest Centroid (NC) clustering and corrected to final species hypotheses by an iterative linear discriminant analysis algorithm. The evaluation of Seifert's and Csoz's data sets arrived at fully congruent conclusions. NC-Ward and NC-K-means clustering disagreed from the final species hypothesis in only 1.9 and 1.9% of the samples in Seifert's data set and by 1.1 and 2.1% in Csoz's data set which is a strong argument for heterospecificity. The type series of *T. crassispinus* and *T. crasecundus* sp. n. were allocated to different clusters with  $p = 0.9851$  and  $p = 0.9912$  respectively. The type series of the junior synonym *T. slavonicus* (Seifert, 1995) was allocated to the *T. crassispinus* cluster with  $p = 0.9927$ . *T. crasecundus* sp. n. and *T. crassispinus* are parapatric species with a long contact zone stretching from the Peloponnisos peninsula across Bulgaria northeast to the southern Ukraine. There is no indication for occurrence of interspecifically mixed nests or intraspecific polymorphism. However, a significant reduction of interspecific morphological distance at sites with syntopic occurrence of both species indicates local hybridization. The results are discussed within the context of the Pragmatic Species Concept of Seifert (2014). The taxonomic description and a differential diagnosis of *T. crasecundus* sp. n. are given.

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#### **The systematics of the *Trichogramma minutum* species complex (Hymenoptera : Trichogrammatidae), a group of important North American biological control agents: the evidence from reproductive compatibility and allozymes**

Pinto, JD; Platner, GR; Stouthamer, R. 2003

Two North American *Trichogramma*, *Trichogramma minutum* Riley, and *Trichogramma platneri* Nagarkatti (the *T. minutum* complex), cannot be distinguished morphologically and their species status has been questioned. Both are commercially available for biological control and are important parasitoids of the eggs of Lepidoptera in agricultural ecosystems. Results are reported of an extensive survey of reproductive compatibility and allozymes at the phosphoglucosomutase (Pgm) locus in collections representing 85 localities throughout much of the geographical range of this species complex. In total, 103 reproductive crosses were performed and 161 collections were analyzed electrophoretically. Results support the recognition of *T. minutum* and *T. platneri* as distinct species. The two are distinguishable electrophoretically at the Pgm locus. High levels of reproductive incompatibility are correlated with the allozymic differences. Although *T. platneri* is western in distribution (W of 115degrees longitude) and *T. minutum* is primarily eastern, the two are sympatric in the Pacific Northwest. A low level of interspecific hybridization in laboratory studies notwithstanding, there is no evidence that introgression occurs in the zone of sympatry. This study continues to stress the importance of species recognition and the inadvisability of using *T. minutum* and *T. platneri* interchangeably in biological control. (C) 2003 Elsevier Science (USA). All rights reserved.

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#### **A native and an introduced parasitoid utilize an exotic gall-maker host**

Cooper, WR; Rieske, LK. 2011

*Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) is non-native to North America and induces formation of galls on petioles and leaves of all chestnut (*Castanea* spp., Fagales: Fagaceae). We investigated the interactions between the gall wasp *D. kuriphilus*, a native parasitoid, *Ormyrus labotus* (Hymenoptera: Ormyridae), and a non-native parasitoid, *Torymus sinensis* (Hymenoptera: Torymidae). Galls were collected monthly from May to August and in January from four locations in the United States consisting of orchard-grown hybrid chestnuts (Hiram, OH and Meadowview, VA), suburban-grown ornamental Chinese chestnuts (*C. mollissima*) (Broadview Heights, OH), or forest-grown American chestnuts (*C. dentata*) (Bowling Green, KY). Parasitoids were removed from galls and *T. sinensis* and *O. labotus* were identified using PCR-markers. The relative abundance of each parasitoid was compared in relation to collection date, habitat, presence of alternative hosts, and gall characteristics. *T. sinensis* was collected from each location and date, and was dominant in the orchard and suburban locations. However, relatively more *O. labotus* were collected within the forest, which had significant oak component and alternative cynipid hosts. *O. labotus* was only collected in spring and early summer, indicating the use of different summer and winter hosts. Observations suggest that in addition to parasitizing *D. kuriphilus*, *O. labotus* hyperparasitizes *T. sinensis*. *T. sinensis* has a longer ovipositor than *O. labotus*, and parasitized larger galls. This study improves our understanding of interactions between an invasive gall wasp, an introduced parasitoid, and native parasitoids, and illustrates novel relationships that may form as exotic species expand their geographic range.

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#### **DNA barcoding of new world cicada killers (Hymenoptera : Crabronidae)**

Hastings, JM; Schultheis, PJ; Whitson, M; Holliday, CW; Coelho, JR; Mendell, AM. 2008

We are engaged in a comprehensive study of cicada killers (*Sphecius* spp.), including their behavioral ecology. At one location, we observed interactions among three putative species of *Sphecius*, and used DNA barcoding to help clarify relationships among them. For this, we sequenced a fragment of the mitochondrial cytochrome c oxidase subunit I gene. During our study, a new taxonomic key of New World cicada killers, based on morphology, was published, and we expanded the barcoding project to test the congruence between barcodes and this key. In general, barcoding evidence supports morphological distinctions among species; sequence divergences between individuals of different species were within the range expected for congeneric Hymenoptera. However, two conflicts between barcoding and morphological evidence were noted. 1) Haplotypes of *Sphecius grandis* Say fall into two highly divergent clades, suggesting they are cryptic species. 2) Two clades of *S. convallis* Patton were found, and the sequences of one clade are virtually identical to those of *S. speciosus* Drury, suggesting that this clade of *S. convallis* is conspecific with *S. speciosus*. Alternative explanations are possible for this result, including hybridization and introgression between the two species. We conclude that our DNA barcoding evidence should be interpreted with caution, but that it has generated interesting questions we hope to resolve with field research coupled with analysis of suitable nuclear gene sequences.

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#### **Genetic architecture of the *Tetragonula carbonaria* species complex of Australian stingless bees (Hymenoptera: Apidae: Meliponini)**

Brito, RM; Francisco, FO; Ho, SYW; Oldroyd, BP. 2014

A species complex is a group of closely related species whose ecological or morphological boundaries are sufficiently vague that delimiting one species from another is difficult. In Australia, a group of four stingless bee species *Tetragonula carbonaria* Smith, *Tetragonula hockingsi* Cockerell, *Tetragonula mellipes* Friese, and *Tetragonula davenporti* Franck - form a species complex in which gross morphology is clinal and overlapping. The species are most readily distinguished by the morphology of their brood combs. Here we genetically characterize bees sampled in areas where the species do and do not have contact. Our data corroborate previous evidence that *T. hockingsi* and *T. carbonaria* are genetically distinct and that there are two genetically distinct groups of *T. hockingsi* - one in the north and the other in the south of Queensland. Curiously, northern populations of *T. hockingsi*, which are allopatric to *T. carbonaria*, are genetically closer to *T. carbonaria* than are southern populations of *T. hockingsi*, which are in sympatry with *T. carbonaria*. We detected three hybrid colonies that appear to have arisen because of anthropogenic movement of *T. hockingsi* colonies from north to south of Queensland where males mated with local *T. carbonaria* queens. We discuss the status of *T. davenporti*, a recently described species cryptically similar to *T. hockingsi* from south-east Queensland. (C) 2014 The Linnean Society of London,

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#### **BaitFisher: A Software Package for Multispecies Target DNA Enrichment Probe Design**

Mayer, C; Sann, M; Donath, A; Meixner, M; Podsiadlowski, L; Peters, RS; Petersen, M; Meusemann, K; Liere, K; Wagele, JW; Misof, B; Bleidorn, C; Ohl, M; Niehuis, O. 2016

Target DNA enrichment combined with high-throughput sequencing technologies is a powerful approach to probing a large number of loci in genomes of interest. However, software algorithms that explicitly consider nucleotide sequence information of target loci in multiple reference species for optimizing design of target enrichment baits to be applicable across a wide range of species have not been developed. Here we present an algorithm that infers target DNA enrichment baits from multiple nucleotide sequence alignments. By applying clustering methods and the combinatorial 1-center sequence optimization to bait design, we are able to minimize the total number of baits required to efficiently probe target loci in multiple species. Consequently, more loci can be probed across species with a given number of baits. Using transcript sequences of 24 apoid wasps (Hymenoptera: Crabronidae, Sphecidae) from the 1KITE project and the gene models of *Nasonia vitripennis*, we inferred 57,650, 120-bp-long baits for capturing 378 coding sequence sections of 282 genes in apoid wasps. Illumina reduced-representation library sequencing confirmed successful enrichment of the target DNA when applying these baits to DNA of various apoid wasps. The designed baits furthermore enriched a major fraction of the target DNA in distantly related Hymenoptera, such as Formicidae and Chalcidoidea, highlighting the baits' broad taxonomic applicability. The availability of baits with broad taxonomic applicability is of major interest in numerous disciplines, ranging from phylogenetics to biodiversity monitoring. We implemented our new approach in a software package, called BaitFisher, which is open source and freely available at <https://github.com/cmayer/BaitFisher-package.git>.

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#### **Phylogenetic analysis of nuclear and mitochondrial genes reveals evolutionary relationships and mitochondrial introgression in the sertifer species group of the genus *Neodiprion* (Hymenoptera : Diprionidae)**

Linnen, CR; Farrell, BD. 2008

*Neodiprion Rohwer* (Hymenoptera: Diprionidae) is a Holarctic genus of conifer-feeding sawflies with a remarkable amount of inter- and intraspecific diversity in host use, behavior, and development. This variation is thought to play a central role in *Neodiprion* diversification, but speciation hypotheses remain untested due to a lack of a robust phylogenetic estimate. Here, we utilize sequence data from three nuclear genes (CAD, ANL43, EF1 alpha) to obtain a phylogenetic estimate for the genus. These analyses suggest that: (1) North American and Eurasian *Neodiprion* are monophyletic sister clades, (2) the sertifer group is paraphyletic with respect to the monophyletic lecontei group, and (3) on at least two occasions, dispersal from eastern to western North America proceeded via southern host bridges. Based on these results and host biogeography, we revise a previous scenario for the evolution of *Neodiprion* and suggest maximum ages for the genus and for the lecontei group (25 My and 14 My, respectively). In addition, because a previous study reported rampant mitochondrial introgression in the lecontei group, we assess its prevalence in the sertifer group. Analysis of three mitochondrial genes (COI, tRNA-leucine, and COII) reveals that mito-nuclear discordance is prevalent in the sertifer group, and patterns of species monophyly are consistent with those expected under frequent mitochondrial introgression. As was the case for lecontei group species, we find that introgression appears to be most pronounced between species that occasionally share hosts, suggesting that divergent host use is an important barrier to gene flow in *Neodiprion*. Finally, we suggest that the lack of phylogenetic resolution and prevalence of species non-monophyly in the non-Pinus feeding *Neodiprion* may result from the rapid divergence (possibly with gene flow) of these species following their entry into a novel adaptive zone. (C) 2008 Elsevier Inc. All rights reserved.

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#### **OVERWINTERING OF AFRICANIZED, EUROPEAN, AND HYBRID HONEY-BEES (HYMENOPTERA, APIDAE) IN THE ANDES OF VENEZUELA**

VILLA, JD; RINDERER, TE; COLLINS, AM. 1993

The potential of Africanized honey bees, *Apis mellifera* L., to survive the cold temperatures and confinement of winter was studied at 4,100 m above sea level in the Andes of Venezuela. The first experiment was conducted through the rainy-season "winter" of 1986 using Africanized (A) and European (E) colonies. In 1986, temperature conditions only rarely allowed honey bee flight. Under these conditions, 13 of 14 A colonies died within 18 wk compared with 4 of 15 E colonies. In 1987, European x Africanized (E x A) hybrid colonies were included in a larger experiment with treatments with various initial adult and brood populations. Higher maximum temperatures during this second experiment allowed worker flight almost daily; colonies in one treatment were confined with screens to test the published hypothesis that flight from A colonies during cold weather causes bees to leave their hives and die, causing the colonies to dwindle. All screened A colonies had died by week 10, while screened E and E x A colonies were alive through week 14. In treatments involving free-flying colonies, there were fewer differences between bee types in colony survival, size, brood production, final adult population, or food consumption. E x A colonies had intermediate values for most traits, suggesting that any differences will be reduced with hybridization as Africanized bees expand their range into areas with high-density populations of European bees.

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#### **Suppression subtractive hybridization analysis reveals expression of conserved and novel genes in male accessory glands of the ant *Leptothorax gredleri***

Oppelt, A; Humann, FC; Fuessl, M; Azevedo, SV; Antonio, DSM; Heinze, J; Hartfelder, K. 2010

Background: During mating, insect males eject accessory gland proteins (Acps) into the female genital tract. These substances are known to affect female post-mating behavior and physiology. In addition, they may harm the female, e. g., in reducing its lifespan. This is interpreted as a consequence of sexual antagonistic co-evolution. Whereas sexual conflict abounds in non-social species, the peculiar life history of social insects (ants, bees, wasps) with lifelong pair-bonding and no re-mating aligns the reproductive interests of the sexes. Harming the female during mating would negatively affect male fitness and sexual antagonism is therefore not expected. Indeed, mating appears to increase female longevity in at least one ant species. Acps are presumed to play a role in this phenomenon, but the underlying mechanisms are unknown. In this study, we investigated genes, which are preferentially expressed in male accessory glands of the ant *Leptothorax gredleri*, to determine which proteins might be transferred in the seminal fluid. Results: By a suppression subtractive hybridization protocol we obtained 20 unique sequences (USs). Twelve had mutual best matches with genes predicted for *Apis mellifera* and *Nasonia vitripennis*. Functional information (Gene Ontology) was available only for seven of these, including intracellular signaling, energy-dependent transport and metabolic enzyme activities. The remaining eight USs did not match sequences from other species. Six genes were further analyzed by quantitative RT-PCR in three life cycle stages of male ants. A gene with carboxy-lyase activity and one of unpredicted function were significantly overexpressed in accessory glands of sexually mature males. Conclusions: Our study is the first one to investigate differential gene expression in ants in a context related to mating. Our findings indicate that male accessory glands of *L. gredleri* express a series of genes that are unique to this species, possibly representing novel genes, in addition to conserved ones for which functions can be predicted. Identifying differentially expressed genes might help to better understand molecular mechanisms involved in reproductive processes in eusocial Hymenoptera. While the novel genes could account for rapidly evolving ones driven by intra-sexual conflict between males, conserved genes imply that rather beneficial traits might get fixed by a process described as inter-sexual cooperation between males and females.

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#### **Egg Parasitoids of *Dalbulus maidis* (Hemiptera: Cicadellidae) Within Maize Agroecosystems and in the Edge Zones of Maize Fields, and on Maize Varieties During the Wet Season in Mexico**

Becerra-Chiron, IM; Moya-Raygoza, G. 2018

Little is known about *Dalbulus maidis* (DeLong) egg parasitoids within maize fields, in the edge zones that surround these fields, and the parasitism on *D. maidis* eggs

oviposited on different maize varieties. The objectives of the present study were first to understand which egg parasitoid species attack *D. maidis* eggs within maize fields and in the surrounding edge zones, and second, to compare parasitism on two maize varieties (land race Ancho-pozolero and hybrid Tigre-Asgrow) during the maize-growing wet season. We used maize plants with sentinel eggs to attract the egg parasitoids in two consecutive wet seasons, in 2016 and 2016. In 2016, *Anagrus* sp. (Hymenoptera: Mymaridae) and *Paracentrobia* sp. (Hymenoptera: Trichogrammatidae) parasitized *D. maidis* eggs within the maize field and on its edges. However, much more parasitism was seen within the maize agroecosystem than in the maize edge zones. In 2016, two Mymaridae species, *Anagrus columbi* Perkins and *Anagrus* sp., and two Trichogrammatidae genera, *Pseudoligotoma* sp. and *Paracentrobia* sp., attacked the *D. maidis* eggs laid on maize hybrids but not those oviposited on the maize land race. Our findings indicate that parasitism of corn leafhopper eggs differs with agroecosystem location and maize varieties.

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#### Four species within the supercolonial ants of the *Tapinoma nigerrimum* complex revealed by integrative taxonomy (Hymenoptera: Fointicidae)

Seifert, B; D'Eustacchio, D; Kaufmann, B; Centorame, M; Lorite, P; Modica, MV. 2017

The West and Central Mediterranean ants known for 50 years under the name *Tapinoma nigerrimum* (NYLANDER, 1856) have attracted attention because of their efficient chemical weapons, impressive supercolonies and potential to limit the spreading of the Argentine Ant *Linepithema humile* (MAYR, 1868). The paper shows that the *T. nigerrimum* complex consists of at least four clearly separable species which differ in morphology of all castes, colony demography, geographic distribution, invasive potential and mtDNA data. Species delimitation by means of Nest Centroid Clustering, considering 20 quantitative phenetic characters in 159 nest samples, resolved four coincident clusters in both female and male castes which are classified as *T. nigerrimum*, *T. magnum* MAYA, 1861, *T. ibericum* SANTOSCHI, 1925, and *T. darioi* sp.n. The exploratory data analyses NC-Ward clustering and NC-k-means clustering showed a mean disagreement from the final species hypothesis between 0 and 2.7% in workers on the nest sample level, whereas the classification error of a linear discriminant analysis was 4.2% in 533 worker individuals. The four phenetic clusters were basically confirmed by analysis of the COI segment of mtDNA with the smallest mean K2p genetic distance of 1.8% observed in *T. darioi* sp.n. against *T. magnum*, and the largest one of 4.0% in *T. nigerrimum* against *T. ibericum*. These data suggest a species divergence between late Pliocene and early Pleistocene (3.3 - 1.5 Ma). The mtDNA haplotypes of nine phenotypically ideal *T. darioi* sp.n. supercolonies, found at three sites in southern France, and Italy were placed within the *T. magnum* cluster. Among four alternative scenarios discussed for these mismatches, hybridization events in the younger evolutionary history with subsequent unidirectional genomic purging of nuDNA was proposed to be the most likely explanation. *Tapinoma nigerrimum* is monodomous to moderately polydomous with aggression between neighbouring colonies, whereas *T. magnum*, *T. ibericum*, and *T. darioi* sp.n. are supercolonial with a potential to become invasive pest ants through introduction by human commerce. For Europe north of 48 N, *T. magnum* could establish populations in nine cities in Germany, Belgium, and the Netherlands, whereas *T. ibericum* is known so far from one site in South England only, and *T. darioi* sp.n. from one city in the Netherlands. The differential zoogeography and biology of the four species and ways of species delimitation are outlined and discussed. *Tapinoma darioi* sp.n. is described as new.

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#### Pollen transport differs among bees and flies in a human-modified landscape

Rader, R; Edwards, W; Westcott, DA; Cunningham, SA; Howlett, BG. 2011

Aim Dispersal distances of insect pollinators are critical in defining their contribution to landscape-wide pollen movement and ultimately gene flow in natural and agricultural systems. We ask whether bee and fly pollinator taxa differ in their dispersal distances and transport of viable pollen in a human-modified system. Location Canterbury and Otago region, South Island, New Zealand. Methods We captured pollen-carrying insects travelling outside of a model mass-flowering agricultural crop, Brassica rapa, using insect flight intercept traps at five distances (0, 100, 200, 300 and 400 m) from the pollen source. We examined pollen loads and pollen viability to determine whether pollen transport distance and viability differ among pollinator taxa. Results A total of 5453 insects were collected of which 717 individuals from 26 insect taxa were positively identified as dispersing pollen up to 400 m from the source. These taxa consisted of four species from two bee families (Hymenoptera: Apidae and Halictidae), and eight species from four fly families (Diptera: Bibionidae, Stratiomyidae, Syrphidae and Tachinidae). Apidae generally carried higher pollen loads and more viable pollen than most fly taxa. Taxa in the fly families Stratiomyidae and Syrphidae, however, carried pollen to 400 m, which is further than both bee families. Main conclusions A diverse array of wild and managed flower visitors can transport viable pollen from a pollen source to at least 400 m. Knowledge of the differences in transport distances among generalist pollinators in human-modified environments is crucial to understand the potential extent to which (1) pollen transport can facilitate gene flow and (2) unwanted hybridization may occur between crops and related weeds.

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#### Multivariate analysis of honeybee populations, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), from western central Africa: morphometrics and pheromones

Radloff, SE; Hepburn, HR. 1997

Morphometric characters and sting pheromones of worker honeybees, *Apis mellifera* Linnaeus, were analysed by multivariate methods to characterize their populations along a transect through three ecological-climatological zones in Cameroon. There are three distinct homogeneous populations and two zones of hybridization. These bees are designated as *A. m. adansonii* Latreille whose area of distribution is intruded by an *A. m. monticola*-like montane group of bees and a third group, *A. m. jemenitica* Ruttner. The delineation of the hybrid zones is supported by intercolonial variance spectra and these significant asymmetries are coincident with transitions between the ecological-climatological zones.

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#### FORAGING BY RED IMPORTED FIRE ANTS, *SOLENOOPSIS INVICTA* (HYMENOPTERA; FORMICIDAE) ON TURFGRASSES

Reinert, JA; McCoy, JE. 2010

Red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera; Formicidae) is a major pest in urban landscapes including residential/commercial lawns, sports fields, golf courses, parks, and highway rights-of-way. Foraging preferences for various turfgrass clippings were investigated under controlled lab conditions. Among bermudagrass (*Cynodon* sp.) cultivars, clippings of 'Tifway' and 'Baby' were 7 times more preferred than clippings of 'Tifton 10' and 'GN1'. The Texas bluegrass x Kentucky bluegrass hybrid (*Poa pratensis* L. x *P. arachnifera* Torr.), TXKY 00-34-2 had 5 times more foraging ants on it than TXKY 01-59-9. Among the zoysiagrasses (*Zoysia japonica*), 'El Toro' was only 2 times more preferred than 'Crown'. For St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze), 'BitterBlue' was 3.4 times more preferred than 'Floratum'. On the buffalograss cultivars (*Buchloe dactyloides* (Nutt.) Engelm.), there were 2 and 4 times more ants foraging 'Texoka' than either 'Prairie' or 'Bison', respectively. After foraging for 5 h on clippings of the 5 or 6 cultivars in each replicate, the number of ants on each grass was bermudagrass (169.3) > zoysiagrass (137.5) = blue-grass hybrids (136.8) > St. Augustinegrass (127.1) > buffalograss (34.5).

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#### A comparison of pollinator fig wasp development in figs of *Ficus montana* and its hybrids with *Ficus asperifolia*

Ghana, S; Suleman, N; Compton, SG. 2015

Figs (Moraceae) and pollinator fig wasps (Hymenoptera: Agaonidae) have a highly specific mutualistic relationship but fig wasps occasionally enter atypical hosts, and this can lead to hybrid fig trees and the potential for gene flow between species. Many fig trees are dioecious, with fig wasp offspring developing in galled ovules inside figs on male trees, whereas seeds develop only in figs on female trees. We generated experimental hybrids between the Asian *Ficus montana* Blume and a closely related African species *Ficus asperifolia* Miquel. Male F1s were sterile if entered by *Kradibia tentacularis* (Grandi) (Agaonidae), the pollinator of *F. montana*,



because its offspring always failed to develop, without ovule enlargement. As with the F1s, figs on most male backcross plants [*F. montana* x (*F. montana* x *F. asperifolia*)] also aborted shortly after pollinator entry, resulting in a higher turnover of figs than with *F. montana*, although the times taken for the figs to reach receptivity were similar. Pollinator larvae nonetheless consistently managed to develop inside the figs of one backcross plant and also occasionally in a few figs from another backcross individual. In these figs, galled ovules developed as normal, whereas in figs that aborted the galled ovules failed to enlarge. The sex ratio of *K. tentacularis* progeny in the backcross figs was female biased and did not differ from that in *F. montana* figs. *Sycoscapter spec.* (Hymenoptera: Pteromalidae), a parasitoid of *K. tentacularis*, was able to lay eggs and developed normally inside male backcross figs where its host was present.

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#### Light at the end of the tunnel: Integrative taxonomy delimits cryptic species in the *Tetramorium caespitum* complex (Hymenoptera: Formicidae)

Wagner, HC; Arthofer, W; Seifert, B; Muster, C; Steiner, FM; Schlick-Steiner, BC. 2017

Species delimitation is fundamental for many biological studies; its importance extends from regional faunistics over behavioral research to the reconstruction of evolutionary history. However, species delimitation in the Palearctic *Tetramorium caespitum* species complex (formerly *Tetramorium caespitum* / *impurum* complex) has stayed ambiguous over a century. A 2006 study argued for the presence of eight Western Palearctic cryptic species but did not draw formal taxonomic conclusions due to multiple unresolved issues. Using 1428 nest samples in an in-depth integrative-taxonomy approach, we here revise the European species of the complex. Unsupervised analyses provide independent species hypotheses based on molecular and morphological disciplines. Following the unified species concept, we show the presence of ten clearly distinguishable European species differing in mitochondrial DNA, nuclear DNA, morphology of workers and males, and ecology. We explain the evolution of the observed mitochondrial-nuclear discordances by peripatric speciation, historical introgression, and recent hybridization, and that of morphological-nuclear discordances by interspecific similarity and intraspecific variability, that is, morphological crypsis. Based on the final species hypotheses, we confirm *T. caespitum* (UNNAEus, 1758), *T. impurum* (FOERSTER, 1850), *T. indocile* SANTSCH, 1927, *T. hungaricum* ROSZLER, 1935, and *T. alpestre* STEINER, SCHLICK-STEINER & SEIFERT, 2010 as good species. We raise *T. fusciclava* CONSANI & ZANGHERI, 1952 stat.n., *T. staerckei* KRATOCHVIL, 1944 sp.rev., and *T. immigrans* SANTSCH, 1927 stat.n. (as an introduced species also in the Americas) to species rank and synonymize *T. semilaeve* var. *kutteri* SANTSCH, 1927 under *T. indocile* and *T. staerckei* var. *gregori* KRATOCHVIL, 1941 under *T. impurum*. Finally, we newly describe *T. breviscapus* sp.n. and *T. caucasicum* sp.n. We present dichotomous determination keys to workers and males and make freely available an online identification key to workers at <https://webapp.uibk.ac.at/ecology/tetramorium/>. Of relevance to resolving other highly complicated taxonomic problems, we highlight that no single data type was sufficient to disentangle the final species boundaries, which underlines the importance of integrating multiple data sources in taxonomy.

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

Beukeboom, LW; van den Assem, J. 2001

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

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#### Olfactory response of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) to volatiles induced by transgenic maize

Nascimento, PT; Fadini, MAM; Rocha, MS; Souza, CSF; Barros, BA; Melo, JOF; Von Pinho, RG; Valicente, FH. 2021

Plants not only respond to herbivorous damage but adjust their defense system after egg deposition by pest insects. Thereby, parasitoids use oviposition-induced plant volatiles to locate their hosts. We investigated the olfactory behavioral responses of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) to volatile blends emitted by maize (*Zea mays* L.) with singular and stacked events after oviposition by *Spodoptera frugiperda* Smith, 1797 (Hymenoptera: Trichogrammatidae) moths. Additionally, we examined possible variations in gene expression and on oviposition-induced volatiles. We used a Y-tube olfactometer to test for the wasp responses to volatiles released by maize plants oviposited by *S. frugiperda* and not-oviposited plants. Using the real-time PCR technique (qRT-PCR), we analyzed the expression of lipoxigenase and three terpene synthase genes, which are enzymes involved in the synthesis of volatile compounds that attract parasitoids of *S. frugiperda*. Olfactometer tests showed that *T. pretiosum* is strongly attracted by volatiles from transgenic maize emitted by *S. frugiperda* oviposition (VTPRO 3, more than 75% individuals were attracted). The relative expression of genes TPS10, LOX e STC was higher in transgenic hybrids than in the conventional (isogenic line) hybrids. The GC-MS analysis revealed that some volatile compounds are released exclusively by transgenic maize. This study provides evidence that transgenic hybrids enhanced chemical cues under oviposition-induction and helped to increase *T. pretiosum* efficiency in *S. frugiperda* control. This finding shows that among the evaluated hybrids, genetically modified hybrids can improve the biological control programs, since they potentialize the egg parasitoid foraging, integrating pest management.

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#### An Updated List of Ants of Alabama (Hymenoptera: Formicidae) with New State Records

Macgown, JA; Booher, D; Richter, H; Wetterer, JK; Hill, JG. 2021

A 2005 review compiled a list of 155 ant species plus the hybrid fire ant *Solenopsis invicta* X *richteri* from Alabama. Here, we add an 38 additional species based on both published and unpublished records and revised identifications raising the total to 193 species and one hybrid. Of these, 32 species and one hybrid are considered exotic to the state. We present county level maps of species richness and collection efforts for native and exotic ants highlighting areas of special concern for conservation and invasion risks.

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#### HYBRIDIZATION IN THE SOCIAL PARASITIC ANT GENUS *EPIMYRMA* (HYMENOPTERA, FORMICIDAE)

JESSEN, K; KLINKICHT, M. 1990

#### Molecular characterization of *Apis mellifera* colonies from Argentina: genotypic admixture associated with ecoclimatic regions and apicultural activities

Agra, MN; Conte, CA; Corva, PM; Cladera, JL; Lanzavecchia, SB; Palacio, MA. 2018

The European honeybee, *Apis mellifera* L. (Hymenoptera: Apidae), is considered as a main contributor to pollination of important crops and to honey production. Originally, beekeeping in Argentina was performed in an extended area covering the north and central region of the country and involving *A. mellifera* of European origin. Later, honeybees of African origin entered South America through Brazil and hybridized with European genetic resources, giving rise to Africanized populations that are characterized by a more aggressive behavior among other unfavorable traits. In this study, a genetic characterization of 396 honeybee colonies from the most



important apicultural region of Argentina is presented in order to provide an updated description of population structure and genetic diversity of commercial and feral colonies. Diversity was analyzed using mitochondrial (COI-COII region) and nuclear (eight microsatellites) markers. Three European (M4, C1, C2J) and three African (A1, A4, A30) haplotypes were detected. European haplotypes were mostly found in commercial apiaries, whereas African haplotypes were detected at high frequencies in feral colonies. Microsatellite data were analyzed to estimate population genetic variability at the province level and to evaluate genetic admixture. A high level of hybridization between Africanized and European honeybees was detected with a significant latitudinal cline from north to south. Extensive population admixture resulted in the definition of four clusters that included both feral and commercial colonies and that are explained not only by geographical distribution and degree of Africanization but also by human influence through beekeeping activities.

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#### **A preliminary list of the ants (Hymenoptera : Formicidae) of Alabama, USA**

MacGown, JA; Forster, JA. 2005

One hundred and fifty-four species of Formicidae, plus the hybrid fire ant *Solenopsis invicta* x *richteri*, are reported from Alabama, U.S.A. Fifty-nine new state records are presented and county distributions are given for each species.

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#### **Genetic variation in foundation species governs the dynamics of trophic interactions**

Valencia-Cuevas, L; Mussali-Galante, P; Cano-Santana, Z; Pujade-Villar, J; Equihua-Martinez, A; Tovar-Sanchez, E. 2018

Various studies have demonstrated that the foundation species genetic diversity can have direct effects that extend beyond the individual or population level, affecting the dependent communities. Additionally, these effects may be indirectly extended to higher trophic levels throughout the entire community. *Quercus castanea* is an oak species with characteristics of foundation species beyond presenting a wide geographical distribution and being a dominant element of Mexican temperate forests. In this study, we analyzed the influence of population ( $H_e$ ) and individual ( $H_L$ ) genetic diversity of *Q. castanea* on its canopy endophagous insect community and associated parasitoids. Specifically, we studied the composition, richness ( $S$ ) and density of leaf-mining moths (Lepidoptera: Tischeridae, Citheraniidae), gall-forming wasps (Hymenoptera: Cynipidae), and canopy parasitoids of *Q. castanea*. We sampled 120 trees belonging to six populations (20/site) through the previously recognized gradient of genetic diversity. In total, 22 endophagous insect species belonging to three orders (Hymenoptera, Lepidoptera, and Diptera) and 20 parasitoid species belonging to 13 families were identified. In general, we observed that the individual genetic diversity of the host plant ( $H_L$ ) has a significant positive effect on the  $S$  and density of the canopy endophagous insect communities. In contrast,  $H_e$  has a significant negative effect on the  $S$  of endophagous insects. Additionally, indirect effects of  $H_L$  were observed, affecting the  $S$  and density of parasitoid insects. Our results suggest that genetic variation in foundation species can be one of the most important factors governing the dynamics of tritrophic interactions that involve oaks, herbivores, and parasitoids.

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#### **Insect Visitors and Abundance of Four Species of *Apis* on Sunflower *Helianthus annuus* L. in Pakistan**

Ali, H; Owayss, AA; Khan, KA; Alqarni, AS. 2015

Sunflower (*Helianthus annuus* L.) is an open pollinated crop and the world's fourth important source of edible oil. Two fields, one of them adjacent to an apiary with 40 hives, were sampled weekly for two months. A total of fifteen species of insect pollinators were recorded. Species of the order Hymenoptera were the most abundant (91%), followed by those of Lepidoptera (6%) and Diptera (3%). The relative abundance of four *Apis* species, i.e. *Apis mellifera*, *A. dorsata*, *A. cerana* and *A. florea*, varied on hourly and weekly basis. The peak densities of all honeybee species were recorded at 12:00 pm and 02:00 pm while minimum densities were recorded at 08:00 am and 04:00 pm. *Apis mellifera* was the most recorded honeybee species compared to other *Apis* spp. during the study. In both fields the differences were significant.

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#### **RECOMBINANT MALES IN THE PARASITIC WASP MUSCIDIFURAX-RAPTORELLUS [HYMENOPTERA, PTEROMALIDAE]**

LEGNER, EF. 1991

The identification of recombinant males in *Muscidifurax raptorellus* Kogan & Legner, secured from virgin hybrid females, formed by crossing cohorts from solitary and gregarious populations, supports chromosomal inheritance of gregarious oviposition behavior. Examination of parasitization behavior in female progeny that had recombinant male fathers, indicated the existence of recombinant males. Such males also were able to elicit immediate phenotypic changes in their female mating partners, at intensities expected from their genetic character, as previously observed with original parental males in this species. Progeny originating from F1 mothers and recombinant fathers demonstrated the highest levels of heterosis, as measured by increased parasitization rates and numbers of eggs laid. Although extranuclear inheritance has not been eliminated, its influence on phenotypic changes in progeny seems minimal.

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

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

To recognize one's mate is essential for all sexually reproducing animals. In insects, mate recognition is often based on chemical cues such as hydrocarbons which are distributed over the insect's cuticle. In the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae), interspecific mating possibly occurs in microsympatry between *Nasonia vitripennis* Walker and *Nasonia giraulti* Darling despite post-zygotic isolation mechanisms preventing hybridization. Males of *N. vitripennis* are known to equally court con- and heterospecific females, which they recognize by means of cuticular hydrocarbons. A recent study surprisingly showed that this might not be the case in *N. giraulti*, leaving open how males of this species achieve the recognition of mating partners. In this study, we investigated chemical mate recognition in *N. giraulti* in more detail and compared observed behaviors with behaviors of *N. vitripennis* by conducting experiments with both species concurrently and under the same experimental conditions. We disentangled the role of female-derived non-polar cuticular lipids - i.e., cuticular hydrocarbons - and more polar cuticular lipids in the ability of males to recognize con- and heterospecific females. In addition, we tested whether females of the two species discriminate similarly between con- and heterospecific males. We demonstrate that, in contrast to *N. vitripennis*, males of *N. giraulti* prefer live conspecific females over heterospecific ones. Furthermore, in contrast to *N. vitripennis*, mate recognition in *N. giraulti* males is not based on cuticular hydrocarbons, but rather involves other chemical messengers, presumably more polar cuticular lipids. In both species, discrimination against heterospecific males decreases with female age.

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#### **Invasion of Japan by exotic leafminers *Liriomyza* spp. (Diptera: Agromyzidae) and its consequences**

Abe, Y. 2017

Identifying patterns and causes of species displacement is important from the viewpoints of ecology and evolutionary biology as this phenomenon affects community structure. Here I review the species displacement between *Liriomyza trifolii* (Burgess) and *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Japan.

These two species and *Liriomyza huidobrensis* (Blanchard) originated from the New World and are considered to have invaded Japan from around 1990 to the early 2000s. During this period, *L. trifolii* was apparently displaced by *L. sativae*, but the direction of displacement in Japan has been contrary to that observed between the same two species in the USA and China. While the displacement of *L. sativae* by *L. trifolii* in these two countries can be attributed to the lower insecticide susceptibility of *L. trifolii* there, species displacement in the opposite direction in Japan is probably due to the relatively high fecundity of *L. sativae* and differential effects of the introduced parasitoid *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) on both *Liriomyza* species, except in the south of the country.

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#### Relationship of Imported Fire Ant (Hymenoptera: Formicidae) Integument Coloration to Cuticular Hydrocarbon and Venom Alkaloid Profiles

Pandey, M; Addesso, KM; Alexander, LW; Youssef, NN; Oliver, JB. 2021

Red imported fire ant (*Solenopsis invicta* Buren; RIFA) and black imported fire ant (*Solenopsis richteri* Forel (Hymenoptera: Formicidae); BIFA) are considered distinct species with introgression via a reproductively functional hybrid (HIFA). The RIFA and BIFA common names are based on relative coloration. Due to human color perception variation, using color to identify RIFA, BIFA or HIFA is challenging. Fire ant identification traditionally involves molecular or chemical techniques, but a colorimetric test could allow rapid and low-cost identification. In this study, ant integument coloration was measured by spectrophotometer, and color attributes were compared to a combined gas chromatography (GC) index derived from cuticular hydrocarbon and venom alkaloid indices. Significant Pearson Correlation coefficients were found for colony GC index versus color attributes red to green (*a*), *blue to yellow* (*b*), chroma (*C*), and *hue* (*h*), but not lightness (*L*). *The RIFA colonies were distinct from BIFA for four of five color attributes and plots of the ab\* and Ch horizontal axis of the Lab\* and LCh\* color spaces. Color attributes for HIFA indices were not distinct from BIFA and RIFA parental species, but HIFA ab and Ch plots were distinct from RIFA and a b plots from BIFA. Color attributes a\*, b\*, and C\* increased and h\* decreased with GC index in a sinusoidal pattern. In conclusion, most RIFA and BIFA color attributes were distinct and ab and Ch color axes plots had potential to distinguish HIFA from parental species, but coloration of HIFA indices was variable and complicated identifications among HIFA phenotypes, RIFA and BIFA.*

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#### Genetic diversity analysis of the invasive gall pest *Leptocybe invasa* (Hymenoptera: Apodemidae) from China

Peng, X; Wang, HT; Guo, CH; Hu, P; Xu, L; Zhou, J; Ding, ZR; Yang, ZD; Yue, BS; Yue, BS; Yue, BS. 2021

*Leptocybe invasa* Fisher et LaSalle is a global invasive pest that seriously damages Eucalyptus plants. Studying the genetic diversity, genetic structure and introgression hybridization of *L. invasa* in China is of great significance for clarifying the breeding strategy, future invasion and diffusion trends of *L. invasa* in China and developing scientific prevention and control measures. Genetic diversity and phylogenetic analyses of 320 *L. invasa* female adults from 14 geographic populations in China were conducted using 10 polymorphic microsatellite loci (SSRs) and mitochondrial DNA cytochrome oxidase I gene sequences (COIs). (1) The Bayesian phylogenetic tree and haplotype network diagram showed that only haplotype Hap3 existed in *L. invasa* lineage B in China, while haplotypes Hap1 and Hap2 existed in lineage A, among which haplotype Hap2 was found for the first time. The nucleotide and haplotype diversities of lineage A were higher than those of lineage B. (2) The SSR genetic diversity of the Wuzhou Guangxi, Ganzhou Jiangxi and Panzhihua Sichuan populations was higher than that of the other 11 populations, and the SSR genetic diversity of lineage A was higher than that of lineage B. (3) The AMOVA analysis of mitochondrial COI data showed that 75.55% of the variation was among populations, and 99.86% of the variation was between lineages, while the AMOVA analysis of nuclear SSR data showed that 35.26% of the variation was among populations, and 47.04% of the variation was between lineages. There were obvious differences in the sources of variation between the COI and SSR data. (4) The optimal K value of COI and SSR data in structure analysis was 2, and PCoA analysis also divided the dataset into two obvious categories. The UPMGA phylogenetic tree based on SSR data clustered 14 geographic species into two groups. The results of genetic structure analysis supported the existence of two lineages, A and B, in China. (5) Structural analysis showed that there was obvious introgressive hybridization in Wuzhou Guangxi, Ganzhou Jiangxi, Panzhihua Sichuan and other populations. These results suggest that lineage introgressive hybridization has occurred in the *L. invasa* population in China. The introgressive hybridization degree and genetic diversity of lineage A are obviously higher than those of lineage B. Lineage introgressive hybridization may be the driving force for further *L. invasa* invasion and diffusion in China in the future.

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#### Distribution of hybrid imported fire ants (Hymenoptera : Formicidae) and some native ant species in relation to local environmental conditions and interspecific competition in Mississippi forests

Menzel, TO; Nebeker, TE. 2008

Studies of interactions between imported fire ants and other ant species (Hymenoptera: Formicidae) have generally addressed populations in open areas where imported fire ant densities are highest. Forest habitats harbor a different assemblage of ant species and interactions between ant species in forests are occurring under different environmental conditions. We have asked three questions about the distribution of hybrid imported fire ants and native ant species in a Mississippi forest: 1) Is their distribution influenced primarily by interspecific competition or by local conditions? 2) Are the communities at locations with and without hybrid imported fire ants different? and 3) Which native ant species are using the same resources as hybrid imported fire ants? To answer these questions, ant species were collected at baits, along with the metrics for a set of local conditions, over a representative sample of forest habitats. Data were analyzed using permutation tests comparing the observed characteristics of the ant community to those expected by random processes. The community of ant species collected at baits was not structured competitively and their distribution was strongly associated with the distribution of local conditions. The assemblage of ant species was different at locations with hybrid imported fire ants from the assemblage at locations without them, but the two groups of locations were equal in richness when samples were standardized by rarefaction. Of the 28 species collected, 14 occurred with hybrid imported fire ants. One species, *Aphaenogaster carolinensis* Wheeler, was negatively associated, and another, *Monomorium minimum* (Buckley), was positively associated with hybrid imported fire ant occurrence.

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#### Tritrophic interaction of parasitoid *Lysiphlebus testaceipes* (Hymenoptera : Aphidiidae), greenbug, *Schizaphis graminum* (Homoptera : Aphididae), and greenbug-resistant sorghum hybrids

Dogramaci, M; Mayo, ZB; Wright, RJ; Reese, JC. 2005

Interactions of the parasitoid *Lysiphlebus testaceipes* (Cresson) and the greenbug, *Schizaphis graminum* (Rondani), on greenbug-resistant Cargill 607E' (antibiosis), Cargill 797' (primarily tolerance), and -susceptible Golden Harvest 510B' sorghum, *Sorghum bicolor* (L.) Moench, were tested using three levels of biotype I greenbug infestation. The parasitoid infestation rate was 0.5 female and 1.0 male *L. testaceipes* per plant. For all three greenbug infestation levels, the parasitoid brought the greenbug under control (i.e., prevented the greenbugs from killing the plants) on both resistant hybrids, but it did not prevent heavy leaf damage at the higher greenbug infestation rates. At the low greenbug infestation rate (50 greenbugs per resistant plant when parasitoids were introduced), greenbugs damaged 5 and 18% of the total leaf area on Cargill 797' and Cargill 607E', respectively, before greenbugs were eliminated. Leaf damage was higher for the intermediate infestation study (120 greenbugs per plant), 21% and 30% leaf area were damaged on the resistant sorghum hybrids Cargill 797' and Cargill 607E', respectively. At the high greenbug infestation rate (300 greenbugs per plant), heavy damage occurred: 61% on Cargill 607E' and 75% on Cargill 797'. The parasitoids did not control greenbugs on the susceptible sorghum hybrid Golden Harvest 510B'. *L. testaceipes* provided comparable control on both greenbug-resistant hybrids. This study supports previous studies indicating that *L. testaceipes* is effective in controlling greenbugs on sorghum with antibiosis resistance to greenbugs. Furthermore, new information is provided indicating that *L. testaceipes* is also effective in controlling greenbugs on a greenbug-tolerant hybrid.

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#### Sequence data reflect the introduction pathways of the *Sirex* woodwasp parasitoid, *Ibalia leucospoides* (Ibaliidae, Hymenoptera)

Hurley, BP; Fitza, KNE; Wingfield, MJ; Slippers, B. 2020

The parasitoid wasp *Ibalia leucospoides* is native to the northern hemisphere and has been introduced to the southern hemisphere as a biological control agent for the invasive woodwasp *Sirex noctilio*. Two subspecies of the parasitoid, *Ibalia leucospoides leucospoides* (Palearctic distribution) and *Ibalia leucospoides ensiger* (Nearctic distribution), were introduced and are reported to have hybridized. Despite extensive records of the numbers and origins of the wasps imported into the southern hemisphere, nothing is known regarding their current population diversity. We investigated the genetic variation of *I. leucospoides* in its native and introduced ranges using mitochondrial (COI) and nuclear (ITS) markers. Mitochondrial DNA diversity in the introduced range was limited, with only five haplotypes, although sequence divergence between these haplotypes was high. Similarly, the ITS rDNA sequences revealed multiple clades present in the introduced range. These results reflect introductions from a wide geographical range but where genetic bottlenecks have possibly reduced the genetic diversity. The data further reflect the origin of the *I. leucospoides* populations in South America and South Africa from New Zealand or Australia. We found no evidence of hybridization between the two subspecies of the parasitoid in its introduced range, and no evidence that *I. leucospoides ensiger* has established outside its native range.

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#### Bee associates of flowering *Astragalus* and *Onobrychis* genebank accessions at a Snake River site in eastern Washington

Clement, SL; Griswold, TL; Rust, RW; Hellier, BC; Stout, DM. 2006

A 3 yr survey of the bee associates of flowering accessions of *Astragalus* and *Onobrychis* species was conducted in open-pollinated field nurseries at Central Ferry, Washington to gain insight into the potential for bee-mediated inter-accession out-crossing in these nurseries. Such nurseries are used by genebank managers at the USDA-ARS Western Regional Plant Introduction Station (WRPIS), Pullman, Washington, to multiply seed of accessions low in viability and/or supply. Fifty-two species of bees (Hymenoptera) in the Andrenidae (4 species), Apidae (18), Colletidae (1), Halictidae (5), and Megachilidae (24) were recorded. Species-rich genera were *Bombus* (8 species) and *Osmia* (16). One undescribed species of *Osmia* was collected. The bee fauna was more diverse on *Astragalus* (45 bee species) compared to *Onobrychis* (29 species). Among the 24 *Astragalus* and 13 *Onobrychis* species censused, bee diversity was highest on *A. bungeanus* Boiss. (24 bee species), *A. chaborasicus* Boiss. & Hausskn. (24 species), *A. miser* var. *serotinus* (A. Gray ex J.G. Cooper) Barney (14 species), *A. cicer* L. (13 species), *A. schistosus* Boiss. & Hohen. (12 species), *A. onobrychis* L. (11 species), *A. podolobus* Boiss. & Hohen. (11 species), and *Onobrychis viciifolia* Scop. (10 species). The presence of a rich bee fauna in WRPIS nurseries at Central Ferry, including documented pollinators of the allogamous species *A. cicer* and *O. viciifolia*, could complicate efforts to prevent hybridization and maintain genetic integrity of genebank accessions of allogamous plant species. Our results support a 1993 decision by WRPIS curators to discontinue the practice of clustering accessions of single species of *Astragalus* and *Onobrychis* in open-pollinated nurseries to regenerate seed.

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#### Comparison of parasitic mites in Russian-Hybrid and Italian honey bee (Hymenoptera : Apidae) colonies across three different locations in north Carolina

Tarpy, DR; Summers, J; Keller, JJ. 2007

The most economically important parasites of honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), colonies are the parasitic mites *Varroa destructor* Anderson & Trueman and *Acarapis woodi* (Rennie). Research has shown that mite-tolerant stocks are effective means to reduce mite infestations within colonies, but it is unclear whether the stocks available commercially are viable means of mite control because they are likely to be genetic hybrids. We compared colonies of a standard commercial stock ("Italian") with those of a commercially purchased mite-tolerant stock ("Russian") for their levels of varroa and "tracheal" mites (*A. woodi*) over the course of 2 yr in three different geographic locations. We were unable to detect significant infestations of tracheal mites; thus, we were unable to adequately compare the stocks for their tolerance. In contrast, we found significant differences in the levels of varroa mites within and among colonies located across the three different study sites for both years. By the end of the first year, we found statistically significant differences between the stocks in varroa mite intensity (mites per adult bee), such that Russian-hybrid colonies tended to have a significantly lower proportion of parasitized adult bees than Italian colonies. In the second year, we found statistically significant differences between the stocks in varroa mite load (daily mite drop), such that Russian-hybrid colonies tended to have lower total numbers of mites than Italian colonies. These findings suggest that beekeepers may benefit by incorporating commercially purchased mite-tolerant stocks into their existing integrated pest management programs.

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#### Infectivity and virulence of *Nosema ceranae* (Microsporidia) isolates obtained from various *Apis mellifera* morphotypes

Porrini, LP; Porrini, MP; Garrido, MP; Muller, F; Arrascaeta, L; Iriarte, PFJ; Eguaras, MJ. 2020

The infection of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), by the microsporidian *Nosema ceranae* is one of the factors related to the increase in colony losses and the decrease in honey production observed in recent years. However, these effects seem to differ depending on the climate zone. The range and prevalence of *N. ceranae* have increased significantly in the last decades, with different consequences in northern and southern temperate areas. The existence of various isolates of *N. ceranae* from distant geographical areas, which probably exhibit different degrees of virulence, could explain the different responses of the bee to the infection. The aim of this work was to compare the effects of two *N. ceranae* isolates from different host populations from Argentina on honey bee survival at two ages post-eclosion. Using cage experiments, we compared the development of infection of worker bees through the estimation of daily bee mortality and spore counts. Host subspecies identity analysis showed a strong similarity with *Apis mellifera scutellata* morphotype for the northern region, with a greater hybridization between subspecies with European origin toward the central and southern regions. Genetic characterization of isolates from the three regions indicated only the presence of *N. ceranae*. Infected bees survived longer than control bees, and bees infected at 5 days had a lower survival than those infected at 72 h with isolates from the three regions. These differences in survival matched the development of the *N. ceranae* infection, with differences in spore loads for infected bees at 5 days. Our studies showed that *Nosema* infection and survival varied among the different ages post emergence of workers, and both increased as the honey bee aged. These differences in susceptibility to infection could be related to the immune response of bees of different ages or to changes in the composition and succession of the intestinal microbiota throughout its ontogeny.

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#### An integrative approach to species discrimination in the *Anagrus atomus* group sensu stricto (Hymenoptera: Mymaridae), with a description of a new species

Nugnes, F; Bernardo, U; Viggiani, G. 2017

Egg parasitoids of the genus *Anagrus* Haliday (Hymenoptera: Mymaridae) are natural enemies of many pests around the world. We used an integrative approach to characterize some species belonging to the *Anagrus atomus* group, using specimens reared from leafhoppers infesting some Lamiaceae. Starting from morphological identifications based on available keys, we carried out a multi-locus genetic characterization using phylogenetic and species delimitation analyses, and integrated it with biological and morphometric evidence. This approach revealed the existence of a new species, *A. nepetellae* sp. nov., here described along with its phenological traits. The approach also allowed us to characterize the expected taxonomic stability of other putative species within the group. We propose two new species groups called the *atomus* group sensu stricto and the *vilis* group, with the goal of streamlining the taxonomy of the *atomus* group. We also present a key for the identification of females of the European species of the *atomus* group sensu stricto. Some specimens show heterozygosity in 28S-D2 sequences, suggesting hybridization between *A. atomus* and *A. nepetellae*, which, if it is common between other species as well, could partly explain the taxonomic problems in the genus *Anagrus*. DNA sequencing of specimens reared by the same biofactory at different times suggests involuntary contamination leading to the displacement of a

laboratory strain of *A. atomus* by *A. nepetellae*, which may have resulted from undetected partial reproductive compatibility.

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#### Style length variation in male and female figs: development, inheritance, and control of pollinator oviposition

Ghana, S; Suleman, N; Compton, SG. 2017

Fig trees (*Ficus* spp., Moraceae) depend on female fig wasps to transport their pollen and as a reward provide nutrition for pollinator fig wasp larvae. Most pollinators are host specific, but natural plant hybrids occur if atypical hosts are pollinated. Female fig wasps lay their eggs into ovaries of female flowers in receptive figs by inserting their ovipositors through the styles, and relative style and ovipositor lengths are believed to largely determine their ability to oviposit. In dioecious fig trees, flowers in receptive male figs have short styles and support larval development, whereas flowers in receptive female figs have long styles and only develop into seeds. Using the dioecious fig tree species *Ficus montana* Burm. f. and *Ficus asperifolia* Miq., we recorded the comparative ontogeny of style lengths to determine whether style growth in the two sexes is coordinated with the attraction of pollinators, and how maximum style lengths relate to the ovipositor lengths of their pollinators. F1 hybrids between these species produce viable seeds, but no pollinator offspring. We examined style lengths in F1s and backcrosses, relative to those of their parents, and whether coordination of style growth with pollinator attraction is disrupted. The ovipositor of *Kradibia tentacularis* (Grandi) (Hymenoptera: Agaonidae), the pollinator of *F. montana*, could reach some of the ovaries in female figs, but fails to lay eggs there. Styles in male F1 figs were intermediate in length between those of the two parents, but in female F1 figs styles grew longer than in either parent. Maximum style lengths in F1s were partially decoupled from receptivity, especially in female figs. Our results illustrate how timing of stigmal growth is coordinated with pollinator attraction, and that this coordination can be disrupted in hybrid individuals.

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#### Wolbachia endosymbionts distort DNA barcoding in the parasitoid wasp genus *Diplazon* (Hymenoptera: Ichneumonidae)

Klopfstein, S; Kropf, C; Baur, H. 2016

Molecular species delimitation has the potential to speed up both discovery and description rates for new species. However, several studies have shown a limited performance of the standard DNA barcoding approach which relies on a single mitochondrial gene, cytochrome oxidase 1 (COI). Besides incomplete lineage sorting or a low substitution rate, distortion of mitochondrial inheritance patterns by bacterial endosymbionts could explain the failure of barcoding. Numerous reviews have discussed this phenomenon, but only few empirical examples exist. In the present study, we examine the effect of *Wolbachia* bacteria on barcoding in the parasitoid wasp genus *Diplazon*. Although integrative taxonomy recognizes 16 species, COI only recovers up to ten. Adding multivariate morphometrics, genotyping a fast-evolving nuclear gene (ITS2) and screening the *Diplazon* species for *Wolbachia*, we show that the failure of DNA barcoding coincides with the presence of the endosymbiont. Two species even share identical COI haplotypes and *Wolbachia* strains, even though ITS2 suggests that they are not closely related. This is one of very few examples of mitochondrial DNA introgression between well-established insect species, facilitated by *Wolbachia*. We review similar reports, provide a list of criteria to identify endosymbiont-mediated introgression, and discuss the prevalence and impact of this phenomenon on insect systematics and evolution.

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#### Microdissection: a tool for bee chromosome studies

Fernandes, A; Scudeler, PES; Diniz, D; Foresti, F; Campos, LAD; Lopes, DM. 2011

The emergence of new molecular biology techniques has provided cytogenetics with tools which allow for the elucidation of questions that classical cytogenetics could not answer. Therefore, the present work standardizes a microdissection protocol for cytogenetic studies in bees. This methodology was first used in these insects and may contribute greatly to studies involving chromosomal rearrangements, heterochromatin composition, B chromosomes and others. For this study, the centromeric region of chromosomes in the stingless bee *Tetragonisca fiebrigi* was used for probe synthesis. The results demonstrated that the methodology used was efficient, presenting markings in the centromeric regions of several chromosomes. Hybridization in other sites indicates that the probe was able to detect regions that present homology with its sequence. This indicates that the technique is effective to study chromosomal evolution, genome organization and even the origin of B chromosomes.

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#### BREAKDOWN OF POLLINATOR SPECIFICITY IN AN AFRICAN FIG TREE

WARE, AB; COMPTON, SG. 1992

A single giant-leafed fig tree (*Ficus lutea*) is planted on the Rhodes University campus in Grahamstown, South Africa, some 500 km outside its normal distribution range. Small numbers of fig wasps (Hymenoptera, Agaonidae) which normally pollinate two other *Ficus* species entered and successfully pollinated the figs of this tree. One of the wasp species reproduced successfully. Monitoring of adult fig wasps arriving at the tree established that these alien species were not attracted to *F. lutea*. However, from laboratory studies it appears that once having landed on *F. lutea* figs, these wasps were stimulated to search for the ostiole, through which they gained entrance to the fig cavity. Females of a third pollinator species were also present on the tree, but they failed to initiate ostiole searching behavior when on the figs. Hybrid seeds resulting from the entry of the alien wasps germinated successfully, but did not progress past the cotyledon stage, indicating postgermination deficiencies in the hybrids.

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#### STUDIES ON THE REPRODUCTIVE-BIOLOGY OF WHITE YAM (*Dioscorea rotundata* POIR)

SEGNOU; FATOKUN, CA; AKORODA, MO; HAHN, SK. 1992

Investigations were conducted on the identification and distribution of yam natural pollinators, flowering pattern and hybridization of white yam (*Dioscorea rotundata* Poir.) at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, in order to provide informations for producing hybrid seeds needed for yam genetic improvement. Insects belonging to the Coleoptera (48.0%), Diptera (20.4%), Hymenoptera (20.0%), Hemiptera (5.8%), and Thysanoptera (5.8%) were caught around yam plants and presumed to be pollinators: they entered open-receptive-flowers and their presence on yam plants coincided with the duration of the flowering period. Studies on the flowering pattern revealed that: (i) Male and female clones differed in their periods of flower initiation as well as the flowering duration; (ii) Seed production and conventional breeding methods are feasible if staggered planting, water and soil fertility could be managed to ensure that flowering periods of males and females nick and also extend flowering. Artificial pollinations within white yam and between white yam and yellow yam (*D. cayensis* Lam) using the camel hair brush method were successful, but resulted in a relatively low fruit set (31.8% maximum). This method was more effective than natural pollination (19.2%) and can thus supplement it for the production of hybrid seeds for introducing genetic variation in yam breeding populations.

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#### HYBRIDIZATION IN PRINCIPAL PARASITIDS OF SYNANTHROPIC DIPTERA - THE GENUS *MUSCIDIFURAX* (HYMENOPTERA, PTEROMALIDAE)

LEGNER, EF. 1988

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**Selitrichodes neseri (Hymenoptera: Eulophidae) recovered from Leptocybe invasa (Hymenoptera: Eulophidae) galls after initial release on Eucalyptus (Myrtaceae) in Brazil, and data on its biology**

Masson, MV; Tavares, WD; Lopes, FD; de Souza, AR; Ferreira, PJ; Barbosa, LR; Wilcken, CF; Zanuncio, JC. 2017

Blue gum chalcid, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae), causes galling damage to *Eucalyptus* species (Myrtaceae) in various regions of the world, but has been controlled effectively by its primary parasitoid, *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae). The objectives of this study were to evaluate the recovery of *S. neseri* after its initial release on *Eucalyptus* plants in Brazil and to provide data on its biology. *Selitrichodes neseri* was imported from South Africa to Brazil for the biological control of *L. invasa*, in Mar 2015, and recovered from Aug 2015 to Dec 2016. Successful recovery of this parasitoid shows its potential to become established in the field. Seedlings of 2 hybrids obtained from crosses between rose gum *Eucalyptus grandis* W. Hill. ex Maiden and flooded gum *Eucalyptus urophylla* S. T. Blake and between *Eucalyptus* sp. and (river red gum *Eucalyptus camaldulensis* Dehnh. x *E. grandis*) showed potential as hosts for culture of *S. neseri* on *L. invasa* in the laboratory because up until the adult parasitoid emergence these seedlings did not wilt. When reared at 25.1 to 26.0 degrees C, the total number of parasitoids and the proportion of male parasitoids were highest, relative to hosts reared at 26.1 to 27.0 degrees C. Peak emergence of *S. neseri* occurred 28 d after parasitism.

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**ESTIMATIONS OF NUMBER OF ACTIVE LOCI, DOMINANCE AND HERITABILITY IN POLYGENIC INHERITANCE OF GREGARIOUS BEHAVIOR IN MUSCIDIFURAX-RAPTORELLUS [HYMENOPTERA, PTEROMALIDAE]**

LEGNER, EF. 1991

Estimates of the minimum number of genetic loci governing expressions of gregarious oviposition in *Muscidifurax raptorellus* Kogan & Legner were enabled by the verification of recombinant males, which were secured from virgin hybrid females, formed by crossing cohorts from solitary and gregarious populations. Examinations of parasitization behavior in female progeny that had F1 male fathers indicated the presence of hybrids among the males. Estimates of gene number, made on the basis of variances in P1, F1, F2 and backcross progeny, and by observing behavior in second and third order backcrosses, ranged from two to 19, with most between two and five. However, backcrossing data suggested that at least eight loci were actively segregating for this characteristic. Semi-dominance of the solitary trait ( $D = 0.63$  to  $0.84$ ), and unequal gene effects probably caused these gene estimates to be lower than the actual number. Estimates of the coefficient of heritability, in the broad sense based on parental and F1 and F2 variances indicated that variability of gregarious behavior in the experimental environment was influenced > 60 % by genotypic factors, offspring-parent regression analyses gave estimates > 38 %.

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**HYBRIDIZATION AND HOST SUITABILITY OF 2 BIOTYPES OF TRIOXYS-PALLIDUS (HYMENOPTERA, APHIDIIDAE)**

MESSING, RH; ALINIAZEE, MT. 1988

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**Population genetic analyses of complex global insect invasions in managed landscapes: a *Leptocybe invasa* (Hymenoptera) case study**

Dittrich-Schroder, G; Hoareau, TB; Hurley, BP; Wingfield, MJ; Lawson, S; Nahrung, HF; Slippers, B. 2018

Increased rates of movement and the accumulation of insects establishing outside their native range is leading to the 'global homogenization' of agricultural and forestry pests. We use an invasive wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), as a case study to highlight the rapid and complex nature of these global invasions and how they can complicate management options. To trace the invasion history of *L. invasa* globally, we characterised the genetic diversity within and between populations from its origin and invaded regions using mitochondrial and nuclear markers. Three mitochondrial Haplogroups were identified, of which two are likely different species that appear to have been independently introduced into different parts of the world. One type (Mitochondrial Haplogroup 1) occurs globally, and is the exclusive type found in Europe, the Middle East, South America and most of Africa. The second type (Mitochondrial Haplogroup 2) co-occurs with the first-type in Laos, South Africa, Thailand and Vietnam, while a third type (Mitochondrial Haplogroup 3) occurs exclusively in Australia, its native range. The distinction of the two invasive Haplogroups was supported by analysis of newly developed simple sequence repeat (microsatellite) markers in populations from 13 countries. Further analyses using clustering methods and approximate Bayesian computation suggested the occurrence of hybridisation in the Laos population and revealed that an unsampled population was the origin of Mitochondrial Haplogroup 1. The analyses also showed little genetic differentiation within the invasive populations, suggesting a limited original introduction from a very small population followed by rapid, global range expansion in a stepwise fashion. Results of this study should provide some guidelines for characterizing invasion pathways of new invasive insect pests.

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**Discrimination of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) and *T. beneficus* Yasumatsu et Kamijo and their hybrids by allele-specific PCR**

Yara, K; Kunimi, Y. 2009

*Torymus sinensis* and *Torymus beneficus* (Hymenoptera: Torymidae) are, respectively, introduced and indigenous parasitoid wasps that attack the invasive chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in Japan. *Torymus beneficus* has two emergence types in spring, here designated early-spring and late-spring. It is very difficult to distinguish these *Torymus* species and emergence types of *T. beneficus* by their morphological and ecological characteristics. We designed four allele-specific primers for the internal transcribed spacer 1 region of nuclear ribosomal DNA, and developed three kinds of allele-specific PCR. These methods enabled us to distinguish the species, emergence type, and F(1)s. This is the first report of the use of molecular markers to distinguish *T. sinensis*, the late-spring strain of *T. beneficus*, and their F(1)s.

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**Repetitive DNAs in *Melipona scutellaris* (Hymenoptera: Apidae: Meliponidae): chromosomal distribution and test of multiple heterochromatin amplification in the genus**

Piccoli, MCA; Bardella, VB; Cabral-de-Mello, DC. 2018

*Melipona* bees are remarkable due to the high contrast in heterochromatin amounts, making this group interesting for studying repetitive DNA amplification. Here, we performed the first efforts for the chromosomal localization of different repetitive DNAs in *M. (Michmelia) scutellaris* and tested for unique or multiple heterochromatin amplification in *Melipona* subgenera. Our data revealed enrichment of repetitive DNAs in chromosomal heterochromatic arms demonstrated by C (0) t-DNA and DOP-PCR probe hybridization, although microsatellites and multigene families were located at terminal euchromatic regions. Analysis using C (0) t-DNA probe from *M. scutellaris* showed positive hybridization only in *Michmelia* species, suggesting monophyletic amplification and sharing of heterochromatin sequences between species. However, the subgenus *Melikerria*, with a high amount of heterochromatin, probably underwent independent heterochromatin amplification or experienced sequence modification.

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

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

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

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## **A FAMILY OF HIGHLY REPEATED DNA-SEQUENCES OF THE FERN SAWFLY HEMITAXONUS-JAPONICUS COMPLEX (HYMENOPTERA, TENTHREDINIDAE)**

SONODA, S; YAMADA, T; NAITO, T; NAKASUJI, F. 1992

A family of highly repeated DNA sequences of the fern sawfly. *Hemitaxonus japonicus* (*Polystichum tripterum* race) has been cloned. Hybridization analysis of the EcoRI, SacI and Sau3AI digests of the genomic DNA isolated from *H. japonicus* (*Polystichum tripterum* race) against the recombinant plasmid, pYS354, containing tandemly repeated sequences showed ladders of each basic monomer unit having a length of ca. 270-bp. In addition to pYS354, the other two types of clones that also contain tandemly repeated sequences isolated from *H. japonicus* (*Polystichum tripterum* race) were cloned and the nucleotide sequences were compared with pYS354. Nucleotide sequences of these repeated sequences showed high homology between each other with highly AT-rich sequences. The presence of the homologous tandemly repeated sequences in the genome of ten species belonging to six genera of Tenthredinidae was analyzed by hybridization against pYS354 as a probe. Among them, only PP-race, the oligophagous population of *H. japonicus* and the sibling species, *H. sasayamensis*, possessed such tandemly repeated DNA sequences.

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## **Ants with Attitude: Australian Jack-jumpers of the *Myrmecia pilosula* species complex, with descriptions of four new species (Hymenoptera: Formicidae: Myrmecinae)**

Taylor, RW. 2015

The six known "Jack-jumper" species *Myrmecia pilosula* Fr. Smith 1858, *M. croslandi* Taylor 1991, *M. banksi*, *M. haskinsorum*, *M. imaii* and *M. impatens* spp.n. are reviewed, illustrated and keyed. *Myrmecia imaii* is known only from southwest Western Australia, the others variously from southeastern Australia and Tasmania. These taxa were previously confused under the name *M. pilosula* (for which a lectotype is designated). Previous cytogenetical findings, which contributed importantly to current taxonomic understanding, are summarized for each species. Eastern and Western geographical races of the widespread *M. pilosula* are recognized. *Myrmecia croslandi* is one of only two eukaryote animals known to possess a single pair of chromosomes ( $2n = 2 \times 3$  or  $4$ ). *Myrmecia impatens* is evidently an allopolyploid ( $n = 5$  or  $14$ ,  $2n = 19$ ) sperm-dependent gynogenetic hybrid between *M. banksi* and an element of the eastern race of *M. pilosula*, or their immediate ancestry. The sting-injected venom of these ants can induce sometimes fatal anaphylaxis in sensitive humans.

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## **Dimorphic antennal systems in gynandromorphic honey bees, *Apis mellifera* L. (Hymenoptera : Apidae)**

Brockmann, A; Bruckner, D. 1999

Gynandromorphic honey bees, *Apis mellifera* (Hymenoptera: Apidae), were examined to determine characteristic morphological and anatomical features of the antennal system. The antennae of gynandromorphic individuals are predominantly worker or drone-like. Hybrid antennae, composed of female and male tissues, occur only rarely (7 out of 188 examined antennae). Depending on the mosaic pattern of the head, both antennae can be drone-like or worker-like, or one can be drone-like and the other worker-like. Examination of the antennal lobes of six characteristic specimens revealed that antennal lobes, which are innervated by drone-like antennae, always have drone-specific enlarged tracts and macroglomerular complexes, whereas antennal lobes innervated by worker-like antennae always are composed of normally sized glomeruli. Thus, there is a strict correlation between the sexual morphology of the antennae and the sexual organization of the antennal lobe neuropil. In one antennal lobe, innervated by a hybrid antenna, we found a hypertrophied glomerulus, certainly homologous to one of the macroglomerular complexes in drone-like antennal lobes. (C) 1999 Elsevier Science Ltd. All rights reserved.

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## **Genomes of the willow-galling sawflies *Euura lappo* and *Eupontania aestiva* (Hymenoptera: Tenthredinidae): a resource for research on ecological speciation, adaptation, and gall induction**

Michell, C; Wutke, S; Aranda, M; Nyman, T. 2021

Hymenoptera is a hyperdiverse insect order represented by over 153,000 different species. As many hymenopteran species perform various crucial roles for our environments, such as pollination, herbivory, and parasitism, they are of high economic and ecological importance. There are 99 hymenopteran genomes in the NCBI database, yet only five are representative of the paraphyletic suborder Symphyta (sawflies, woodwasps, and hornets), while the rest represent the suborder Apocrita (bees, wasps, and ants). Here, using a combination of 10X Genomics linked-read sequencing, Oxford Nanopore long-read technology, and Illumina short-read data, we assembled the genomes of two willow-galling sawflies (Hymenoptera: Tenthredinidae: Nematinae: Euurina): the bud-galling species *Euura lappo* and the leaf-galling species *Eupontania aestiva*. The final assembly for *E. lappo* is 259.85 Mbp in size, with a contig N50 of 209.0 kbp and a BUSCO score of 93.5%. The *E. aestiva* genome is 222.23 Mbp in size, with a contig N50 of 49.7 kbp and a 90.2% complete BUSCO score. De novo annotation of repetitive elements showed that 27.45% of the genome was composed of repetitive elements in *E. lappo* and 16.89% in *E. aestiva*, which is a marked increase compared to previously published hymenopteran genomes. The genomes presented here provide a resource for inferring phylogenetic relationships among basal hymenopterans, comparative studies on host-related genomic adaptation in plant-feeding insects, and research on the mechanisms of plant manipulation by gall-inducing insects.

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## **Multivariate analysis of honeybees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), of the Horn of Africa**

Radloff, SE; Hepburn, HR. 1997

Morphometric characters and sting pheromones of worker honeybees, *Apis mellifera* Linnaeus, were analysed by multivariate methods to delineate the honeybee populations of the Horn of Africa. Four discrete and statistically homogeneous populations were identified: *A. m. jemenitica* Ruttner, *A. m. bandasii* Mogga, *A. m. sudanensis* Rashad in Ethiopia and an unclassified group in southwestern Somalia. Areas of high intercolonial Variance are interpreted as zones of hybridization between the populations.

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#### **Defensive behavior of africanized honeybees (Hymenoptera: Apidae) in Dourados-Mato Grosso do Sul, Brazil**

Faita, MR; Carvalho, RMMC; Alves, VV; Chaud-Netto, J. 2014

African bees were introduced in Brazil in 1956, in an attempt to improve honey production. The accidental hybridization between African and European breeds originated africanized bees, which are very well adapted to the local climate. That bee poly-hybrid has an initial production of honey 70 % more than Europeans. However, African and africanized bees were much more defensive than European subspecies, which required the development of appropriate management techniques. Beekeepers in southern Mato Grosso do Sul learned to work with africanized bees. The aim of this study was to evaluate the defensive behavior of Africanized bees in Dourados MS, using a ball of black leather and artificial enemy. There were is recorded, the time to deliver the first bite, the time it takes to enrage (attack the enemy with great intensity), the distance from the pursuit of the enemy after the first attack and the number of bites left in the beanbag. The results indicate a significant concentration of colonies of bees with different intensity and defensive behavior, but similar to that presented by africanized bees in the 60/70, particularly in the region of Ribeirao Preto (SP). It appears, in terms of their defensive behavior, bees in the southern region of the state, did not suffer influence by bees of European origin.

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#### **Variability and genetic components of innate fruit odour recognition in a parasitoid of *Drosophila***

Campan, E; Couty, A; Carton, Y; Pham-Delegue, MH; Kaiser, L. 2002

This study aims to investigate the genetic variability of olfactory responses to odours from the host habitat, involved in host selection by insect parasitoids. The probing response of females to fruit and non-fruit odours was studied in *Leptopilina boulardi* Barbotin et al. (Hymenoptera, Figitidae), a parasitoid of the frugivorous *Drosophila melanogaster* larvae. Responses of two strains (from Nasrallah, Tunisia, and from Brazzaville, Congo) and their hybrids were compared. Probing is a searching mode by repeated ovipositor insertions into the breeding substrate to locate digging larvae. Most Brazzaville females exhibited innate ovipositor probing into agar in response to the fruit aromas (banana, orange, pear, prickly pear), whereas this was rare in the Nasrallah strain. These four fruits are all breeding sites for *D. melanogaster*, whereas mushroom is not. Mushroom odour did not trigger any probing response in either strain. In addition, the latency of the probing response was shorter in Brazzaville females. Results from hybrid females (F1 and Backcross) showed that both probability to probe in response to a fruit aroma and latency of the response were strongly heritable. Regarding the probability to probe, there was an apparent complete dominance of Nasrallah characteristics, whereas additive inheritance was found in the Brazzaville lineage. The contrast between reciprocal F1 hybrids suggested a strong interaction with non-chromosomal factors. It is suggested that a cytoplasmic factor in the Nasrallah strain decreases females' propensity to probe in response to significant odours. The latency of probing appeared to be under a chromosomal influence. Thus, this study identifies a strong genetic component in the responses to odours from the host habitat. It also provides information on the genetic basis of the two characters studied.

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#### **Alfalfa leaf cutting bee (Hymenoptera : Megachilidae) pollination of oilseed rape (*Brassica napus* L.) under isolation tents for hybrid seed production**

Soroka, JJ; Goerzen, DW; Falk, KC; Bett, KE. 2001

In this 3-yr field study, the activities of alfalfa leafcutting bees (*Megachile rotundata* Fabricius) (LCB) at varying stocking levels were monitored in shade cloth isolation tents containing male-sterile and male-fertile rows of oilseed rape *Brassica napus* L. to determine optimum conditions for hybrid seed production. Bumble bees, *Bombus impatiens* Cresson, were similarly tested in 1 yr; their pollination activities were minimal, and weight of seed produced on female lines in tents containing bumble bees was similar to that in tents without pollinators. Leafcutting bee activity, as measured by the number of female bees at the entrance to tunnels in the hive, at first increased, then decreased with increasing stocking rate. Duration of time spent visiting flowers did not vary with stocking rate, but was longer on male-fertile than on male-sterile flowers. Production of hybrid seed within tents varied with leafcutting bee stocking rate, with the highest seed yield achieved at stocking rates equivalent to three charges at weekly intervals of 400 000 leafcutting bees per hectare. At optimum LCB stocking rates, the method described provided sufficient seed quantity for small-plot multi-location field evaluation of oilseed rape hybrids.

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#### **FIRST REPORT OF THE PARASITOID *GANASPIS BRASILIENSIS* IHERING (HYMENOPTERA: FIGITIDAE) IN MEXICO**

Gonzalez-Cabrera, J; Cordoba-Urtiz, EG; Moreno-Carrillo, G; Sanchez-Gonzalez, JA; Arredondo-Bernal, HC. 2020

To develop biological programs for the spotted wing drosophila, *Drosophila suzukii* (Matsumura), the Mexican government placed sentinel traps with larvae and pupae of *D. melanogaster* Meigen from December 2017 to May 2018 in berry crops of Jalisco, Mexico. From those explorations. *Ganaspis brasiliensis* Ihering was identified as a first report in Mexico, although its presence in America has been noted in Brazil, Guadeloupe and Panama. This local parasitoid could contribute to the protection of the national berry industry by its field release or its hybridization with an exotic population highly specialized on *D. suzukii*.

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#### **Mating preference in the commercially imported bumblebee species *Bombus terrestris* in Britain (Hymenoptera : Apidae)**

Ings, TC; Raine, NE; Chittka, L. 2005

Commercial trade of bumblebees in Europe results in different subspecies of *Bombus terrestris* Linnaeus 1758 being shipped into regions where they are not native. Although previous studies have shown that these subspecies will interbreed, none have assessed mating preference of the different populations. This study examines the mating preferences between two geographically isolated populations of *B. terrestris* which have unnaturally been brought together through the commercial trade in bumblebees. Under controlled choice conditions, mating between commercially imported *B. t. dalmatinus* (from South Eastern Europe) and native British *B. t. audax* was non-random. Commercially imported gynes (unfertilised queens) preferred to mate with males from the same population (71% of matings). In light of the continued escape of imported gynes and males, these results indicate that there is a possibility of establishment of South Eastern European *B. t. dalmatinus* in Britain, and that hybrids will also occur. The ecological risks of such an establishment are discussed.

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#### **A taxonomic revision of the Palaearctic members of the *Formica rufa* group (Hymenoptera: Formicidae) - the famous mound-building red wood ants**

Seifert, B. 2021

A revision of the Palaearctic members of the *Formica rufa* group, the famous mound-building red wood ants, is presented based on Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) and on genetic information from studies published in cooperation with others. Standardized morphological character systems were described numerically to allow objective hypothesis formation by exploratory data analyses and testing by hypothesis-driven data analyses. NUMOBAT data were recorded in a total of 1200 samples with 5500 worker individuals and 410 gynes. Comparative tables to workers and gynes of all species and the most frequent hybrids and a key to the workers are presented. Considering 54 available names, the survey recognized 13 good species, 32 junior synonyms and eight names not interpretable to species level (incertae sedis). The ratio of junior synonyms against the number of recognized species is elevenfold the ratio found in a revision of Palaearctic *Lasius s.str.* conducted by the same author in 2020 with basically the same methodology. Excessive name production in the *F. rufa* group is partly result of the big attention these eye-catching ants have received by naturalists but it also reflects the enormous difficulties to reasonably interpret a multitude of phenotypes. These difficulties are caused by extraordinary frequency of reticulate evolution, particular mechanisms for the evolution of deviating local populations, and intraspecific polymorphism with differences sometimes being larger than those between species. Hybridization and introgression were shown or made plausible in 46% of the 13 recognized species with regional hybridization frequencies of above 20% in three species. The author assumes that the evolutionary history of *rufa* group ants will turn out as extremely reticulate comparable with the situation in *Heliconius* butterflies or Darwin Finches once whole genome analyses will be available for all species. The 13 species of the *F. rufa* group were assigned to four species complexes: (a) the *F. rufa* complex with *F. rufa* LINNAEUS, 1761 and *F. polycтена* FOERSTER, 1850; (b) the *F. lugubris* complex with *F. lugubris* ZETTERSTEDT, 1838, *F. helvetica* sp.n., *F. paralugubris* SEIFERT, 1996, *F. aquilonia* YARROW, 1955 and *F. ussuriensis* sp.n.; (c) the *F. pratensis* complex with *F. pratensis* RETZIUS, 1783 and *F. kupyanskayae* BOLTON, 1995 and (d) the *F. truncorum* complex with *F. truncorum* FABRICIUS, 1804, *F. dusmeti* EMERY, 1909, *F. frontalis* SANTSCHE, 1919 and *F. sinensis* WHEELER, 1913. *Formica yessensis* WHEELER, 1913 is recognized as a new junior synonym of *F. truncorum*. Special sections describe the situation in six hybrid combinations. Comments on species incertae sedis and unavailable names are given in a final chapter.

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#### Structural and transcriptional features of *Bombus terrestris* satellite DNA and their potential involvement in the differentiation process

Rouleux-Bonnin, F; Bigot, S; Bigot, Y. 2004

A unique satellite DNA family was characterized in the genome of the bumble bee, *Bombus terrestris*. Sequence analysis revealed that it contains two wide palindromes of about 160 and 190 bp, respectively, that span 75% of the repeated unit. One feature of this satellite DNA is that it accounts for different amounts of genomic DNA in males and females. The DNA curvature and bendability were determined by migration on PAGE and by computer analysis. It has been correlated with the presence of dA/dT stretches repeated in phase with the helix turn and with the presence of the deformable dinucleotide CA-TG embedded in some of these A-T-rich regions. Transcription of the satellite DNA was also analyzed by Northern blot hybridization and RT-PCR. Multimeric transcripts spanning several satellite DNA units were found in RNA samples from males, workers, and queens. These transcripts resulted from a specific transcription occurring on one DNA strand in the embryos or on both DNA strands in imagoes. The involvement of DNA curvature in the organization of the satellite DNA and the function of the satellite transcripts is discussed.

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

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

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

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#### Persistence conditions of symmetric social hybridogenesis in haplo-diploid hymenoptera

Yamauchi, A; Yamamura, N. 2006

In eusocial Hymenoptera species, females variably develop into either alate females (queens) or workers, and in most cases, caste differentiation is determined environmentally. Recently, however, female castes in two harvester ant species, *Pogonomyrmex rugosus* and *P. barbatus*, were found to be determined genetically in hybrid zones of these two species. In the hybrid populations, homozygous females (e.g. AA or BB) and heterozygous females (AB) develop into alate females and workers, respectively. This genetic caste determination system is called symmetric social hybridogenesis (SSH). It is clear that populations with SSH can persist only if all four genotypes (AA and BB females, and A and B males) coexist simultaneously. However, it is not obvious that these populations are always persistent when the four genotypes simultaneously exist. Here, we examined the stability and persistence of an SSH population using a simple mathematical model. According to the analysis of the model, the SSH population persists only when some conditions are satisfied: (1) each female mates with more than two males, and (2) male production increases less steeply than linearly with increasing numbers of workers in a colony, and alate female production increases more steeply than linearly with increasing numbers of workers, or (T) male production increases more steeply than linearly with increasing numbers of workers in a colony, and alate female production increases much more steeply than male production. Therefore, it is not obvious that SSH populations are maintained and are stable for long periods. We discuss whether these conditions are satisfied in real SSH populations. (c) 2005 Elsevier Ltd. All rights reserved.

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#### HYBRIDIZATION AND INBREEDING EFFECTS ON GENOME COADAPTATION IN A HAPLO-DIPLOID HYMENOPTERA - COTHONASPIS-BOULARDI (EUCOILIDAE)

BIEMONT, C; BOULETREAU, M. 1980

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#### REPETITIVE DNA-SEQUENCE FAMILIES IN HEMITAXONUS-MINOMENSIS AND H-ATHYRII (HYMENOPTERA, TENTHREDINIDAE)

SONODA, S; YAMADA, T; NAITO, T; NAKASUJI, F. 1995

Families of the repetitive DNA sequences from *Hemitaxonus minomensis* and *H. athyrii* were characterized. pHMS family and pHME family in *H. minomensis* consist of tandemly arranged arrays whose basic repeat units are 260 bp and 330 bp, respectively. pHAE family in *H. athyrii* consists of a tandemly arranged array whose

basic repeat unit is 380 bp. pHMS family and pHME family occupy approximately 4.8% and 0.07% of the genome of *H. minomensis*, respectively. By contrast, in *H. athyrii*, pHAE family comprise 0.04% of the genome. Nucleotide sequence comparison of these three repetitive families showed very little homology. Southern blot hybridization using six species of *Hemitaxonus* showed that these repetitive families are species specific.

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#### **Repellency of callicarpenal and intermedeol against workers of imported fire ants (Hymenoptera : Formicidae)**

Chen, J; Cantrell, CL; Duke, SO; Allen, ML. 2008

Callicarpenal and intermedeol are two insect-repellent terpenoids isolated from leaves of American beautyberry (*Callicarpa americana* L.; Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.). The repellency of these two terpenoids against workers of red imported fire ants, *Solenopsis invicta* Buren, black imported fire ants, *Solenopsis richteri* Forel, and a hybrid of these two species was evaluated using digging bioassays. In a multiple choice digging bioassay using two colonies from each species and their hybrid, callicarpenal showed significant repellency at concentration as low as 50 ppm against both red imported fire ant colonies and 6.25 ppm against all black imported fire ant and hybrid colonies. Intermedeol showed significant repellency at concentration as low as 1.50 ppm against both red imported fire ant colonies and 6.25 ppm against all black imported fire ant and hybrid colonies. In total, 15 colonies, five colonies from each species and the hybrid, were tested on callicarpenal and intermedeol at 50 ppm in a two-choice digging bioassay. Both callicarpenal and intermedeol showed repellency against all colonies, and intermedeol showed significantly greater repellency than callicarpenal against both species and their hybrid.

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#### **Behavioral and genetic characteristics of a new species of *Nasonia***

Raychoudhury, R; Desjardins, CA; Buellesbach, J; Loehlin, DW; Grillenberger, BK; Beukeboom, L; Schmitt, T; Werren, JH. 2010

*Nasonia* (Hymenoptera: Pteromalidae) is a genus of parasitoid wasps, which is fast emerging as a model system for evolutionary, genetic, developmental and host-endosymbiont interaction studies. In this study, we report a new species, *Nasonia oneida*, distinguish its behavioral, genetic and morphological features, and characterize its pre-mating and post-mating isolation with the other *Nasonia* species. Phylogenetic analyses indicate that *N. oneida* is the sister species to *Nasonia giraulti* with its own uniquely distinct cuticular hydrocarbon profiles, behavioral characteristics and subtle morphological differences. An important characteristic of *N. oneida* is the strong mate discrimination shown by the females against all the other *Nasonia* species. A genetic analysis of this phenotype by interspecies hybridization indicates that this strong discriminating phenotype is recessive. A formal species description of *N. oneida* Raychoudhury & Desjardins is also provided. *Heredity* (2010) 104, 278-288; doi:10.1038/hdy.2009.147; published online 20 January 2010

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#### **Morphometric identification of honey bee subspecies reveals a high proportion of hybrids within a Mediterranean protected area**

Ropars, L; Affre, L; Geslin, B. 2021

The importance of natural protected areas for the preservation of locally adapted subspecies (or local genotype) of honey bees has been recently emphasized in the literature. In western Europe, initiatives have emerged to protect the native subspecies i.e., the Western European dark bee, *Apis mellifera mellifera* (Hymenoptera: Apidae). Here, we investigated the honey bee subspecies diversity in a Mediterranean protected area, Calanques National Park, near Marseille, France. We found that the population of honey bees is mainly composed of hybrids between *Apis mellifera mellifera* and *Apis mellifera intermissa*. These hybrids might have a better resistance to the Varroa mite and a better adaptation to arid Mediterranean climate. Before promoting the native honey bee subspecies, *Apis mellifera mellifera*, it appears essential to identify honey bee subspecies used by beekeepers in protected areas.

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#### **Species-diagnostic single-nucleotide polymorphism and sequence-tagged site markers for the parasitic wasp genus *nasonia* (Hymenoptera : Pteromalidae)**

Niehuis, O; Judson, AK; Werren, JH; Hunter, WB; Dang, PM; Dowd, SE; Grillenberger, B; Beukeboom, LW; Gadau, J. 2007

Wasps of the genus *Nasonia* are important biological control agents of house flies and related filth flies, which are major vectors of human pathogens. Species of *Nasonia* (Hymenoptera: Pteromalidae) are not easily differentiated from one another by morphological characters, and molecular markers for their reliable identification have been missing so far. Here, we report eight single-nucleotide polymorphism and three sequence-tagged site markers derived from expressed sequenced tag libraries for the two closely related and regionally sympatric species *N. giraulti* and *N. vitripennis*. We studied variation of these markers in natural populations of the two species, and we mapped them in the *Nasonia* genome. The markers are species-diagnostic and evenly spread over all five chromosomes. They are ideal for rapid species identification and hybrid recognition, and they can be used to map economically relevant quantitative trait loci in the *Nasonia* genome.

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#### **BIOCHEMICAL AND BEHAVIORAL EVIDENCE FOR HYBRIDIZATION BETWEEN FIRE ANTS, SOLENOPSIS-INVICTA AND SOLENOPSIS-RICHTERI (HYMENOPTERA, FORMICIDAE)**

VANDERMEER, RK; LOFGREN, CS. 1989

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#### **Variety-specific population density and infestation levels of apple sawfly (*Hoplocampa testudinea* Klug) in two differently managed apple orchards in Lithuania**

Tamosiunas, R; Valiuskaite, A; Surviliene, E; Duchovskiene, L; Rasiukeviciute, N. 2014

An investigation of apple sawfly (*Hoplocampa testudinea* Klug 1816) (Hymenoptera: Symphyta: Tenthredinidae) populations was conducted in conventionally and organically managed apple orchards of Institute of Horticulture of Lithuanian Research Centre for Agriculture and Forestry in 2010-2013. The aim of this investigation was to examine population density of apple sawfly in conventionally and organically managed apple orchards by means of white sticky traps Rebel (R) Bianco, determine relationship between population density and infestation levels of fruits, and evaluate susceptibility of various apple cultivars to this pest. Density of apple sawfly populations varied considerably between years, management systems and apple cultivars. Strong or moderate dependence of infestation on trap catches was determined in six out of seven cultivars studied in organic orchard. In conventional orchard cultivars 'Noris', 'Auksis' and 'Lobo' suffered significantly higher damage and cultivars 'Lodel', 'Ligol' and 'Spartan' could be attributed to a moderately susceptible group of cultivars. Reliable damage levels could not be determined for conventional orchard. In organic orchard, cultivars 'Aldas', 'Rubinola', 'Rajka', were classified as highly susceptible, 'Lodel' and 'Vitos' - as moderately susceptible and 1 cultivar - 'Enterprise' as of low susceptibility. Control thresholds for six cultivars was defined at 5% infestation level: 'Aldas' - 13 sawfly trap(-1), 'Rubinola' - 11 sawfly trap(-1), 'Vitos' - 23 sawfly trap(-1), 'Lodel' - 21 sawfly trap(-1), 'Rajka' 11 sawfly trap(-1) and 'Enterprise' - 34 sawfly trap(-1). No reliable damage threshold for hybrid No. 18501 was determined.

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## Biological and physiological response of a tropical parasitoid, *Tetrastichus brontispae* (Ferriere) following exposure to low temperature

Lu, BQ; Tang, ZZ; Bellis, GA; Wright, MG; Jin, QA; Peng, ZQ; Wen, HB; Wan, J; Li, YQ. 2016

*Tetrastichus brontispae* FerriSre (Hymenoptera: Eulophidae) is an important natural enemy of the coconut leaf beetle *Brontispa longissima* (Gestro), a serious invasive pest on palm plants. Exposure to low temperature (2 A degrees C) for 4, 24 and 48 h had an adverse effect on biological performance (survival, parasitism rate) of *T. brontispae* with immature parasitoids (eggs, larvae, and pupae) being more sensitive to low temperature than adults. Exposure to 2 A degrees C for 24 h reduced the water content and increased the concentration of glycerol and trehalose, and activity of superoxide in *T. brontispae* adults. Analysis of suppression subtractive hybridization indicated the integrative controlling and transcriptome regulation of the networks in the cold stress response of *T. brontispae*. Our study suggests that the allocation of energy reserves to either prevent or repair cold-induced damage may consume resources that could otherwise be invested in reproduction and consequently its ability to exert control on *B. longissima*.

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## GEOGRAPHICAL OVERLAP OF 2 MITOCHONDRIAL GENOMES IN SPANISH HONEYBEES (*APIS-MELLIFERA-IBERICA*)

SMITH, DR; PALOPOLI, MF; TAYLOR, BR; GARNERY, L; CORNUET, JM; SOLIGNAC, M; BROWN, WM. 1991

Restriction enzyme cleavage maps of mitochondrial DNA from the Spanish honeybee, *Apis mellifera iberica* (Hymenoptera: Apidae), were compared with those from the European subspecies *A. m. mellifera*, *A. m. ligustica*, and *A. m. carnica*, and the African subspecies *A. m. intermissa* and *A. m. scutellata*. The mitochondrial DNA (mtDNA) of the two African subspecies can be distinguished by restriction fragment polymorphisms revealed by Hinf I digests. Two distinct mtDNA types were found among Spanish honeybees: a west European *mellifera*-like type, which predominates in the north of Spain, and an African *intermissa*-like type, which predominates in the south. Spain appears to be a region of contact and hybridization between the two subspecies *A. m. intermissa* and *A. m. mellifera*, which respectively represent African and west European honeybee lineages. This natural boundary between European and African honeybee populations in the Old World may provide a model for predicting the eventual outcome of the colonization of North America by introduced African honeybees.

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## Wolbachia is present in *Apis mellifera capensis*, *A-m. scutellata*, and their hybrid in Southern Africa

Hoy, MA; Jeyaprakash, A; Alvareza, JM; Allsopp, MH. 2003

*Apis mellifera capensis*, *A. m. scutellata* and their hybrids were screened by a sensitive Long PCR protocol for *Wolbachia* because this endosymbiont has been implicated in causing thelytoky in other Hymenoptera. *Wolbachia* was found in all workers of *A. m. capensis* examined, and in workers and drones of *A. m. scutellata* and in hybrid workers of these two subspecies. Cloning and sequencing indicated that all contained the same unique *Wolbachia* strain, named wCap-B1, which belongs to the Con Group because it displayed less than 2.5% sequence divergence from the reference strain from *Tribolium confusum*. wCap-B1 is closely related to *Wolbachia* from *Diaphorina citri*, *Solenopsis invicta*, *Coleomegilla maculata lengi*, *Plutella xylostella*, and *Bemisia tabaci*. Because no sequence differences were found among these bee populations, infection with this *Wolbachia* strain is unlikely to be associated with thelytoky in *A. m. capensis*.

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## Molecular differentiation of two strains of the parasitoid *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae) using specific PCR primers

Zhu, YC; Dowdy, AK; Baker, JE. 2004

Two strains (Sav and Bam) of the parasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) showed different sensitivity to organophosphate insecticides. By using polymerase chain reaction (PCR) and DNA sequencing, we demonstrated clear molecular difference between these two strains. DNA markers that are specific for the Barn strain were developed, and PCR-generated DNA fragments were cloned and sequenced. Two DNA fragments unique to the Barn strain contained 365 and 584 nucleotides. A pair of specific primers was designed from each fragment. PCR-amplification of the DNA from individual wasps generated fragments of the expected sizes only in the Barn strain. Studies conducted on F1 and F2 hybrids produced from crossing and backcrossing between resistant and susceptible strains indicated that these DNA markers are located on mitochondria and inherited exclusively maternally. Probes developed from these fragments may be used in assessing genetic information of natural populations and in studies on physiological or biochemical differences between the strains of this beneficial insect.

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## Inverse dispersal patterns in a group of ant parasitoids (Hymenoptera: Eucharitidae: Oaseminae) and their ant hosts

Baker, AJ; Heraty, JM; Mottern, J; Zhang, JX; Hines, HM; Lemmon, AR; Lemmon, EM. 2020

When postulating evolutionary hypotheses for diverse groups of taxa using molecular data, there is a tradeoff between sampling large numbers of taxa with a few Sanger-sequenced genes or sampling fewer taxa with hundreds to thousands of next-generation-sequenced genes. High taxon sampling enables the testing of evolutionary hypotheses that are sensitive to sampling bias (i.e. dating, biogeography and diversification analyses), whereas high character sampling improves resolution of critical nodes. In a group of ant parasitoids (Hymenoptera: Eucharitidae: Oaseminae), we analyse both of these types of datasets independently (203 taxa with five Sanger loci, 92 taxa with 348 anchored hybrid enrichment loci) and in combination (229 taxa, 353 loci) to explore divergence dating, biogeography, host relationships and differential rates of diversification. Oaseminae specialize as parasitoids of the immature stages of ants in the subfamily Myrmicinae (Hymenoptera: Formicidae), with ants in the genus *Pheidole* being their most common and presumed ancestral host. A general assumption is that the distribution of the parasite must be limited by any range contraction or expansion of its host. Recent studies support a single New World to Old World dispersal pattern for *Pheidole* at c. 11-27 Ma. Using multiple phylogenetic inference methods (parsimony, maximum likelihood, dated Bayesian and coalescent analyses), we provide a robust phylogeny showing that Oaseminae dispersed in the opposite direction, from Old World to New World, c. 24-33 Ma, which implies that they existed in the Old World before their presumed ancestral hosts. Their dispersal into the New World appears to have promoted an increased diversification rate. Both the host and parasitoid show single unidirectional dispersals in accordance with the presence of the Beringian Land Bridge during the Oligocene, a time when the changing northern climate probably limited the dispersal ability of such tropically adapted groups.

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## A new form of arthropod phenoloxidase is abundant in venom of the parasitoid wasp *Pimpla hypochondriaca*

Parkinson, N; Smith, I; Weaver, R; Edwards, JP. 2001

We have recently identified phenoloxidase (PO) activity among several biologically active factors in Venom from the parasitoid wasp *Pimpla hypochondriaca*. We have now isolated three genes, designated POI, POII and POIII, from a cDNA Library made from venom-producing glands and found that their products are related to pro-phenoloxidases (PPOs), which are expressed as proenzymes in haemocytes and which mediate immune processes in arthropods. This is the first report of PO as a venom constituent. Amino acid sequence comparisons between the three *Pimpla* POs and PPOs revealed several notable differences, including the absence of sequences which specify the site of proteolytic activation in insect PPOs and the unprecedented occurrence of signal peptide sequences. NH2-terminal amino acid analysis of PO purified from venom yielded a peptide sequence matching the predicted mature NH2 termini of POI and POII, confirming the authenticity of the signal peptide and indicating that proteolytic processing, other than to remove the signal peptide, does not occur in the wasp. Expression of POI, analysed by Northern



hybridization, was approximately uniform from the time of adult emergence to day 6 post-emergence, after which it declined. A novel means of host immune suppression, mediated by the unregulated activity of venom PO in the haemocoel, is proposed. (C) 2001 Elsevier Science Ltd. All rights reserved.

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#### **Identification of *Torymus sinensis* and *T. beneficus* (Hymenoptera : Torymidae), introduced and indigenous parasitoids of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera : Cynipidae), using the ribosomal ITS2 region**

Yara, K. 2006

The parasitoid wasps *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae) are introduced and indigenous natural enemies, respectively, of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), an invasive pest of chestnuts in Japan. *T. beneficus* has two emergence types in spring, here tentatively designated as the early-spring strain and late-spring strain. It is very difficult to distinguish these two *Torymus* species accurately according to their morphological and ecological characteristics. Although the sequences of internal transcribed spacer 2 of the nuclear ribosomal DNA (ITS2) of these parasitoids are very similar, we have developed a pair of primers for amplifying a part of ITS2 that distinguishes species and emergence type. By performing high-resolution electrophoresis of PCR products amplified by this specific primer pair, we have succeeded in accurately identifying *T. beneficus* (early-spring strain) and some *T. sinensis* parasitoids. However, this technique was discovered to be inapplicable to identify other *T. sinensis* and *T. beneficus* (late-spring strain) parasitoids with the same ITS2 genotype. In spite of this problem, the ITS2 marker appears to be more powerful than any other molecular markers so far reported, since it is applicable to detection of *T. beneficus*, *T. sinensis*, and their hybrids. (c) 2005 Elsevier Inc. All rights reserved.

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#### **Extracted Venom and Cuticular Compounds of Imported Fire Ants, *Solenopsis* spp., and Chemotaxonomic Applications Across a Persistent Hybrid Zone**

DeFauw, SL; Rojas, MG; Morales-Ramos, JA; Boykin, DL. 2010

Characterization of cuticular hydrocarbons permits basic distinctions among colonies of *Solenopsis invicta* Buren, *Solenopsis richteri* Forel and their hybrids (Hymenoptera: Formicidae); thus, providing opportunities to investigate details of landscape ecology for this species complex and to assess levels of invasiveness. We introduce an alternative method for the cluster analysis of cuticular molecules of imported fire ants that is complementary to a widely-used method based on calculating cuticular hydrocarbon and venom alkaloid indices. Results from this GC-MS method were analyzed using various hierarchical and normal mixtures clustering methods to test the stability of group membership. Principal component and discriminant analyses were used to produce three-dimensional views of group separation. The relative proportions of 12 intensity peaks served to differentiate imported fire ant hybrids into four assemblages: hybrids closely allied with *S. invicta*, hybrids close to *S. richi*, a 'core' hybrid grouping, and an 'outlier' hybrid group. The most influential peaks of the assemblage (based on F-values) included 3 peaks with the piperidine structural motif and an alkane. Use of 3 peaks identified by stepwise linear discriminant analysis resulted in misclassification of 5% of the ant colonies, whereas use of 4 peaks resulted in the misclassification of 2.5%. Thus, this GC-MS method and multivariate assessment of biochemical data may facilitate the finer-scale distinction of hybrid colonies in terms of their surficial semiochemical complexity and 'alliance' with parental species. Application of these techniques would be especially useful in refining biological control strategies.

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#### **Suitability of Hybrid and Landrace Maize Plants Within Conventional and Organic-Polyculture Maize Agroecosystems for Hosting Parasitic Wasps**

Moya-Raygoza, G; Figueroa-Bautista, P. 2021

Polycultures and conventional monoculture have different effects on insect pests and natural enemies. Little is known about the parasitoid response to landrace and hybrid plants damaged by insect pests within organic-polyculture agroecosystems vs. monoculture ones. The aim of the present study was to compare the suitability of these agroecosystems for hosting parasitic wasps by evaluating the parasitism rate, parasitoid abundance, and species composition on landrace and hybrid maize races within organic-polyculture and conventional maize agroecosystems. This study was performed within the Mesoamerican region where maize was originally domesticated and where the maize insect pest *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) originated. Maize plants of landrace and hybrid varieties were used to attract egg parasitoids of *D. maidis* within each of the two agroecosystems; conventional maize monoculture and an organic-polyculture of landrace maize, beans, and squash, which also included weed plants. Four treatments were conducted in this field experiment: 1) landrace-within organic agroecosystem, 2) hybrid-within organic agroecosystem, 3) landrace-within conventional agroecosystem, and 4) hybrid-within conventional agroecosystem. A community of parasitic wasps, of which the micro-wasp *Paracentrobia* sp. (Hymenoptera: Trichogrammatidae) was the most abundant member, was found attacking the *D. maidis* eggs in both agroecosystems. The parasitism rate and parasitoid abundance were similar among the four treatments. However, the abundance of parasitoids was greater in the conventional maize monoculture. Bait plants (landrace and hybrid maize) within each agroecosystem did not show differences in either parasitoid abundance or parasitism rate.

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#### **Developmental stability and pollination**

Moller, AP. 2000

A number of different insect species (ranging from Diptera, Lepidoptera and Coleoptera to Hymenoptera) have been shown to discriminate between small degrees of asymmetry when visiting flowers or flower-like models. Such preferences for symmetry may have an innate basis. Small degrees of bilateral or radial asymmetry of flowers are considered to represent a measure of developmental instability, since deviations from perfect symmetry reflect the inability to maintain developmental precision during ontogeny. Environmental factors causing increased asymmetry in leaves and flowers include radioactive radiation, ultraviolet radiation, excess artificial fertilizer, various pollutants, extreme saline conditions, herbivory and competition. Genetic factors that contribute to increased asymmetry in plants include homozygosity, hybridization, mutation and quantitative genetic differences among individuals. Insect preferences for symmetric flowers increase reproductive success of both pollen donors and recipients by affecting seed set and embryo abortion. The ability of insects to discriminate between flowers of superior and inferior quality is hypothesized to depend on the level of developmental instability of the perceptive apparatus of insects. Hence, asymmetry of insects may have consequences for plant reproductive success and mating patterns.

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#### **Occurrence of Phorid Fly (Diptera: Phoridae) Parasitoids of Imported Fire Ants (Hymenoptera: Formicidae) in Georgia**

Gardner, WA; Peeler, HB; Diffie, SK. 2013

Ten releases of *Pseudacteon* decapitating flies (Diptera: Phoridae), a classical biological control agent of imported fire ants (Hymenoptera: Formicidae) in the southern United States, were made at 8 different sites in Georgia (USA) over a period spanning 2000 - 2012. Intensive monitoring and survey activities were initiated in 2008 to delineate the dispersal and occurrence of the *Pseudacteon* spp. in the state. Results show that all 159 counties in Georgia have at least 1 species of *Pseudacteon*; *P. curvatus* Borgmeier is established in 129 of those counties, *P. tricuspidatus* Borgmeier is established in 70 counties, and *P. obtusus* Borgmeier is established in the county in which it was released. Both *P. curvatus* and *P. tricuspidatus* occur together in 39 counties, and all 3 of the *Pseudacteon* species occur in the 1 county in which all 3 species were released. These results help target supplemental or additional releases of *P. tricuspidatus*, *P. curvatus*, and *P. obtusus* as well as releases of other candidate *Pseudacteon* species.

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## Gene copy number and differential gene expression in haploid and diploid males of the stingless bee, *Melipona quadrifasciata*

Borges, AA; Humann, FC; Tavares, MG; Campos, LAO; Hartfelder, K. 2012

Complementary sex determination in Hymenoptera implies that heterozygosity at the sex locus leads to the development of diploid females, whereas hemizygosity results in haploid males. Diploid males can arise through inbreeding. In social species, these pose a double burden on colony fitness, from significant reduction in its worker force and through being less viable and fertile than haploid males. Apart from being "misfits", diploid males are of interest to assess molecular correlates for possibly ploidy-related bionomic differences. Herein, we generated suppression subtractive cDNA libraries from newly emerged haploid and diploid males of the stingless bee *Melipona quadrifasciata* to enrich for differentially expressed genes. Gene Ontology classification revealed that in haploid males more DEGs were related to stress responsiveness, biosynthetic processes, reproductive processes and spermatogenesis, whereas in diploid ones differentially expressed genes were associated with cellular organization, nervous system development and amino acid transport were prevalent. Furthermore, both libraries contained over 40 % ESTs representing possibly novel transcripts. Quantitative RT-PCR analyses confirmed the differential expression of a representative DEG set in newly emerged males. Several muscle formation and energy metabolism-related genes were under-expressed in diploid males. On including 5-day-old males in the analysis, changes in transcript abundance during sexual maturation were revealed.

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## Two distinct populations of cavity-nesting honey bees (Hymenoptera: Apidae) in South Sulawesi, Indonesia

Hadisoesoilo, S; Otis, GW; Meixner, M. 1995

Two distinct cavity-nesting morphs of bees were collected in South Sulawesi, Indonesia. Morphometric analyses indicate that the smaller, darker morph corresponds to *Apis cerana*. The other morph is larger, with yellowish clypeus and legs. Discriminant analysis of the morphometric data yields an ellipse for this second morph that is distinct at the 95% confidence interval from those for the other recognized Asian cavity-nesting honey bees, *A. cerana* and *A. koschevnikovi*. Preliminary evidence indicates that the two morphs are largely allopatric in South Sulawesi, but they are sometimes found in close proximity (e.g., 12 km). If subsequent study demonstrates little or no hybridization between the two forms, the larger "yellow" morph should be referred to as *Apis nigrocincta* F. Smith, 1861, based on the similarity to the type specimen collected by A. R. Wallace in Sulawesi.

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## Phylogenetics and genetic diversity of the *Cotesia flavipes* complex of parasitoid wasps (Hymenoptera: Braconidae), biological control agents of lepidopteran stemborers

Muirhead, KA; Murphy, NP; Sallam, N; Donnellan, SC; Austin, AD. 2012

The *Cotesia flavipes* complex of parasitoid wasps (Hymenoptera: Braconidae) are economically important for the biological control of lepidopteran stem-boring pests associated with gramineous crops. Some members of the complex successfully parasitize numerous stem-borer pest species, however certain geographic populations have demonstrated variation in the range of hosts that they parasitize. In addition, the morphology of the complex is highly conserved and considerable confusion surrounds the identity of species and host-associated biotypes. We generated nucleotide sequence data for two mtDNA genes (COI, 16S) and three anonymous nuclear loci (CfBN, CfCN, CfEN) for the *C. flavipes* complex. To analyze genetic variation and relationships among populations we used (1) concatenated mtDNA and nDNA data, (2) a nDNA multilocus network approach, and (3) two species tree inference methods, i.e. Bayesian estimation of species trees (BEST) and Bayesian inference of species trees from multilocus data with \*BEAST. All phylogenetic analyses provide strong support for monophyly of the complex and the presence of at least four species, *C. chilonis* (from China and Japan), *C. sesamiae* (from Africa), *C. flavipes* (originating from the Indo-Asia region but introduced into Africa and the New World), and *C. nonagriæ* (from Australia and Papua New Guinea). Haplotype diversity of geographic populations relates to historical biogeographic barriers and biological control introductions, and reflects previous reports of ecological variation in these species. Strong discordance was found between the mitochondrial and nuclear markers in the Papua New Guinea haplotypes, which may be an outcome of hybridization and introgression of *C. flavipes* and *C. nonagriæ*. The position of *Cotesia flavipes* from Japan was not well supported in any analysis and was the sister taxon to *C. nonagriæ* (mtDNA, BEAST), *C. flavipes* (nDNA) or *C. flavipes* + *C. nonagriæ* (BEST) and, may represent a cryptic species. The concatenated five gene phylogenetic analyses did not support the overall separation and monophyly of clades associated with different host species, although some clades did show specific host associations, possibly due to localized host availability, rather than host specificity. Our results provide a framework for assessing whether distinct lineages represent cryptic species, and for examining parasitoid-host evolution and compatibility more generally. Given the limitations of morphological based identification for members of this complex, molecular identification is recommended prior to any biological control introductions. (C) 2012 Elsevier Inc. All rights reserved.

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

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

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

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## Characterization of a highly conserved satellite DNA from the parasitoid wasp *Trichogramma brassicae*

Landais, I; Chavigny, P; Castagnone, C; Pizzol, J; Abad, P; Vanlerberghe-Masutti, F. 2000

An EcoRI satellite DNA has been isolated, cloned and sequenced from *Trichogramma brassicae*, a minute parasitic wasp. This repeated family represents 16% of the genome. The monomer is 385 base pairs (bp) long and has an A+T content of 64.5%. The average nucleotide sequence variability among 12 randomly chosen monomers is extremely low (0.5%), suggesting that the amplification of the monomer into a high-copy-number family occurred recently. An EcoRI satellite DNA probe has been developed and used, at high stringency, as an identification tool to unambiguously discriminate *T. brassicae* from nine other *Trichogramma* species. However, at a lower stringency, a hybridization signal can be detected in two closely related *Trichogramma* species, and, using PCR assay, the presence of the *T. brassicae* EcoRI monomer has been detected in several other species of *Trichogramma*. These results argue in favor of the 'library' model of satellite DNA evolution that predicts that related species share a number of low-copy satellite sequences, some of which could be amplified into a major satellite family in each of the species. Furthermore, this *T. brassicae* EcoRI satellite DNA sequence exhibits particular internal features such as a long inverted repeat that can form a dyad structure. Such sequence motifs seem to be a common characteristic of satellite DNAs, suggesting that they could result from selective forces acting on repetitive DNA. (C) 2000 Elsevier Science B.V. All rights reserved.

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**A molecular marker distinguishes the subspecies *Melipona quadrifasciata quadrifasciata* and *Melipona quadrifasciata anthidioides* (Hymenoptera : Apidae, Meliponinae)**

Waldschmidt, AM; de Barros, EG; Campos, LAO. 2000

The stingless bee species *Melipona quadrifasciata* includes two subspecies, *Melipona quadrifasciata anthidioides* and *Melipona quadrifasciata quadrifasciata*. The morphological difference between the two subspecies is the presence of three to five continuous yellow stripes on the terga on the 3rd to 6th segments in workers and males of *M. q. quadrifasciata*, and two to five interrupted bands in *M. q. anthidioides*. We identified a DNA marker which is present in *M. q. quadrifasciata* and absent in *M. q. anthidioides*. Only one among the *M. q. quadrifasciata* colonies did not present the marker. It was also absent in bees collected in northern Minas Gerais State (Brazil), despite their morphological resemblance to *M. q. quadrifasciata*. The marker can be used for studying the genetic structure of the hybridization zone formed by the intercrossing of the two subspecies.

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***Glycaspis brimblecombei* (Hemiptera: Psyllidae) attack patterns on different *Eucalyptus* genotypes**

Tuller, J; Oliveira, KN; Silva, JO; de Faria, ML; do Espirito-Santo, MM; Serrao, JE; Zanuncio, JC. 2017

Background. The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), an eucalypt insect pest from Australia, was reported in Brazil in 2003. This study evaluated damage patterns of this pest on *Eucalyptus camaldulensis* Dehn (Myrtaceae) and its hybrids *E. urophylla* X *E. camaldulensis* (urocam) and *E. urophylla* X *E. grandis* (urograndis). In addition, parasitism rates of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) on *G. brimblecombei* collected on different eucalypt genotypes are reported. Methods. Plantation plots of three eucalypt genotypes were evaluated over one year. The eucalypt leaves were collected and examined for attack by *G. brimblecombei*. Nymph parasitism of *G. brimblecombei* by *P. bliteus* was recorded. Results. Damage by *G. brimblecombei* was lower on the hybrid genotypes and on the adaxial surface of the eucalypt leaves. *G. brimblecombei* egg and nymph density were negatively correlated with monthly rainfall. Nymph parasitism of *G. brimblecombei* by *P. bliteus* was low (2.9%) independent of genotype and did not vary throughout the year. Discussion. Our data indicate the use of less susceptible eucalypt genotypes (e. g., hybrids) as an alternative to *G. brimblecombei* management. Because of the current low mortality rates for *G. brimblecombei* resulting from *P. bliteus* parasitism, biological control with this natural enemy is not recommended as a management strategy for *G. brimblecombei*.

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**A preliminary bibliographic survey of the insects found in poultry houses from the Neotropical Region, with remarks on selected taxa shared with native birds' nests**

Di Iorio, O; Turienzo, P. 2011

Species of insects associated to the habitat of *Gallus gallus* (Aves: Phasianidae) in the Neotropical Region belong to 144 identified species (42 Coleoptera; 14 Diptera; 17 Hymenoptera; 9 Siphonaptera; 1 Lepidoptera; 56 Hemiptera (one hybrid); 5 Dermaptera); 33 identified to genus (21 Coleoptera; 3 Diptera; 7 Hymenoptera; 1 Hemiptera; 1 Blattaria); 37 identified to family (23 Coleoptera; 9 Diptera; 2 Hymenoptera; 1 Lepidoptera; 2 Hemiptera); and 6 to order (2 Coleoptera; 1 Hymenoptera; 1 Siphonaptera; 1 Psocoptera; 1 Dermaptera). Most of the insects are haematophagous ectoparasites (Hemiptera; Siphonaptera; Diptera), detritivores (Coleoptera; Lepidoptera; Diptera; Blattaria), predators (Coleoptera; Diptera; Hemiptera; Dermaptera; Hymenoptera), and parasitoids (Hymenoptera). A total of 46 native American species and/or subspecies of Triatominae (Hemiptera: Reduviidae) were found in chicken houses, from which 18 were also found in birds' nests. It was recently observed that other insects from poultry houses, generally exotic species, had colonized native American birds' nests. Comments and remarks of selected taxa also found in birds' nests from Argentina and other countries are provided.

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**Modern Maize Hybrids Have Lost Volatile Bottom-Up and Top-Down Control of *Dalbulus maidis*, a Specialist Herbivore**

Coll-Araoz, MV; Hill, JG; Luft-Albarracin, E; Virla, EG; Fernandez, PC. 2020

Following damage by herbivores, many plants release volatiles that dissuade future conspecifics from feeding. In many crop plants however, induced volatiles mediating this kind of interactions among plants, herbivores and also their natural enemies have been altered through the process of domestication. The selection of crops for increased yield may have gone at a cost of defense, possibly including defense-related volatiles. *Dalbulus maidis* (Hemiptera: Cicadellidae), a specialist leafhopper that only feeds on Zeaspp., is a vector of Corn Stunt Spiroplasma, a serious maize disease. Here, we compared the volatiles released following *D. maidis* attack by a maize landrace and two maize hybrids of temperate and tropical background. Also, we performed behavioral assays with the leafhopper contrasting healthy non-attacked maize seedlings versus attacked seedlings. The maize landrace produced more than 6-fold larger quantities of induced volatiles compared to the maize hybrids after herbivory. Corn leafhopper females were able to detect and significantly preferred the odors of healthy seedlings over the attacked ones only in the landrace. They did not discriminate between the attacked and non-attacked hybrids. Additionally, we found that the attraction of the parasitoid wasp *Anagrus virlai* (Hymenoptera: Mymaridae) to its host was diminished in the tested hybrids. The parasitoid was able to detect the odors of the attacked landrace, however it was unable to discriminate between healthy and attacked maize hybrid plants. These results suggest that those more domesticated germplasms may have lost the ability not only to release volatiles that avoid colonization of future herbivores, but also to attract their natural enemies in a tritrophic system.

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**The introduced English wasp *Vespula vulgaris* (L.) (Hymenoptera : Vespidae) newly recorded invading native forests in Tasmania**

Matthews, RW; Goodisman, MAD; Austin, AD; Bashford, R. 2000

The social wasp *Vespula vulgaris* (L.), an introduced species that has caused extreme ecological damage in New Zealand, is reported from southern Tasmanian forests for the first time. In mainland Australia, this wasp has been present in the Melbourne area since 1958 and our retrospective analysis places it in Hobart since 1995. In the present paper, we document *V. vulgaris* in natural areas in southern Tasmania, well away from human habitation. Malaise trap samples collected since 1997 from nine sites at the Warra Long-term Ecological Research area in southern Tasmania revealed the widespread presence of both *V. vulgaris* and *V. germanica* (F.), another introduced species of concern. Analysis of microsatellite DNA markers showed no evidence of hybridisation between the two species. The potential impact of this newest social insect threat to Australian native biota is discussed.

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***Sergey* gen. n., a new doryctine genus from temperate forests of Mexico and Cuba (Hymenoptera, Braconidae)**

Martinez, JJ; Lazaro, RNM; Pedraza-Lara, C; Zaldivar-Riveron, A. 2016

The new doryctine genus *Sergey* gen. n. is described with four new species (*S. cubaensis* Zaldivar-Riveron & Martinez, sp. n., *S. coahuilensis* Zaldivar-Riveron & Martinez, sp. n., *S. tzeltal* Martinez & Zaldivar-Riveron, sp. n., *S. tzotzil* Martinez & Zaldivar-Riveron, sp. n.) from temperate forests of Mexico and Cuba. Similar to many other doryctine taxa, the new genus has a considerably elongated, petiolate basal sternal plate of the first metasomal tergite, although it can be distinguished from these by having the mesoscutum sharply declivous anteriorly with sharp anterolateral edges. The described species have been characterised molecularly based on two mitochondrial (COI, cyt b) and one nuclear (28S) gene markers. Based on the mitochondrial gene genealogies reconstructed, the evidence suggests the

existence of incomplete lineage sorting or hybridization in the populations from Chiapas and Oaxaca assigned to *S. tzeltal* sp. n.

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#### **Parasitoid-host matching between the little decapitating fly *Pseudacteon curvatus* from Las Flores, Argentina and the black fire ant *Solenopsis richteri***

Porter, SD; Briano, JA. 2000

Matching biotypes of potential biocontrol agents to target host populations can greatly improve the effectiveness of control. This study was designed to determine if the fly *Pseudacteon curvatus* Borgmeier from Las Flores, Buenos Aires Province, Argentina prefers its natural host, the black fire ant, *Solenopsis richteri* Forel. We found that *P. curvatus* strongly preferred *S. richteri* from Argentina, *S. richteri* from the United States, and hybrid (*S. richteri* x *S. invicta*) fire ants from the United States when each was tested against *S. invicta* from the United States. The time to pupation of developing parasitoids was 10% and 21% longer in hybrid and red fire ants than in black fire ants. Parasitism rates, however, were not significantly different among these ant hosts in no-choice parasitism tests.

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#### **Sugarcane Aphid Population Growth, Plant Injury, and Natural Enemies on Selected Grain Sorghum Hybrids in Texas and Louisiana**

Brewer, MJ; Gordy, JW; Kerns, DL; Woolley, JB; Rooney, WL; Bowling, RD. 2017

In response to the 2013 outbreak of sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), on sorghum, *Sorghum bicolor* (L.), in North America, experiments were conducted at three southern U.S. grain sorghum production locations (Corpus Christi, TX; Winnsboro, LA; Rosenberg, TX). The objectives were to authenticate yield decline on susceptible hybrids (2014 and 2015) and to measure aphid population growth and natural enemy prevalence on susceptible and resistant hybrids with similar genetic background (2014). Yield decline on susceptible hybrids (Tx 2752/Tx430 and DKS53-67) was more substantial when aphid population growth accelerated quickly and peaked above 300 aphids per leaf (50 to nearly 100% yield decline). Location and year variation in maximum aphid density and cumulative aphid-days was high, with doubling time values on the susceptible hybrids ranging between 3.9 and 7.9 d. On resistant Tx2752/Tx2783, leaf injury and yield decline were not seen or less severe than on its paired susceptible Tx2752/Tx430. Aphids declined on Tx2752/Tx2783 after initial colony establishment (Corpus Christi) or took about 60% longer to double in population size when compared with Tx2752/Tx430 (Winnsboro). The predominant natural enemy taxa were aphelinid mummies (Hymenoptera: Aphelinidae), ladybird beetles (Coleoptera: Coccinellidae), and sryphid flies (Diptera: Syrphidae), and they were more prevalent during flowering than prior to flowering. They were generally responsive to changes in aphid density of both susceptible and resistant hybrids, but variability points to need for further study. In future research, full season observations should continue as well as more detailed study of potential compatibility of sorghum resistance and biological control.

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#### **Current genetic status of honey bees in Anatolia in terms of thirty polymorphic microsatellite markers**

Karabag, K; Tunca, RI; Tuten, E; Dogaroglu, T. 2020

Turkey, having three phytogeographical floristic regions, is a natural bridge among three continents. A lot of subspecies and ecotypes of honey bees have been reported within Turkey. However, hybridization due to informal cultivation and uncontrolled migratory beekeeping practices are thought to affect the genetic diversity of local honey bee populations, and this may result the loss of allele combinations resulting from long evolutionary processes. Numerous identification and conservation studies on honey bee subspecies have been conducted in many countries to determine the loss of genetic variability. On this basis, genetic causes and phylogenetic relationships of four common honey bee subspecies [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera carnica* Pollmann, 1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] from five provinces (Artvin, Duzce, Hatay, Kirlareli and Muya) selected based on their importance in apicultural activities were studied using 30 microsatellite loci in 2018. The genetic distances of populations ranged from 0.30 to 0.70. Genetic variation was 8.96% among the populations, 44.9% among the individuals within the populations and 46.1% for all individuals. Further genetic researches on the honey bee populations will be of advantage for anticipating potential future problems.

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#### **Isolation and characterization of two families of satellite DNA with repetitive units of 135 bp and 2.5 kb in the ant *Monomorium subopacum* (Hymenoptera, Formicidae)**

Lorite, P; Carrillo, JA; Aguilar, JA; Palomeque, T. 2004

Analyzing the satellite DNA in the ant species *Monomorium subopacum* we found two unrelated families of satellite DNA. Because these satellite DNA families were isolated using the two enzymes *HaeIII* and *EcoRI* we called the two families *HaeIII* and *EcoRI* family, respectively. The *HaeIII* family proved to be organized in a 135-bp basic unit repeat, the *EcoRI* family in a 2.5-kb basic unit repeat. The latter represents perhaps the longest satellite DNA isolated up to now in insects. The *HaeIII* family apparently comprises about 10% of the total genomic DNA whereas the *EcoRI* family represents only about 1-2%. A comparative analysis of the two satellite DNA sequences showed no homology between the two families although both sequences possessed long A and T stretches. Eight of the 34 chromosomes showed hybridization with the *HaeIII* family and hybridization signals are visible in six chromosomes with the *EcoRI* family. Analysis of the electrophoretic mobility of satellite DNA on non-denaturing polyacrylamide showed that the *HaeIII* family is only slightly curved. However, the unit of the *EcoRI* satellite DNA family has curvature, especially the first 1000 bp of the monomeric repeat, in which this DNA is AT rich and has numerous A and T stretches. There are also internal inverted subrepeats in each family. The sequences of satellite DNA families found in *Monomorium subopacum* are different from the sequences of other satellite DNAs cloned in insects, including other species of ants. Copyright (C) 2003 S. Karger AG, Basel.

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#### **GEOGRAPHICAL VARIATION IN HABITAT CHOICE AND HOST SUITABILITY IN THE PARASITOID *ASOBARA-RUFESCENS***

KRAAIJEVELD, AR; VOET, S; VANALPHEN, JJM. 1994

In the Netherlands, *Asobara rufescens* (Forster) (Hymenoptera: Braconidae) is a parasitoid of drosophilid larvae in decaying plant material. In several places in the Mediterranean, parasitoids looking very similar to *A. rufescens* were collected on fermenting substrates a hybridization experiment showed that the parasitoids were indeed *A. rufescens*. In an olfactometer Portuguese *A. rufescens* do not have a preference for either the odour of yeast or decaying leaves, while their Dutch conspecifics prefer the odour of decaying leaves. The survival probability of Portuguese *A. rufescens* in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), a species typical for fermenting substrates, is much higher than the survival probability of Dutch *A. rufescens* in this host species. It is hypothesized that decaying plant material may be unsuitable for drosophilid larvae during part of the year in the Mediterranean, forcing *A. rufescens* there to broaden its microhabitat choice. The use of fermenting substrates brings *A. rufescens* in contact with its sibling *A. tabida* Nees, a species typical for fermenting substrates in most of Europe. Portuguese *A. rufescens* appear to be genetically isolated from *A. tabida*. In the Netherlands, where the two species occupy different microhabitats, there is only a premating barrier.

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#### **No evidence of queen thelytoky following interspecific crosses of the honey bees *Apis cerana* and *Apis mellifera***

Gloag, R; Tan, K; Wang, Y; Song, W; Luo, W; Buchman, G; Beekman, M; Oldroyd, BP. 2017

The human-mediated dispersal of species over geographical boundaries can bring previously isolated sister taxa into contact. Interspecific mating between closely related species may then occur, with various outcomes ranging from hybridization to reproductive interference. In the case of the Eastern honey bee *Apis cerana* and the Western honey bee *Apis mellifera*, an additional possible reproductive outcome has been posited: interspecific sperm triggers queens to produce daughters from unfertilized eggs via thelytokous parthenogenesis. Such an outcome would go unnoticed in natural population mergers, as queens, which are polyandrous, are likely to mate with both conspecific and interspecific males. We performed reciprocal crosses between *A. mellifera* and *A. cerana* via artificial insemination, plus control inseminations of saline (five queens per species per treatment), and genetically assessed the sex and origin of any resulting offspring. Neither *A. cerana* nor *A. mellifera* queens produced viable female brood after receiving interspecific semen, indicating a high cost of interspecific mating for both species. In two *A. cerana* colonies headed by cross-inseminated queens, workers responded by activating ovaries and laying eggs that were mainly male but occasionally female (i.e. thelytokous, 2% of brood), despite the queen's continued presence in the nest. We conclude that thelytoky is not a consistent response to interspecific mating by queens of *A. mellifera* or *A. cerana*. Rather, at least in *A. cerana*, when colonies are faced with "mis-mated" queens, it may be up to the workers to secure the reproductive future of the colony.

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#### Foraging behaviour of honeybees (*Apis* spp.) (Hymenoptera: Apidae) in hybrid seed production of Indian mustard (*Brassica juncea*)

Maity, A; Chakrabarty, SK; Yadav, JB. 2014

Pollination of entomophilous crops by honeybees is considered as one of the effective and inexpensive method for improving the crop yield and its quality. In most of the crops, effective pollination is prerequisite for fruitful fertilisation. Hence, we investigated the differential foraging activity of honeybees in relation to aspects of crop management including sowing time and parental row ratio in the hybrid seed production of the first Indian mustard hybrid NRCHB 506. *Apis* spp. (half of which being *Apis dorsata*) was the dominant pollinators in our study. The pollinator abundance was maximum in the forenoon in male line (19.1) and in the afternoon in female line (13.9). Pollen collectors (8.7) outnumbered the nectar collectors (7.2) in male line and the reverse in female line. Pollinator abundance decreased in rows distantly positioned from the male parent row. Air temperature was positively correlated ( $r = 0.631$ ) and relative humidity negatively correlated ( $r = -0.736$ ) with honeybee foraging. Warmer weather conditions during flowering period showed higher number of honeybee visiting the hybrid seed production plots. This study would help the seed producers to standardise the planting geometry and date of sowing to promote maximum pollinator abundance at peak flowering period resulting in higher hybrid seed yield.

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#### Distribution and rapid range expansion of the introduced willow sawfly *Nematus oligospilus* Forster (Hymenoptera: Tenthredinidae) in Australasia

Caron, V; Ede, F; Sunnucks, P; O'Dowd, DJ. 2014

The willow sawfly, *Nematus oligospilus* Forster (Hymenoptera: Tenthredinidae), has been introduced inadvertently across temperate regions in the Southern Hemisphere, including New Zealand and Australia, where it has dispersed extremely rapidly. A host specialist herbivore, at high population densities it can defoliate and damage introduced willows (*Salix* spp.), many species of which are invasive weeds in Australasia. In this study, we show that in just 10 years, the distribution of *N. oligospilus* has expanded across most of south-eastern Australia and south-western Western Australia and, in less than 15 years across both the North and South Islands of New Zealand. The relative population density of *N. oligospilus* varied widely within both geographic regions. The distribution of *N. oligospilus* was still expanding in Australia where high densities resulted in widespread willow defoliation. In contrast, the distribution of the willow sawfly had reached fully across both the North and South Islands in New Zealand, but its density had declined from the early stage of invasion. All willow taxa surveyed were utilized by the willow sawfly, but host preferences were apparent, especially for the *S. fragilis*/*S. x rubens* hybrid complex. Many factors, including natural enemies, willow range expansion and environmental conditions are likely to affect the long-term population dynamics and spread of *N. oligospilus* in Australia.

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#### Genetic divergence between the sympatric queen morphs of the ant *Myrmica rubra*

Leppanen, J; Seppa, P; Vepsalainen, K; Savolainen, R. 2015

Pairs of obligate social parasites and their hosts, where some of the parasites have recently diverged from their host through intraspecific social parasitism, provide intriguing systems for studying the modes and processes of speciation. Such speciation, probably in sympatry, has also been propounded in the ant *Myrmica rubra* and its intraspecific social parasite. In this species, parasitism is associated with queen size dimorphism, and the small microgyne has become a social parasite of the large macrogyne. Here, we investigated the genetic divergence of the host and the parasite queen morphs in 11 localities in southern Finland, using nuclear and mitochondrial markers of queens and workers. We formulated and tested four speciation-related hypotheses that differed in the degree of genetic divergence between the morphs. The queen morphs were genetically distinct from each other with little hybridization. In the nuclear data, when localities were nested within queen morphs in the hierarchical amova, 39% of the genetic variation was explained by the queen morph (standardized  $F(CT)=0.63$ , uncorrected  $F-CT=0.39$ ), whereas 18% was explained by the locality ( $F(SC)=0.39$ ,  $F-SC=0.29$ ). This result corroborated the hypothesis of advanced sympatric speciation. In contrast, the mitochondrial DNA could not settle between the hierarchical levels of locality and queen morph, thus substantiating equally the hypotheses of incipient and advanced sympatric speciation. Together, our results support the view that the microgynous parasite has genetically diverged from its macrogynous host to the level of a nascent species.

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#### Survival of imported fire ant (Hymenoptera : Formicidae) species subjected to freezing and near-freezing temperatures

James, SS; Pereira, RM; Vail, KM; Ownley, BH. 2002

Survival at low temperatures is an important parameter determining distribution of imported fire ants in the United States. Supercooling points and survival at low temperatures, and the effects of species, individual size, and *Thelohania solenopsae* Knell, Allen & Hazard (Microsporida: Thelohaniidae) infection on these parameters, were examined. We tested *Solenopsis richteri* Forel, *S. richteri* X *invicta* hybrid, and *Solenopsis invicta* Buren. Great variation was observed in the supercooling points, which are not an appropriate measure of cold hardiness for imported fire ants. When exposed to near-freezing temperatures above their supercooling points, fire ants died at different rates depending on the species and *T. solenopsae*-infection status. Extended exposure to 4degreesC resulted in both the hybrid and *S. invicta* infected with *T. solenopsae* having significantly lower mortality rates than either the *S. richteri* or the uninfected *S. invicta*. At 0.5degreesC, the hybrids had significantly lower mortality than the uninfected *S. invicta*, but mortalities for *S. richteri* and *T. solenopsae*-infected *S. invicta* were not significantly different from each other or the hybrid. Ant mortality was 100% for all ant types after 7 d at -4degreesC. The uninfected *S. invicta* was consistently less cold-tolerant than the other ant types. The hybrid fire ants and the *T. solenopsae*-infected *S. invicta* had the lowest mortalities. These results support the hypothesis that extended cold injury causes winter kill of fire ants, and may partially explain the distribution of fire ant species in the United States.

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

Radloff, SE; Hepburn, HR. 1999

Multivariate morphometric analyses were performed on measurements of nine characteristics of 4011 worker bees from natural populations of the Drakensberg mountains and surrounding areas in southern Africa between 28 degrees and 34 degrees S latitude and 25 degrees and 31 degrees E longitude. A discriminant function analysis yielded three morphoclusters: group 1 comprising an unnamed population at higher than 1500 m altitude in the mountains; group 2 consisting of



bees considered to be *A. m. scutellata* x *A. capensis* hybrids, and group 3 consisting of *A. m. scutellata* surrounding the mountains at lower (less than 1500 m) altitudes. The bees of group 1 show size similarities to disjunct populations of other large Afromontane bees, *A. m. monticola*. Group 2 hybrid bees occur in an area of marked climatic variability and manifest significantly high variances in their morphometrics, pheromones and DNA characteristics. It is suggested that differences in swarming seasons among these populations effectively cause temporal reproductive isolation between the group 1 mountain bees and those *A. m. scutellata* below the escarpment.

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#### First report of *Bombus terrestris xanthopus* Kriechbaumer on the Italian peninsula (Hymenoptera: Apidae)

Quaranta, M; Felicioli, A. 2012

A male specimen of *Bombus terrestris* (L. 1758) showing an intermediate colour between the subspecies *xanthopus* Kriechbaumer 1870 endemic to Corsica and the subspecies *terrestris* ubiquitous in the mainland was found within samples collected in 1999 on the Tuscan coast. Hybrid specimens between these subspecies are common on some of the islands of the Tuscan archipelago, while others only have pure populations. The finding is significant as it could signal the spread of the subspecies *xanthopus* to the mainland. We consider and discuss the circumstances of the finding.

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#### Phorid flies in Alabama: A tale of two species

Graham, LCF; Porter, SD; Bertagnolli, VE. 2003

Two species of phorid fly have been released at 11 sites in Alabama and have been recovered from 9 sites. *Pseudacteon tricuspidatus* Borgmeier (Diptera: Phoridae) was released in South Alabama in populations of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), and *Pseudacteon curvatus* Borgmeier was released in North Alabama in hybrid fire ant populations (*Solenopsis invicta* x *Solenopsis richteri* Forel). The number of mounds per hectare and mound size was recorded for all release sites and for control sites, if established. Data from the three oldest sites are presented. Mound numbers decreased at two release sites, but increased at the third. Further analysis and data collection are needed to determine the long-term effect of phorid flies on Alabama fire ants.

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#### Susceptibility of eucalyptus species and hybrids to the gall wasp *Leptocybe invasa* (Hymenoptera: Eulophidae) in northern Misiones, Argentina

Eskiviski, ER; Schapovaloff, ME; Dummel, DM; Fernandez, MM; Aguirre, FL. 2018

Aim of study: To analyze the susceptibility of *Eucalyptus* and hybrids species to *Leptocybe invasa* through field assays. Area of study: The north of the Argentine province of Misiones (Colonia Delicia). Material and methods: A total of 11 *Eucalyptus* species and 2 hybrids were surveyed for damage and severity of *L. invasa* infestation. Six evaluations were made during an annual period. Main results: The susceptibility ranking to *L. invasa* from highest to lowest was *E. tereticornis* > *E. propinqua* > *E. dunni* > *E. camaldulensis* > *E. grandis* > *E. major* > *E. longistrata* > *E. grandis* x *E. camaldulensis*. However, *E. moluccana*, *E. urophylla* x *E. grandis* and *E. urophylla* were tolerant to *L. invasa*. Research highlights: This study suggests that in Misiones, *E. tereticornis* is the most sensible eucalyptus species to gall wasp attack, whereas other species and hybrids presented low damage levels or tolerance to *L. invasa* region.

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#### Deceptive strategy in *Dactylorhiza* orchids: multidirectional evolution of floral chemistry

Wroblewska, A; Szczepaniak, L; Bajguz, A; Jedrzejczyk, I; Talalaj, I; Ostrowiecka, B; Brzozko, E; Jermakowicz, E; Mirski, P. 2019

Background and Aims The deception strategies of orchids remain poorly understood, especially in regard to the chemical compounds emitted from their flowers and their interaction with various taxonomic groups of pollinators. We investigated the phylogenetic relationships and compared the variation of floral chemical compounds between food-deceptive *Dactylorhiza* taxa (*D. incarnata* var. *incarnata* and *D. incarnata* var. *ochroleuca*, *D. fuchsii* and *D. majalis*) from populations in north-eastern Poland. We propose a model of the evolution of deception based on floral chemical signals in this genus. Methods A Bayesian approach based on polymorphic plastid DNA (trnL, trnF and psbC-trnK), internal transcribed spacer (ITS) sequences and flow cytometry data was applied to confirm the taxonomic status of the studied orchids. We also identified and classified the pollinators and flower visitors in each *Dactylorhiza* population to the taxonomic level and compared our results with literature data. The chemical composition of pentane and diethyl ether extracts from the flowers was analysed by gas chromatography-mass spectrometry. Variation of the floral chemical components was visualized by non-metric multidimensional scaling based on Bray-Curtis dissimilarity. Key Results The genetic distinctiveness of *D. incarnata*, *D. fuchsii* and *D. majalis* was confirmed. No hybrids between them were found, but the chloroplast DNA (cpDNA), ITS haplotypes and flow cytometry showed genetic similarity between *D. incarnata* var. *incarnata* and *D. incarnata* var. *ochroleuca*. We determined that *Apis mellifera* (Hymenoptera) was the only shared pollinator of these taxa. *Strangalia attenuata* and *Alosterna tabacicolor* (Coleoptera) and *Volucella pellucens* and *V. bombylans* (Hymenoptera) were observed pollinating *D. fuchsii*. Visualization of the emission rates of the 61 floral chemical compounds detected from pentane extracts (mainly hydrocarbons and aldehydes) and the 51 from diethyl extracts (with abundant groups of benzenoids and non-aromatic acids) strongly differentiated *D. incarnata*, *D. fuchsii* and *D. majalis*, while those of the two varieties of *D. incarnata* (var. *incarnata* and var. *ochroleuca*) were almost identical. Conclusions While the genetic data clearly supported the distinct lineages of *D. incarnata*, *D. fuchsii* and *D. majalis*, the patterns of emission of their flower chemical compounds were more complex within the series of shared compounds (alkanes and aldehydes) and taxon-specific compounds (benzenoids and esters). Their floral bouquet can influence the sexual, social and feeding behaviour of pollinators in different ways. We observed that the floral chemical compounds attracted both shared and species-specific pollinators to *Dactylorhiza*, confirming the multidirectional character of floral chemical signals in these food-deceptive taxa. Reduction of species-specific pollination levels in *Dactylorhiza* orchid taxa may promote hybridization between them.

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#### A gene encoding a polydnavirus structural polypeptide is not encapsidated

Deng, LQ; Stoltz, DB; Webb, BA. 2000

Polydnaviruses are symbiotic viruses associated with some parasitic Hymenoptera that are vertically transmitted as proviruses within wasp genomes. To study this symbiotic association a gene encoding an abundant *Campoletis sonorensis* polydnavirus Virion protein was characterized. This gene is not encapsidated but resides in the wasp genome where it is expressed only during virus replication. Immunolocalization studies detected the encoded 44-kDa protein only in oviduct tissue with ultrastructural studies detecting epitopes between or on virion envelopes. Expression and localization of the 44-kDa protein are consistent with its being a viral structural protein but localization of the gene only within the wasp genome is atypical, raising the possibility that this protein is adventitiously packaged during virion assembly. To address this possibility, quantitative dot blot and genomic Southern blot hybridizations were performed to determine whether the copy number of the p44 gene increased disproportionately during replication, as would be expected for a gene encoding a virion protein. The copy number of the p44 gene increases in tissues supporting virus replication but is unchanged in other tissues, suggesting that this gene is amplified in replicative cells. The data indicate that genes encoding polydnavirus virion proteins may be distributed between wasp and encapsidated viral genomes. (C) 2000 Academic Press.

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#### Differences in reproductive potential of two populations of *Catolaccus grandis* (Hymenoptera : Pteromalidae) and their hybrids

Morales-Ramos, JA; Rojas, MG; King, EG. 2000

A new colony of the boll weevil ectoparasitoid *Catolaccus grandis* was introduced from Guasave, Sinaloa, Mexico to improve vigor of a 12-year-old laboratory reared stock in Weslaco, Texas. The biological characteristics of the introduced colony were compared to those of the Weslaco colony and a crossbreed of these 2 colonies. Developmental time was not significantly different among the 3 colonies, but the preovipositional period of the Sinaloa females was 3 times as long compared to the other 2 colonies. The fecundity, net reproductive rate ( $R_0$ ), and intrinsic rate of increase ( $r(m)$ ) of females from Sinaloa were significantly lower than those of females from Weslaco and the hybrid colony. Generation time (G) and doubling time (DT) were significantly longer in the Sinaloa colony. These characteristics make the Sinaloa population less desirable for mass propagation and release to control boll weevil populations than the Weslaco colony. The biological and population parameters of the hybrid colony were not significantly different from those of the Weslaco population. The implications of the observed results on the mass propagation and release strategies against the boll weevil are discussed and recommendations are presented.

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#### Cell Position During Larval Development Affects Postdiapause Development in *Megachile rotundata* (Hymenoptera: Megachilidae)

Yocum, GD; Rinehart, JP; Kemp, WP. 2014

*Megachile rotundata* (F.) (Hymenoptera: Megachilidae) is the primary pollinator of alfalfa in the northwestern United States and western Canada and provides pollination services for onion, carrot, hybrid canola, various legumes, and other specialty crops. *M. rotundata* females are gregarious, nest in cavities either naturally occurring or in artificial nesting blocks, where they construct a linear series of brood cells. Because of the physical layout of the nest, the age of the larvae within the nest and the microenvironment the individual larvae experience will vary. These interacting factors along with other maternal inputs affect the resulting phenotypes of the nest mates. To further our understanding of in-nest physiology, gender and developmental rates were examined in relationship to cell position within the nest. Eighty-two percent of the females were located within the first three cells, those furthest from the nest entrance. For those individuals developing in cells located in the deepest half of the nest, the sex of the previous bee had a significant effect on the female decision of the gender of the following nest mate. Removing the prepupae from the nest and rearing them under identical conditions demonstrated that position within the nest during larval development had a significant effect on the postdiapause developmental rates, with males whose larval development occurred deeper in the nest developing more slowly than those toward the entrance. No positional effect on postdiapause developmental rates was noted for the females. The cell position effect on male postdiapause developmental rate demonstrates that postdiapause development is not a rigid physiological mechanism uniform in all individuals, but is a dynamic plastic process shaped by past environmental conditions.

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#### Revision of the *Camponotus fulvopilosus* (De Geer) species-group (Hymenoptera: Formicidae)

Robertson, HG; Zachariades, C. 1997

The *Camponotus fulvopilosus* species-group comprises four species: *C. fulvopilosus* (De Geer) (arid and open savanna areas of southern Africa), *C. brevisetosus* Forel (granite outcrops on the Drakensberg escarpment and its foothills), *C. detritus* Emery (dunes of the Namib Desert) and *C. storeatus* Forel (southern Cape). A key to major workers and queens is provided, redescription is provided for major workers, and the biology of each species is reviewed. *Camponotus fulvopilosus* comprises three morphological forms that are parapatrically distributed with discernible hybrid zones. The evolution of the four species is discussed on the basis of their biogeography.

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#### PATTERNS OF HERBIVORY IN THE *QUERCUS-GRISEA* X *QUERCUS-GAMBELII* SPECIES COMPLEX

AGUILAR, JM; BOECKLEN, WJ. 1992

In a recent paper, Whitham proposed the hybrids-as-sinks hypothesis, which posits that hybrid hosts are more susceptible to phytophagous insects than are parental hosts. We test the hybrids-as sinks hypothesis in the *Quercus grisea* Liebm. X *Quercus gambelii* Nutt. hybrid complex. We measure densities of leaf-mining moths (Lepidoptera: Nepticulidae and Gracillariidae) and gall wasps (Hymenoptera: Cynipidae) on parental and hybrid host trees. We find no support for the hybrids-as-sinks hypothesis in the present hybrid complex. Hosts in this complex exhibited a unidirectional herbivore gradient, where hybrid hosts supported intermediate levels of herbivores when compared to parental species. We discuss possible causes affecting patterns of herbivore densities and distribution in this species complex, including host architecture, leaf morphology, and geographic range.

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#### Morphology and number of Ommatidia in the compound eyes of *Solenopsis invicta*, *Solenopsis richteri*, and their hybrid (Hymenoptera : Formicidae)

Baker, GT; Ma, PWK. 2006

The morphology and number of ommatidia of the compound eyes of *Solenopsis invicta*, *Solenopsis richteri* and their hybrid are investigated. Comparisons are made of the morphology and number of ommatidia in *S. invicta*, *S. richteri* and their hybrid. Major workers have significantly more facets than minor workers, male reproductive more than female reproductive, and the reproductives more than workers. Workers and reproductives have significantly more ommatidia in *S. invicta* than in *S. richteri* and significantly fewer in the hybrid than in either species. The major workers of each species have significantly wider facets than the minor workers, and workers have significantly wider facets than reproductives, which showed no difference in their facet diameter. There are no significant differences when the facet diameter is compared between the members of the castes of different species. These morphological and numerical dissimilarities are discussed in connection with known behavioral differences in the castes. (c) 2006 Elsevier GmbH. All rights reserved.

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#### Assessment of Pollen Diversity Available to Honey Bees (Hymenoptera: Apidae) in Major Cropping Systems During Pollination in the Western United States

Topitzhofer, E; Lucas, H; Chakrabarti, P; Breece, C; Bryant, V; Sagili, RR. 2019

Global western honey bee, *Apis mellifera* (L.) (Hymenoptera: Apidae), colony declines pose a significant threat to food production worldwide. Poor nutrition resulting from habitat loss, extensive monocultures, and agricultural intensification is among the several suggested drivers for colony declines. Pollen is the primary source of protein for honey bees; therefore, both pollen abundance and diversity are critical for colony growth and survival. Many cropping systems that employ honey bee colonies for pollination may lack sufficient pollen diversity and abundance to provide optimal bee nutrition. In this observational study, we documented the diversity and relative abundance of pollen collected by honey bees in five major pollinator-dependent crops in the western United States. We sampled pollen from pollen traps installed on honey bee colonies in the following cropping systems-almond, cherry, highbush blueberry, hybrid carrot, and meadowfoam. The pollen diversity was estimated by documenting the number of different pollen pellet colors and plant taxa found in each pollen sample. The lowest pollen diversity was found in almond crop. Relatively higher quantities of pollen collection were collected in almond, cherry, and meadowfoam cropping systems. The information gleaned from this study regarding pollen diversity and abundance may help growers, land managers, and beekeepers improve pollen forage available to bees in these cropping systems.

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## Environmental and genotypic effects on Russian-hybrid and Italian honey bee (*Apis mellifera*) (Hymenoptera : Apidae) foraging behavior

Kreitlow, KL; Tarpy, DR. 2006

Both environment and genotype have a significant influence on the foraging behavior of honey bees (*Apis mellifera*). We compared the colony-level foraging behaviors of two honey bee stocks at three different sites by sampling 50 foragers each from 16 Russian-hybrid and 16 Italian colonies distributed among the Coastal Plain, Piedmont, and Mountain regions of North Carolina. We detected highly significant differences in foraging behavior among the different sites, but we found very few differences between the stocks. Moreover, there were very few environment-genotype interactions on foraging behavior, suggesting that the effect of genotype does not vary significantly depending on the environment in which it is located. These findings suggest that, while there are some notable differences between Russian-hybrid and Italian honey bees, the environment has a relatively larger impact on honey bee foraging behavior for these two stocks.

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## Native Solitary Bees Provide Economically Significant Pollination Services to Confection Sunflowers (*Helianthus annuus* L.) (Asterales: Asteraceae) Grown Across the Northern Great Plains

Mallinger, RE; Bradshaw, J; Varenhorst, AJ; Prasifka, JR. 2019

The benefits of insect pollination to crop yields depend on genetic and environmental factors including plant self-fertility, pollinator visitation rates, and pollinator efficacy. While many crops benefit from insect pollination, such variation in pollinator benefits across both plant cultivars and growing regions is not well documented. In this study, across three states in the northern Great Plains, United States, from 2016 to 2017, we evaluated the pollinator-mediated yield increases for 10 varieties of confection sunflowers, *Helianthus annuus* L. (Asterales: Asteraceae), a plant that is naturally pollinator-dependent but was bred for self-fertility. We additionally measured pollinator visitation rates and compared per-visit seed set across pollinator taxa in order to determine the most efficacious sunflower pollinators. Across all locations and hybrids, insect pollination increased sunflower yields by 45%, which is a regional economic value of over \$40 million and a national value of over \$56 million. There was, however, some variation in the extent of pollinator benefits across locations and plant genotypes, and such variation was significantly related to pollinator visitation rates, further highlighting the value of pollinators for confection sunflowers. Female *Andrena helianthi* Robertson (Hymenoptera: Andrenidae) and *Melissodes* spp. (Hymenoptera: Apidae) were the most common and effective pollinators, while other bees including managed honey bees (Hymenoptera: Halictidae), *Apis mellifera* L. (Hymenoptera: Apidae), small-bodied sweat bees (Hymenoptera: Halictidae), bumble bees *Bombus* spp. (Hymenoptera: Apidae), and male bees were either infrequent or less effective on a per-visit basis. Our results illustrate that wild bees, in particular the sunflower specialists *A. helianthi* and *Melissodes* spp., provide significant economic benefits to confection sunflower production.

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## The influence of nest site selection on the population dynamics of Africanized honey bees in an urban landscape

Birt, AG; Chen, SHV; Baum, KA; Tchakerian, MD; Coulson, RN. 2017

Urban landscapes provide habitat for many species, including domesticated and feral honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). With recent losses of managed honey bee colonies, there is increasing interest in feral honey bee colonies and their potential contribution to pollination services in agricultural, natural, and urban settings. However, in some regions the feral honey bee population consists primarily of Africanized honey bees. Africanized honey bees (AHB) are hybrids between European honey bees and the African honey bee, *Apis mellifera scutellata* Lepeletier, and have generated economic, ecological, and human health concerns because of their aggressive behavior. In this study, we used two long-term datasets (7-10 years) detailing the spatial and temporal distribution of AHB colonies in Tucson, AZ, USA, where feral colonies occupy a variety of cavities including water meter boxes. A stage-structured matrix model was used to elucidate the implications of nest site selection and the effects of colony terminations on the structure and dynamics of the AHB population. Our results suggest that Tucson's AHB population is driven by a relatively small number of source colonies that escape termination (ca. 0.165 colonies per km<sup>2</sup> or 125 colonies in total), although immigrating swarms and absconding colonies from the surrounding area may have also contributed to the stability of the Tucson AHB population. Furthermore, the structure of the population has likely been impacted by the number and spatial distribution of water meter boxes across the city. The study provides an example of how urban wildlife populations are driven by interactions among landscape structure, human management, and behavioral traits conferred by an invasive genotype.

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## Detection of single-base substitution in an esterase gene and its linkage to malathion resistance in the parasitoid *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae)

Zhu, YC; Dowdy, AK; Baker, JE. 1999

*Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) is an important parasitoid of stored-grain insect pests. Partial cDNA sequences of an esterase-like enzyme have been obtained from a malathion-resistant (R) strain and a susceptible (S) strain of this wasp. A single-base substitution in the R strain has been confirmed by using PCR amplification of specific allele (PASA) to amplify genomic DNA extracted from individual resistant and susceptible parents, F-1 hybrids from double reciprocal crosses, and progeny from backcrosses. The R allele appeared to be inherited in a strict Mendelian fashion in both diploid female and haploid male progeny. The esterase fragment co-segregated with resistance in these crosses and backcrosses. Female wasps in a mixed population of *A. calandrae* that survived a malathion screen carried the R allele for the esterase-like enzyme, while those wasps that died did not have the R allele. The single base-pair mutation, guanine in the R strain and thymine in the S strain, presumably results in a tryptophan-to-glycine amino acid substitution in the encoded protein. We do not know how these amino acid substitutions may relate to functional differences in the enzyme. However, this esterase gene or another linked esterase gene may encode the resistance-associated malathion detoxifying activity in the R strain. (C) 1999 Society of Chemical Industry.

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## Pollination effects of the sweat bee *Lasioglossum villosulum trichopse* (Hymenoptera : Halictidae) on genic male-sterile lettuce

Goubara, M; Takasaki, T. 2004

For development of an F-1 hybrid of lettuce cultivars, an efficient pollinator must be found. To evaluate pollination effects of a candidate pollinator, *Lasioglossum villosulum trichopse*, the foraging behavior and pollination ability of this sweat bee were investigated on the flowers of male-fertile (MF) and genic male-sterile (GMS) lettuce. Females of the sweat bee visited three lettuce cultivars for a short time corresponding to each cultivar's full blooming time and showed unique foraging behavior on the MF lettuce flower. The flower heads thus visited by a single pollen-gathering female sweat bee indicated a seed set as high as that of self-pollinated flower heads on a fine day. On the GMS lettuce, the rate of seed set was significantly lower in the bee-pollinated flower heads than that of hand-pollinated flower heads which bloomed on fine days. The low seed set was considered to be associated with weather conditions during the experimental period. This is the first report of successful F-1 hybrid seed production of lettuce using a pollinator insect.

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## GEOGRAPHIC-VARIATION OF PONTANIA-ACUTIFOLIAE (HYMENOPTERA, TENTHREDINIDAE) AND POSSIBILITY OF PARALLEL EVOLUTION OF THE GALL-MAKER AND ITS HOST-PLANT

A new subspecies of gall-making sawfly, *Pontania acutifoliae daphnoides* spp. n., is described from Western Europe. Its populations from the Baltic region are considered to be a separate form. The new subspecies differs from the nominotypical one by longer saw and darker coloration, the form *baltica* differs by shorter antennae and by more roundish eyes, and populations from the Alps are distinguished by very dark coloration and by antennal hollows with sparser hairs. The populations from Druskininkaj, Pskov, and Yurmala occupy intermediate position and possibly the introgressive hybridization has taken place there. The conspecificity of *P. a. acutifoliae* and *P. a. daphnoides* f. *baltica* has been tested by experimental crossing observations on the insects mating behaviour. The ranges of *P. acutifoliae* subspecies and forms are very similar to those of their host plants: *Salix acutifolia*, *S. daphnoides*, and *S. d. pomeranica*. It seems that the divergence of all these geographic forms of the gall-maker and of their host plants was almost simultaneous. Such kind of the parallel evolution seems to be not very common among gall-making sawflies.

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#### **Dataset of wing venation measurements for *Apis mellifera caucasica*, *A. mellifera carnica* and *A. mellifera mellifera* (Hymenoptera: Apidae), their hybrids and backcrosses**

Wegrzynowicz, P; Los, A. 2020

Background Wing venation is used as a tool in honeybee (*Apis mellifera* L., 1758) subspecies identification. The presented dataset concerns nineteen landmarks located at honeybee worker's forewing vein junctions. Landmarks of *Apis mellifera caucasica* Pollmann, 1889, *A. mellifera carnica* Pollmann, 1879 and *A. mellifera mellifera* Linnaeus, 1758, their hybrids and backcrosses were measured. In total, data from 9590 wings were collected. The dataset could be used in geometric morphometric analysis, studies of degree of inheritance of morphological features and, after further development and supplementation with other local subspecies and hybrids, can contribute to in-depth evolutionary research on honeybees. New information Baseline dataset for wing venation of hybrids and backcrosses of *A. mellifera carnica*, *A. mellifera caucasica* and *A. mellifera mellifera*.

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#### **Establishment of *cotesia flavipes* (Hymenoptera : braconidae) in sugarcane fields of Ethiopia and origin of founding population**

Assefa, Y; Mitchell, A; Conlong, DE; Muirhead, KA. 2008

*Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) is used as a classical biological control agent against *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), a serious exotic pest of cereal crops in eastern and southern Africa. This parasitoid has been introduced into several African countries for the control of *C. partellus* in maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.), but it has never been released in Ethiopia. It is hypothesized that it spread into Ethiopia from populations released in Kenya and Somalia to become the predominant parasitoid of *C. partellus* in maize and sorghum fields of the country. In recent surveys conducted in Ethiopia, *C. flavipes* was recovered from *C. partellus* in sugarcane, *Saccharum* L. spp. hybrids, at a site > 2,000 km from the nearest known release sites in Kenya and Somalia. These findings question published hypotheses that estimate the dispersal rate of *C. flavipes* to be 60 km per year in Africa, and they suggest that since its release in Africa this parasitoid has developed strains adapted to searching particular host plants infested by particular stem borers. The anomalies between our results and previous reports evoked the hypothesis that *C. flavipes* in Ethiopian sugarcane might be a different strain. To test this hypothesis, we compared partial COI gene sequences of *C. flavipes* collected from sugarcane in Ethiopia and those of specimens from other African countries to determine the origin of the Ethiopian population. In addition, COI sequences were obtained for *C. flavipes* from other continents. The *C. flavipes* population established in Ethiopian sugarcane is most closely related to the populations released against *C. partellus* in maize in other parts of Africa, which were derived from the original population imported from Pakistan. The dispersal rate of the parasitoid was estimated to be > 200 km per year.

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#### **Comparison of Honey Bee (Hymenoptera: Apidae) Colony Units of Different Sizes as Pollinators of Hybrid Seed Canola**

Ovinge, LP; Hoover, SE. 2018

We compare two different sizes of honey bee colony units: singles (one brood chamber) and doubles (two brood chambers) in hybrid seed canola pollination in southern Alberta in 2014 and 2015. Currently, canola seed production companies only contract double-brood chamber units to pollinate canola in southern Alberta, but it may be advantageous to the industry if singles could also be contracted for pollination, as they are in many other crops. To evaluate the differences between the colony units, we measured population size, nectar and pollen foraging, nectar and pollen load weights, pollen collection, and honey production. The colony populations of both the single- and double-brood chamber hives in this study were highly variable. In 2015, there was no difference between the single- and the double-brood chamber colonies in adult bee populations, and the singles had more sealed brood than did the double-brood chamber colonies. Our findings indicate that in comparison to doubles, on a per-frame basis, singles yield more pollen, more nectar foragers, similar or more pollen foragers, and similar amounts of honey. Therefore, we conclude that singles could be used to provide the same level of pollination services as doubles currently do in hybrid seed canola pollination, and growers should focus on receiving healthy populous colonies, regardless of the number of brood boxes.

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#### **Epidemiology of asexuality induced by the endosymbiotic *Wolbachia* across phytophagous wasp species: host plant specialization matters**

Boivin, T; Henri, H; Vavre, F; Gidoin, C; Veber, P; Candau, JN; Magnoux, E; Roques, A; Auger-Rozenberg, MA. 2014

Among eukaryotes, sexual reproduction is by far the most predominant mode of reproduction. However, some systems maintaining sexuality appear particularly labile and raise intriguing questions on the evolutionary routes to asexuality. Thelytokous parthenogenesis is a form of spontaneous loss of sexuality leading to strong distortion of sex ratio towards females and resulting from mutation, hybridization or infection by bacterial endosymbionts. We investigated whether ecological specialization is a likely mechanism of spread of thelytoky within insect communities. Focusing on the highly specialized genus *Megastigmus* (Hymenoptera: Torymidae), we first performed a large literature survey to examine the distribution of thelytoky in these wasps across their respective obligate host plant families. Second, we tested for thelytoky caused by endosymbionts by screening in 15 arrhenotokous and 10 thelytokous species for *Wolbachia*, *Cardinium*, *Arsenophonus* and *Rickettsia* endosymbionts and by performing antibiotic treatments. Finally, we performed phylogenetic reconstructions using multilocus sequence typing (MLST) to examine the evolution of endosymbiont-mediated thelytoky in *Megastigmus* and its possible connections to host plant specialization. We demonstrate that thelytoky evolved from ancestral arrhenotoky through the horizontal transmission and the fixation of the parthenogenesis-inducing *Wolbachia*. We find that ecological specialization in *Wolbachia*'s hosts was probably a critical driving force for *Wolbachia* infection and spread of thelytoky, but also a constraint. Our work further reinforces the hypothesis that community structure of insects is a major driver of the epidemiology of endosymbionts and that competitive interactions among closely related species may facilitate their horizontal transmission.

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#### **A taxonomic revision of the *Formica subpilosa* RUZSKY, 1902 group (Hymenoptera: Formicidae)**

Seifert, B; Schultz, R. 2009

The members of the West to Central Asian *Formica subpilosa* group were investigated by means of numeric morphology-based alpha-taxonomy (NUMOBAT). 18 phenotypic characters were described on the basis of 201 nest samples comprising 591 worker individuals. Five morphospecies were distinguished: *Formica*

subpilosa RUZSKY, 1902, *F. litoralis* KUZNETZOV-UGAMSKY, 1926, *F. clarissima* EMERY, 1925, *F. pamirica* DLUSSKY, 1965, and *F. kashmirica* STARCKE, 1935. A leave-one-out cross-validation discriminant analysis (LOOCV-DA) separated the first four species with an error indication of 0% and allocated all available type series near to the cluster centres with a-posteriori probabilities of  $p = 1,000$ . The heterospecificity of *Formica kashmirica*, represented only by the type sample and not reasonably testable by a DA, is justified by a character combination not found in any of the other species. *Formica clarissima*, *F. kashmirica*, *F. litoralis*, and *F. pamirica* are elevated to species rank. *Formica litoralis* and *P. pamirica* are parapatric sibling species - there seems to be no morphological convergence with falling geographic distance but the question of possible hybridisation in contact zones cannot be answered by the existing data. *Formica cinerea* var. *bipilosa* KARAVAJEV, 1926 was confirmed to be a junior synonym of *F. subpilosa*. The separation of the Palaearctic *Serv. formica* species with reddish pigmentation into a *Formica cinerea* MAYR, 1853, a *F. rufibarbis* FABRICIUS, 1793, and a *F. subpilosa* group is confirmed by a discriminant analysis. All species are depicted and a simplified identification key is given. Species of this group prefer habitats with exposed alluvial, lacustrine or aeolian soils and sparse plant cover. The *F. subpilosa* group ecologically replaces the *V. cinerea* group in Central Asia.

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#### Molecular and morphological variation among the European species of the genus *Aphidius* Nees (Hymenoptera: Braconidae: Aphidiinae)

Mitrovski-Bogdanovic, A; Mitrovic, M; Milosevic, MI; Zikic, V; Jamhour, A; Ivanovic, A; Tomanovic, Z. 2021

The main objective of the present paper was to analyse and compare the patterns of molecular and morphological divergence of European parasitoid wasps belonging to the diverse genus *Aphidius* Nees, 1818. The maximum likelihood and maximum parsimony trees constructed by including 64 different haplotypes of the barcoding region of mitochondrial cytochrome oxidase subunit I (mtCOI) identified for 33 *Aphidius* species showed identical topology. A high level (99%) of bootstrap support was found for the phylogenetic line consisting of *A. ribis* Haliday, 1834, *A. chaetosiphonis* Tomanovic & Petrovic, 2011 and *A. hortensis* Marshall, 1896, and for the group consisting of *A. colemani* Vierck, 1912, *A. transcaspicus* Telenga, 1958, *A. asteris* Haliday, 1834 and *A. platensis* Brethes, 1913. The remaining lineages on the trees were not significantly supported. We applied the approach of geometric morphometrics to explore morphological divergences in forewing size. A significant difference of mean wing shape was found between *Aphidius* species. The observed low resolution of the mtCOI gene of morphologically and ecologically well-defined *Aphidius* species is probably due to species hybridisation followed by introgression of mtDNA. Despite low resolution of the phylogenetic tree, the permutation test for a phylogenetic signal in wing shape was statistically significant, indicating that phylogenetically more closely related species are more similar than unrelated ones. A clear agreement between molecular and morphological variation was determined only for the two phylogenetically well-resolved groups.

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#### Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach

Seifert, B. 2009

Cryptic species are a major challenge for alpha-taxonomy in ants. Their reliable identification requires the application of elaborate methods such as Numeric Morphology-Based Alpha-Taxonomy or analysis of DNA and cuticular hydrocarbons. Complications caused by intraspecific polymorphism and interspecific hybridisation necessitate integrating these methods in multi-source approaches. The frequency of cryptic species was estimated in three ant genera subject to a thorough analysis as +/- 46% of about 94 Palaearctic *Lasius* species, +/- 43% of about 67 Palaearctic *Formica* species and +/- 52% of about 77 *Cardiocondyla* species worldwide. Similarly high ratios were predicted for other ant genera, although testable data are missing. Cryptic biodiversity is not evenly distributed within the evaluated ant genera. The indicative value of the following investigation methods was assessed in ants: Morphology-Based Alpha-Taxonomy (MOBAT), Numeric MOBAT (NUMOBAT), analysis of nuclear and mitochondrial DNA, cuticular hydrocarbons, pheromones, allozymes, karyotypes, ethology, and ecology. NUMOBAT is arguably the "backbone" of a testable integrative taxonomy, the deciding link to Zoological Nomenclature, the only useable method for DNA-degraded specimens and the only method to examine vouchers in which no damage is allowed. The unacceptably high ratios of paraphyly in mtDNA barcoding forbid its application as primary decision finder. In conclusion, no single method but only an integrative, multi-source alpha-taxonomy offers the most convincing approach towards recognition of real biodiversity.

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#### *Ancylostoma* secreted protein 2. cloning and characterization of a second member of a family of nematode secreted proteins from *Ancylostoma caninum*

Hawdon, JM; Narasimhan, S; Hotez, PJ. 1999

Invading infective third-stage larvae (L-3) of parasitic nematodes execute a series of programmed developmental events in response to a host-specific signal encountered during infection. One of these early events is the release of excretory/secretory products. Using an in vitro feeding assay that mimics these early events of infection, a protein released by in vitro activated larvae of the hookworm *Ancylostoma caninum* was identified. This protein, Ac-ASP-2, was partially sequenced, and the cDNA encoding it isolated by PCR and screening of an *A. caninum* L-3 cDNA library. The Ac-asp-2 cDNA encodes a protein of 219 amino acids that is related to a previously identified protein, Ac-ASP-1, from hookworms. Both molecules are members of an evolutionarily diverse family of molecules that include the venom allergens of the Hymenoptera, and the testes specific proteins/sperm-coating glycoproteins of mammals. Homologues are present in nearly all nematodes tested, as demonstrated by PCR-hybridization and database searching. The Ac-asp-2 mRNA is synthesized in all life history stages, but the gene product is released only by L-3 activated to feed in vitro. The wide distribution of the Ac-asp-2 in nematodes and its release in response to host specific signals suggests that Ac-ASP-2 serves an important function in nematode physiology and development, and possibly in the infective process of parasitic species. (C) 1999 Elsevier Science B.V. All rights reserved.

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#### SPECIES COMPOSITION AND ABUNDANCE OF THE NATURAL ENEMIES OF SUGARCANE APHID, *Melanaphis sacchari* (ZEHNTNER) (HEMIPTERA: APHIDIDAE), ON SORGHUM IN TEXAS

Maxson, EL; Brewer, MJ; Rooney, WL; Woolley, JB. 2019

The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an emergent sorghum pest in the United States. This study was designed to identify which natural enemy species are present in aphid populations in sorghum in Texas, and to track the seasonal population trends of the aphid and its natural enemies on sorghum hybrids that differ in susceptibility to the aphid. From 2015 through 2016, sugarcane aphid and its natural enemies were sampled weekly in plots of aphid-susceptible and partially aphid-resistant sorghum hybrids at two field sites in Nueces County and Burleson County, Texas. In 2015, aphids and natural enemies had greater peak abundance on the susceptible hybrid than on the resistant hybrid. Peak abundance of most natural enemies tended to lag behind that of aphids by one to two weeks. Natural enemy taxa observed at both field sites included two primary parasitoid wasp species (Hymenoptera: Aphelinidae: *Aphelinus nigritus* Howard stat. rev.; Braconidae: *Lysiphlebus testaceipes* (Cresson)), one species of hyperparasitoid (Hymenoptera: Encyrtidae), ten lady beetle species (Coleoptera: Coccinellidae) and three morphospecies of dusky lady beetle (Coccinellidae: Scymninae), three hoverfly species (Diptera: Syrphidae), five green lacewing species (Neuroptera: Chrysopidae), brown lacewings (Neuroptera: Hemerobiidae: Hemerobius), and minute pirate bugs (Hemiptera: Anthracoridae: *Orius insidiosus* Say)). *Aphelinus* and Coccinellidae were the numerically dominant natural enemy groups in all sorghum hybrids, followed by Chrysopidae and Syrphidae. Aphids mummified by *Aphelinus* were hyperparasitized by *Syrphophagus aphidivorus* at a rate of approximately 90%. Natural enemy densities were similarly proportionate to aphid densities on both aphid-susceptible and aphid-resistant plants. Overall, the continuity of natural enemy species composition and population trends supports that these natural enemies responded positively to sugarcane aphid on sorghum.

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## Recent speciation in the *Formica rufa* group ants (Hymenoptera, Formicidae): inference from mitochondrial DNA phylogeny

Goropashnaya, AV; Fedorov, VB; Pamilo, P. 2004

This study examines phylogenetic relationships among six species of the *Formica rufa* group ants (*F. polycтена*, *F. rufa*, *F. lugubris*, *F. paralugubris*, *F. aquilonia*, and *F. pratensis*). The phylogeny based on a 2051 bp fragment of mtDNA including *cyt b*, *tRNA(Ser)*, and *ND1* genes supports the division of the group into three major clusters: one with the species *F. polycтена* and *F. rufa*, one with *F. aquilonia*, *F. lugubris*, and *F. paralugubris*, and the third one with *F. pratensis*. The interspecific divergence estimates (mean 0.98 +/- 0.15% for the main phylogenetic groups) imply that radiation took place during the Pleistocene. Comparison of the divergence estimates among the *F. rufa* group species with divergence estimates among other closely related species of insects suggests that speciation in the group was relatively fast, and the mitochondrial lineages of *F. polycтена* and *F. rufa* have not fully separated. The haplotype tree shows also signs of transfer of mtDNA between species through hybridisation. The distribution of polygyny (multiple queens per nest) along the branches of the tree indicates that the social type characterised by highly polygynous societies and large colonial networks, has originated at least three times. The species *F. aquilonia* and *F. paralugubris* that build such large supercolonies, cluster tightly together with very little nucleotide variation, suggesting that this type of social organisation could be a factor promoting speciation in the ants. (C) 2003 Elsevier Inc. All rights reserved.

## Abundance of the Sugarcane Borer (Lepidoptera: Crambidae) and Foraging Ants (Hymenoptera: Formicidae) in Sugarcane Grown on Organic and Mineral Soils in Florida

Roldan, EL; Beuzelin, JM; VanWeelden, MT; Cherry, RH. 2020

A study was conducted in Florida to determine sugarcane borer, *Diatraea saccharalis* (F.), injury and infestation levels in sugarcane (*Saccharum* spp. hybrids), *D. saccharalis* parasitism rates, and ant foraging activity in 32 commercial fields as affected by soil type (shallow organic vs deep organic vs mineral). In 2017 and 2018, each field was sampled four times during the summer for *D. saccharalis* by inspecting 100 sugarcane stalks and for foraging ants using plastic tubes baited with hot dog at 12 locations. One non-parasitized *D. saccharalis* larva was collected in 2017 and in 2018 out of 12,100 and 12,600 stalks sampled, respectively. Additional sampling of 50 stalks per field in October showed that 0.6% (2017) and 0.1% (2018) of the sugarcane stalks had bored internodes, and one *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) cocoon mass was observed. Seven ant species foraged in sugarcane fields, including the red imported fire ant, *Solenopsis invicta* Buren, which was the most abundant ant. *Solenopsis invicta* was not affected by soil type; however, sugarcane fields on shallow organic soils might represent a more suitable environment. The third most abundant foraging ant, *Nylanderia bourbonica* (Forel), was more abundant in mineral soil fields than in shallow and deep organic soil fields. Results suggest that *D. saccharalis* population levels in Florida sugarcane are extremely low under current production conditions regardless of soil type. In addition, the observation of *C. flavipes*, *S. invicta*, and six other ant species suggest that biological control contributes to these low *D. saccharalis* population levels.

## European corn borer and its parasites overwintering abundance and damages on different corn FAO maturity groups

Lemic, D; Mandic, J; Cacija, M; Drmic, Z; Mrganic, M; Cavlovicak, S; Bazok, R; Viric Gasparic, H. 2019

European corn borer (ECB) is one of the most significant maize pests in the world and also in Croatia. ECB causes yield reduction from 2 to 25%, even more in years favorable for its development. According to estimations, these losses are around 7%. About 90% of the hybrids had some resistance to whorl-leaf feeding (first-generation ECB) and 75% had some resistance to sheath and sheath-collar feeding (second-generation ECB). Along with resistance, modern maize hybrids possess certain level of tolerance. The main aim of this paper was to determine moth eclosion of the overwintering generation and presence of the parasites of ECB larvae during the overwintering as well as to estimate population density of ECB on maize growing area in Croatia. Also, the aim was to establish the differences among maize FAO maturity groups in damage caused by ECB larvae. Estimated overwintering population was over 8,000 moths/ha i.e. more than 4 million larvae of first generation. During the overwintering four different parasites attacked the larvae. Two species belong to the order Hymenoptera (*Cotesia marginiventris* Cresson and *Eriborus terebrans* Gravenhorst) and two species belong to the order Diptera (*Ramonda spathulata* Fallen and *Lydella thompsoni* Herting). One caterpillar predator species *Paragymnomerus spiricornis* Spinola (Hymenoptera) overwinters in maize stalks as well. The highest attack of the first ECB generation was recorded on FAO maturity group 500. The damage from second ECB generation was the highest on FAO groups 400 and 500. The maize of the higher FAO groups has high and robust stems with large number of big leaves. That intensive vegetative growth is a biological characteristic that attracts first generation of ECB to intensifying egg laying. High population level of the first generation may lead to high level of second ECB generation which ultimately caused yield reduced on the hybrids of longer vegetation period (medium-late FAO maturity groups).

## Short-term assessment of Bt maize on non-target arthropods in Brazil

Fernandes, OA; Faria, M; Martinelli, S; Schmidt, F; Carvalho, VF; Moro, G. 2007

Although not yet available for cultivation in Brazil, the effect of Bt maize hybrids on natural enemies and soil dwelling arthropods should be assessed prior to its release to growers. Trials were carried out during one growing season in two different locations with the genetically modified maize hybrids 7590-Bt11 and Avant-ICP4, comparing with their respective non-Bt isogenic hybrids. Arthropods were evaluated through direct observation on plants and pitfall traps. In general, no differences were observed between populations of earwig (Dermaptera: Forficulidae), lady beetles (Coleoptera: Coccinellidae), minute pirate bug (Coleoptera: Anthrenidae), ground beetles (Carabidae), tiger beetles (Cicindelidae), and spiders (Araneae). There was no difference in egg parasitism of *Helicoverpa zea* (Boddie) by *Trichogramma* sp. (Hymenoptera: Trichogrammatidae). Thus, Bt maize hybrids expressing insecticide proteins Cry 1A(b) and VIP 3A do not cause reduction of the main maize dwelling predators and parasitoids.

## Pollen Collection, Honey Production, and Pollination Services: Managing Honey Bees in an Agricultural Setting

Hoover, SE; Ovinge, LP. 2018

Hybrid canola seed production is an important pollination market in Canada; typically both honey bees (*Apis mellifera* L. (Hymenoptera: Apidae)) and Alfalfa Leafcutting bees (*Megachile rotundata* Fab. (Hymenoptera: Megachilidae)) are concurrently managed to ensure pollination in this high-value crop. Beekeepers are paid to provide pollination services, and the colonies also produce a honey crop from the canola. Pollen availability from male-fertile plants is carefully managed in this crop to provide an abundance of pollen to fertilize male-sterile ('female') plants. This abundance of pollen represents an underutilized resource for beekeepers, and an opportunity to diversify the hive-products produced for market in this management system. We used a commercial-style pollen trap to collect pollen from colonies twice weekly for the duration of canola pollination, and compared the honey production and amount of sealed brood in colonies with pollen traps to those without pollen traps. We found that while pollen trapping reduced honey production, there was no negative impact on brood production, and at current market prices, the per-hive revenue was higher in colonies from which pollen was trapped. Pollen trapping honey bee colonies in the context of hybrid canola pollination, therefore, offers beekeepers an opportunity to diversify their products and increase their revenue.

## Pollen collection and foraging force by European and European x Africanized hybrid honey bees (Hymenoptera : Apidae) in mixed genotype colonies are similar

The foraging force and pollen collection of European and European X Africanized hybrid worker honey bees, *Apis mellifera* L., housed in a common nest environment were compared. Significant heterogeneity was found within both genotype populations for the proportion of the bees that foraged, as well as for the proportion of foragers that collected pollen. However, there was not a consistent bias for either genotype to collect pollen or to field a greater proportion of the total foraging population. These results suggest that when sharing a common environment, European X Africanized hybrids and European honey bees do not differ with respect to individual foraging decisions. Results also suggest that the pollinating efficiency of commercial colonies maintained in Africanized areas will probably not diminish as a consequence of introgression of African honey bee genes, if problems associated with their management can be controlled.

#### Isolation and characterization of a baculovirus associated with the insect parasitoid wasp, *Cotesia marginiventris*, or its host, *Trichoplusia ni*

Grasela, JJ; McIntosh, AH; Shelby, KS; Long, S. 2008

A multiple nucleopolyhedrovirus (MNPV) was isolated from *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) larvae that had been stung by the parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). The wild type virus was plaque purified by infecting a *Heliothis subflexa* (BCIRL-HsAM1) cell line and isolating several clones. The mean estimated genomic size of this virus based on PstI, BstEII, Styl, HindIII restriction profiles was estimated to be 106 +/- 2.5 kbp (mean +/- SE). A clone designated as TnMNPV/CmBCL9 was used in bioassays against several lepidopteran pests and in comparative studies with the baculoviruses AcMNPV, AgMNPV, AfMNPV, PxMNPV and HzSNPV of *Autographa californica*, *Anticarsia gemmatilis*, *Anagrapha falcifera*, *Plutella xylostella*, and *Helicoverpa zea*, respectively. Infectivity studies showed that TnMNPV/CmBCL9 was highly infectious for *Heliothis subflexa* and *T. ni*, with an LC50 value 0.07 occlusion bodies/mm(2) in both species and also infectious for *H. zea* and *Heliothis virescens* with LC50 values of 0.22 and 0.27 occlusion bodies/mm(2), respectively. Restriction endonuclease analysis of the isolate and selected baculoviruses revealed profiles that were very similar to AfMNPV but different from the restriction endonuclease profiles of the other baculoviruses. Hybridization studies suggest that the TnMNPV/CmBCL9 was closely related to AfMNPV and AcMNPV-HPP. Further support for this comes from a phylogenetic analysis employing a split-graphs network, comparing the polh, egt, and p10 genes from TnMNPV/CmBCL9 with those from other baculoviruses and suggests that this virus is closely related to the AcMNPV variants, AfMNPV and RoMNPV of *Rachiplusia ou*.

#### A taxonomic revision of the *Cardiocondyla nuda* group (Hymenoptera: Formicidae)

Seifert, B; Okita, I; Heinze, J. 2017

A taxonomic revision of the *Cardiocondyla nuda* species group is presented based on methods of Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) and supplemented by analysis of mtDNA. A total of 258 samples with 571 worker individuals were investigated by the hierarchical and non-hierarchical exploratory data analyses NC-Ward and NC-K-Means clustering considering 16 NUMOBAT characters. Two species are described as new, increasing the number of species in the group to eight. We separate the group into two main clades: the *C. mauritanica* species complex, which is of Oriental and Indo-Australian origin and contains the cryptic species *C. mauritanica* Forel 1890, *C. strigifrons* Viehmeyer 1922, *C. kagutsuchi* Terayama 1999, and *C. itsukii* sp. nov. and the Australasian and Polynesian *C. nuda* species complex with the cryptic species *C. nuda* (Mayr 1866), *C. atalanta* Forel 1915, *C. paranuda* Seifert 2003, and *C. compressa* sp. nov. The mean error of the two NC-clustering methods relative to the controlling linear discriminant analysis was 0.4% in *C. mauritanica*, 2.2% in *C. itsukii*, 0% in *C. strigifrons*, 0% in *C. kagutsuchi*, 1.5% in *C. nuda*, 3.2% in *C. atalanta* and 3.2% in *C. paranuda*-all these data are below the 4% threshold recommended by the Pragmatic Species Concept. The morphologically determined species clusters were confirmed by mtDNA data with a rather strong sequence divergence among the cryptic species of the *C. nuda* complex of 5.6-7.9%. The mean mismatch of two different mtDNA analyses with NUMOBAT clustering was 5.4% in 54 samples of seven species of the *C. nuda* group for which mtDNA data were available. The mismatch thus is smaller than in many other studies of Eumetazoa in general or ants in particular and is probably explained by low frequencies of ancient hybridization and/or incomplete lineage sorting. Comments on zoogeography, colony demography and behavior are given in the species sections and determination keys are provided. *Cardiocondyla ectopia* Snelling 1974 and *Leptothorax caparica* Henin, Paiva & Collingwood 2002 (syn nov.) are synonymized under *Cardiocondyla mauritanica*. *Cardiocondyla nuda sculptinodis* is not a member of the *C. nuda* group and is moved to *C. shuckardi sculptinodis*, a revived combination.

#### Distribution of *Pseudacteon* spp. (Diptera: Phoridae), biological control agents of *Solenopsis* spp. (Hymenoptera: Formicidae), in Louisiana and associated prevalence of *Kneallhazia solenopsae* (Microsporidia: Thelohaniidae)

Meszaros, A; Oi, DH; Valles, SM; Beuzelin, JM; Reay-Jones, FPF; Johnson, SJ. 2014

Phorid flies, *Pseudacteon* spp. (Diptera: Phoridae), have been released in the United States since 1996 as biological control agents for imported fire ants, *Solenopsis invicta* Buren, *Solenopsis richteri* Forel, and their hybrid (Hymenoptera: Formicidae). A statewide survey was conducted in Louisiana during 2009 and 2010 to determine the distribution of *Pseudacteon tricuspidis* Borgmeier and *Pseudacteon curvatus* Borgmeier, and to quantify the potential spatial association between the two species. Additionally, collected phorid flies were tested for the microsporidium *Kneallhazia* (=Thelohania) *solenopsae* (Knell, Allen, and Hazard), another natural enemy of fire ants, to establish an account of the pathogen's distribution and prevalence in phorid fly populations. *P. tricuspidis* and *P. curvatus* have expanded their range from four and three separate release sites, respectively, in Louisiana. By 2010, *P. tricuspidis* and *P. curvatus* occupied approximately 86,600 and 111,900 km(2), respectively. Overall, *P. curvatus* was more abundant than *P. tricuspidis*, with 41.9 +/- 7.3 (SE) and 9.8 +/- 1.5 (SE) females, respectively, collected on average per sampling site in 2009. Spatial Analysis by Distance IndicEs (SADIE) suggested aggregation for the two phorid fly species. *P. tricuspidis* collections were positively associated with *P. curvatus* collections, indicating that gaps and clusters of both species generally overlapped. *K. solenopsae* was detected in 22 of 64 parishes, and among 124 collection sites tested, 19% yielded positive responses for *K. solenopsae*. *P. tricuspidis* males and females and *P. curvatus* females were found to harbor *K. solenopsae*. This study is the first to detect *K. solenopsae* in *P. tricuspidis*. (C) 2014 Elsevier Inc. All rights reserved.

#### Parasitoids of leafmining lepidoptera. in spontaneous growth plants in an organic citrus orchard in Montenegro, RS, Brazil

dos Santos, JP; Dal Soglio, FK; Redaelli, LR; Costa, VA; Foelkel, E. 2007

This work aimed to check if *Phyllocnistis citrella* attacks spontaneous growth plants present in an organic citrus orchard, to identify in these plants other leafmining Lepidoptera and its parasitoids, and to verify if the parasitoids species are the same ones reported for *R. citrella*. The work was conducted in Montenegro, RS, in an organic orchard of the hybrid 'Murcott' (*Citrus sinensis* x *C. reticulata*). Samplings were taken every other week, from May 2003 to May 2004, with a ring of 0.28 m(2) being randomly thrown in the lines and interlines of 30 trees. All the plants with mines inside of the ring were collected. The screening of the material was made in laboratory. Eleven species of leafmining Lepidoptera and 12 species of microhymenopteran parasitoids were found. Some genera identified that this study had been already reported in several regions of the world with parasitizing species *P. citrella*, such as *Chrysocharis*, *Closterocerus*, *Sympiesis* (Hymenoptera: Eulophidae) and *Bracon* (Hymenoptera: Braconidae).

#### Evolution of full-length and deleted forms of the Mariner-like element, Botmar1, in the genome of the bumble bee, *Bombus terrestris* (Hymenoptera : Apidae)

Rouleux-Bonnin, F; Petit, A; Demattei, MV; Bigot, Y. 2005

Mariner-like elements (MLE) are Class II transposable elements that are very widespread among eukaryotic genomes. One MLE belonging to the mauritiana subfamily, named Botmar1, has been identified in the genome of the bumble bee, *Bombus terrestris*. gDNA hybridization with the Botmar1 transposase ORF revealed that about 230 elements are present in each haploid genome of *B. terrestris* that consist entirely of 1.3- and 0.85-kbp elements. The analysis of their sequences revealed that there are two Botmar1 subfamilies of similar ages in the *Bombus terrestris* genome: one is composed entirely of 1.3-kbp elements, whereas the second comprises both completed and deleted elements. Our previous data indicated that the internally deleted form, which correspond to the 0.85-kbp Botmar1-related elements occur in other distantly related hymenopteran genomes. Because the presence of similar 1.3- and 0.85-kbp Botmar1-related elements in some distantly related hymenopteran species cannot be explained by horizontal transfers, the nucleic acid sequence properties of these elements were further investigated. We found that certain structural properties in their nucleic acid sequence might explain the occurrence of 0.85-kbp Botmar1-related elements presenting similarly located internal deletions in hymenopteran genomes.

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#### **Building-Up of a DNA Barcode Library for True Bugs (Insecta: Hemiptera: Heteroptera) of Germany Reveals Taxonomic Uncertainties and Surprises**

Raupach, MJ; Hendrich, L; Kuechler, SM; Deister, F; Moriniere, J; Gossner, MM. 2014

During the last few years, DNA barcoding has become an efficient method for the identification of species. In the case of insects, most published DNA barcoding studies focus on species of the Ephemeroptera, Trichoptera, Hymenoptera and especially Lepidoptera. In this study we test the efficiency of DNA barcoding for true bugs (Hemiptera: Heteroptera), an ecological and economical highly important as well as morphologically diverse insect taxon. As part of our study we analyzed DNA barcodes for 1742 specimens of 457 species, comprising 39 families of the Heteroptera. We found low nucleotide distances with a minimum pairwise K2P distance <2.2% within 21 species pairs (39 species). For ten of these species pairs (18 species), minimum pairwise distances were zero. In contrast to this, deep intraspecific sequence divergences with maximum pairwise distances >2.2% were detected for 16 traditionally recognized and valid species. With a successful identification rate of 91.5% (418 species) our study emphasizes the use of DNA barcodes for the identification of true bugs and represents an important step in building-up a comprehensive barcode library for true bugs in Germany and Central Europe as well. Our study also highlights the urgent necessity of taxonomic revisions for various taxa of the Heteroptera, with a special focus on various species of the Miridae. In this context we found evidence for on-going hybridization events within various taxonomically challenging genera (e.g. *Nabis* Latreille, 1802 (Nabidae), *Lygus* Hahn, 1833 (Miridae), *Phytocoris* Fallen, 1814 (Miridae)) as well as the putative existence of cryptic species (e.g. *Aneurus avenius* (Duffour, 1833) (Aradidae) or *Orius niger* (Wolff, 1811) (Anthocoridae)).

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#### **EFFECT OF GENOTYPE ON CYTOPLASMIC INCOMPATIBILITY BETWEEN 2 SPECIES OF NASONIA**

BREEUWER, JAJ; WERREN, JH. 1993

Cytoplasmically inherited bacteria cause bidirectional incompatibility between two species of the parasitoid wasp genus *Nasonia*. These bacteria belong to the alpha sub-division of the Proteobacteria. Normally, hybrid (female) offspring are not produced in interspecific crosses. Elimination of the cytoplasmically-inherited bacteria by antibiotic treatments restores compatibility, i.e. hybrid females are produced. Bidirectional incompatibility could be caused directly, by differences between the bacteria of the two species, or indirectly, by the different interactions of the bacteria with host species genomes. To distinguish between these two possibilities, genetic crosses were set up to introduce the nuclear genome of each wasp species into the cytoplasmic (microbial) background of the other. These strains were then tested for compatibility to the infected parental species. Individuals from both reciprocal introgressions were only compatible with individuals that had the same cytoplasm, independent of nuclear background. This indicates that compatibility type is independent of the host genotype and is based on differences between cytoplasmic bacteria of the two *Nasonia* species. However, compatibility differences from interactions between the bacteria and the other cytoplasmically inherited elements (e.g. mitochondria) have not been ruled out.

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#### **Range limits in sympatric cryptic species: a case study in *Tetramorium* pavement ants (Hymenoptera: Formicidae) across a biogeographical boundary**

Cordonnier, M; Bellec, A; Dumet, A; Escarguel, G; Kaufmann, B. 2019

In the context of climate changes, factors that determine the distribution patterns of European species of the ubiquitous ant genus *Tetramorium* were investigated. The study took place along a steep North-South climatic gradient across the boundary between the European Continental and Mediterranean biogeographical regions spanning 460 km along the Rhone valley, France. Ants from 1690 *Tetramorium* colonies were collected in 19 sampling zones. Species were identified using an integrative approach based on a two-step process combining nuclear DNA (14 microsatellite markers), morphological examination and mitochondrial DNA cytochrome oxidase I sequencing. The impact of climate on species distribution patterns was tested using bioclimatic variables. Species discrimination was successful, despite a complex situation with cryptic species, inter-specific hybridisation and uneven sampling across species. Our results showed a strong effect of latitude, temperature and rainfall on the distribution of three of four species. *Tetramorium semilaeve* was found only south of the boundary, in warmer and dryer sites; *Tetramorium caespitum* extended north and 70 km south of the boundary and favoured colder sites with strong seasonal variation. *Tetramorium immigrans* was absent from the northernmost sampling zones and favoured warmer and wetter sites. *Tetramorium moravicum* was mostly found close to the boundary, but without significant climatic preferences. The fundamental role of climate as a factor limiting the ranges of these species at a major biogeographical boundary is confirmed. Monitoring range limits of these strongly climate-dependant species may offer exciting insights on the impact of climate changes on species distributions.

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#### **Hybrid Genome Assembly and Evidence-Based Annotation of the Egg Parasitoid and Biological Control Agent *Trichogramma brassicae***

Ferguson, KB; Kursch-Metz, T; Verhulst, EC; Pannebakker, BA. 2020

*Trichogramma brassicae* (Bezdenko) are egg parasitoids that are used throughout the world as biological control agents and in laboratories as model species. Despite this ubiquity, few genetic resources exist beyond COI, ITS2, and RAPD markers. Aided by a *Wolbachia* infection, a wild-caught strain from Germany was reared for low heterozygosity and sequenced in a hybrid de novo strategy, after which several assembling strategies were evaluated. The best assembly, derived from a DBG2OLC-based pipeline, yielded a genome of 235 Mbp made up of 1,572 contigs with an N50 of 556,663 bp. Following a rigorous ab initio, homology-, and evidence-based annotation, 16,905 genes were annotated and functionally described. As an example of the utility of the genome, a simple ortholog cluster analysis was performed with sister species *T. pretiosum*, revealing over 6000 shared clusters and under 400 clusters unique to each species. The genome and transcriptome presented here provides an essential resource for comparative genomics of the commercially relevant genus *Trichogramma*, but also for research into molecular evolution, ecology, and breeding of *T. brassicae*.

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#### **Genomic Characterization and Curation of UCEs Improves Species Tree Reconstruction**

Van Dam, MH; Henderson, JB; Esposito, L; Trautwein, M. 2021

Ultraconserved genomic elements (UCEs) are generally treated as independent loci in phylogenetic analyses. The identification pipeline for UCE probes does not require prior knowledge of genetic identity, only selecting loci that are highly conserved, single copy, without repeats, and of a particular length. Here, we characterized UCEs from 11 phylogenomic studies across the animal tree of life, from birds to marine invertebrates. We found that within vertebrate lineages, UCEs are mostly intronic and intergenic, while in invertebrates, the majority are in exons. We then curated four different sets of UCE markers by genomic category from five different

studies including: birds, mammals, fish, Hymenoptera (ants, wasps, and bees), and Coleoptera (beetles). Of genes captured by UCEs, we find that many are represented by two or more UCEs, corresponding to nonoverlapping segments of a single gene. We considered these UCEs to be nonindependent, merged all UCEs that belonged to a particular gene, constructed gene and species trees, and then evaluated the subsequent effect of merging cogenic UCEs on gene and species tree reconstruction. Average bootstrap support for merged UCE gene trees was significantly improved across all data sets apparently driven by the increase in loci length. Additionally, we conducted simulations and found that gene trees generated from merged UCEs were more accurate than those generated by unmerged UCEs. As loci length improves gene tree accuracy, this modest degree of UCE characterization and curation impacts downstream analyses and demonstrates the advantages of incorporating basic genomic characterizations into phylogenomic analyses.

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#### **Prothoracic gland inactivation in *Heliothis virescens* (F.) (Lepidoptera : Noctuidae) larvae parasitized by *Cardiochiles nigriceps* Viereck (Hymenoptera : Braconidae)**

Pennacchio, F; Falabella, P; Sordetti, R; Varricchio, P; Malva, C; Vinson, SB. 1998

*Heliothis virescens* (F.) last instar larvae parasitized by the endophagous braconid *Cardiochiles nigriceps* Viereck fail to attain the pupal stage, due to a parasitoid-induced alteration of ecdysteroid biosynthesis and metabolism. Currently available information on host prothoracic gland inactivation in this host-parasitoid system is reported here. Prothoracic glands of *H. virescens* mature larvae show a depressed biosynthetic activity, without undergoing gross morphological disruption. The ultrastructure of gland cells is characterized by minor parasitoid-induced changes, with the rough endoplasmic reticulum appearing more developed and electron-dense than in nonparasitized controls. Eventually, the cells of prothoracic glands of parasitized host last instar larvae die but maintain their structural integrity. The inactivation of pupally committed host prothoracic glands is achieved through the disruption of the PTTH signal transduction pathway. The second messenger cAMP appears to be normally produced in response to PTTH stimulation of glands explanted from parasitized host larvae, however the downstream activation of the cAMP-dependent protein kinase does not appear to occur. In fact, a marked underphosphorylation of regulatory target proteins is observed. This underphosphorylation is associated with a significant reduction in general protein synthesis, which appears to be blocked at the translational level, to a redirection of specific protein synthesis and to a drastic suppression of ecdysteroidogenesis. These parameters appeared to be correlated in a kinetic time-course study, confirming their functional link. *C. nigriceps* polydnavirus (CnPDV) plays a major role in the inactivation of pupally committed host prothoracic glands, while putative factors occurring in the host haemolymph do not seem to be of particular importance at that developmental stage. Southern blot hybridization indicates the occurrence of PKI (protein kinase inhibitor)-like genes in the *C. nigriceps* genome, which, in contrast, are undetectable in *H. virescens*. (C) 1998 Elsevier Science Ltd. All rights reserved.

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#### **Efficacy of Nootka Oil as a Biopesticide for Management of Imported Fire Ants (Hymenoptera: Formicidae)**

Addesso, KM; Oliver, JB; O'Neal, PA; Youssef, N. 2017

Recent concerns regarding the impact of traditional synthetic pesticides on nontarget organisms have generated demand for alternative products with lower environmental impact. This demand has led to increasing focus on plant essential oils as sources of new biopesticides. In this study, we demonstrate that the essential oil of the Alaskan yellow cedar, *Cupressus nootkatensis* (D. Don) Spach, has activity against hybrid imported fire ant workers, *Solenopsis invicta* Burén x *Solenopsis richteri* Forel. In digging assays, ants were repelled by nootka oil and digging continued to be suppressed by nearly 50% in nootka oil-treated sand aged 6mo in the laboratory. Higher worker mortality was also observed in contact and fumigation assays compared to control checks. In a field drench test, mortality of mounds treated with nootka oil lagged behind mounds treated with bifenthrin treatment for 7 wk, but both nootka oil and bifenthrin had higher mortality than the untreated check at the end of the 12-wk evaluation period. In a band application evaluation, nootka oil plots maintained a 90-95% reduction in fire ant mounds from the 2nd to 17th wk, when new mounds began to intrude on the field plots. The quarantine-approved bifenthrin band treatment maintained 100% control from the 2nd to 24th wk. Although the formulation tested here did not perform to Federal Imported Fire Ant Quarantine standards, other formulations may enable this product to reach 100% control. In addition, nootka oil could be beneficial in situations where ant suppression rather than complete quarantine elimination is the management goal.

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#### **Attractiveness and effectiveness of an artificial diet fed to hybrid imported fire ants, *Solenopsis invicta* x *richteri* (Hymenoptera : Formicidae)**

Vogt, JT. 2003

Attractiveness of freeze-dried and reconstituted entomophage diet to hybrid fire ants (*Solenopsis invicta* x *richteri*) was investigated in choice tests using freeze-killed, crushed cricket (*Acheta domestica* L.) as a standard. Worker ants were strongly attracted to both crickets and reconstituted diet. Foragers collected approx. 27 times more reconstituted diet than freeze-dried diet, and collected statistically equivalent amounts of artificial diet and crickets (36.0 +/- 7.0 and 26.0 +/- 0.3 mg/h, respectively). Even though workers were strongly attracted to the artificial diet, all measures of colony growth (mean mass of brood, workers, and queen) were at least 30% lower in colonies fed sugar water + artificial diet than in colonies fed sugar water + crickets or sugar water + artificial diet + crickets. While this diet may have some utility as a bait for monitoring fire ants in the field, it offers no advantage over a standard diet of crickets and sugar water for rearing fire ants in the laboratory.

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#### **Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies**

Simmons, AT; Gurr, GM. 2005

1 The cultivated tomato, *Lycopersicon esculentum*, is an economically important worldwide crop. Current pest management techniques rely heavily on pesticides but trichome-based host-plant resistance may reduce pesticide use. 2 A review of the literature is provided on trichomes of wild *Lycopersicon* species and the effects of trichome-based host-plant resistance on arthropods. Solvents have been used to remove glandular trichome exudates and the resulting diminution of their effects quantified. Correlational approaches to assess the relationship between the different trichome types and effects on pests have also been used. 3 Most studies have focused on Lepidoptera and Hemiptera, although some work has included Coleoptera, Diptera and Acarina, and both antibiotic and antixenotic effects have been demonstrated. 4 Natural enemies are a cornerstone of international pest management and this review discusses how the compatibility of this approach with trichome-based host-plant resistance is uncertain because of the reported negative effects of trichomes on one dipteran, one hemipteran and several Hymenoptera. 5 For trichome-based host-plant resistance to be utilized as a pest management tool, trichomes of wild species need to be introgressed into the cultivated tomato. Hybrids between the cultivated tomato and the wild species *Lycopersicon hirsutum* f. *glabratum*, *Lycopersicon pennellii* and *Lycopersicon cheesmanii* f. *minor* have been produced and useful levels of resistance to Acarina, Diptera and Hemiptera pests have been exhibited, although these effects may be tempered by effects on natural enemies. 6 This review proposes that studies on genetic links between fruit quality and resistance, field studies to determine the compatibility of natural enemies and trichome-based host-plant resistance, and a strong focus on *L. cheesmanii* f. *minor*, are all priorities for further research that will help realize the potential of this natural defence mechanism in pest management.

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#### **Comparison of Methods for Species-Tree Inference in the Sawfly Genus *Neodiprion* (Hymenoptera: Diprionidae)**

Linnen, CR; Farrell, BD. 2008

Conifer-feeding sawflies in the genus *Neodiprion* provide an excellent opportunity to investigate the origin and maintenance of barriers to reproduction, but obtaining



a phylogenetic estimate for comparative studies of *Neodiprion* speciation has proved difficult. Specifically, nonmonophyly within and discordance between individual gene trees, both of which are common in groups that diverged recently and/or rapidly, make it impossible to infer a species tree using methods that are designed to estimate gene trees. Therefore, in this study, we estimate relationships between members of the lecontei species group using four approaches that are intended to estimate species, not gene, trees: (1) minimize deep coalescences (MDC), (2) shallowest divergences (SD), (3) Bayesian estimation of species trees (BEST), and (4) a novel approach that combines concatenation with monophyly constraints (CMC). Multiple populations are sampled for most species and all four methods incorporate this intraspecific variation into estimates of interspecific relationships. We investigate the sensitivity of each method to taxonomic sampling, and, for the BEST method, we assess the impact of prior choice on species-tree inference. We also compare species-tree estimates to one another and to a morphologically based hypothesis to identify clades that are supported by multiple analyses and lines of evidence. We find that both taxonomic sampling and method choice impact species-tree estimates and that, for these data, the BEST method is strongly influenced by and branch-length priors. We also find that the CMC method is the least sensitive to taxonomic sampling. Finally, although interspecific genetic variation is low due to the recent divergence of the lecontei group, our results to date suggest that incomplete lineage sorting and interspecific gene flow are the main factors complicating species-tree inference in *Neodiprion*. Based on these analyses, we propose a phylogenetic hypothesis for the lecontei group. Finally, our results suggest that, even for very challenging groups like *Neodiprion*, an underlying species-tree signal can be extracted from multi-locus data as long as intraspecific variation is adequately sampled and methods that focus on the estimation of species trees are used.

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## FERTILIZATION OF HETERO-SPECIFIC INSECT EGGS BY SPERM INJECTION

SAWA, M. 1991

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

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## REPRODUCTION BY VIRGIN QUEEN FIRE ANTS IN QUEENLESS COLONIES - COMPARATIVE-STUDY OF 3 TAXA (SOLENOPSIS-RICHTERI, HYBRID SOLENOPSIS-INVICTA RICHTERI, S-GEMINATA) (HYMENOPTERA, FORMICIDAE)

VARGO, EL; PORTER, SD. 1993

In the fire ant, *Solenopsis invicta*, some winged virgin queens are known to shed their wings (dealate) upon removal of the mated mother queen. These virgin queens then develop their ovaries and begin to lay eggs, thereby foregoing the option of leaving on mating flights and attempting to found their own colonies. Such a response of virgin queens to queenlessness has not been reported for other ants. In order to determine if virgin queens of some other fire ants (subgenus *Solenopsis*) would respond in the same way, experiments were conducted on *S. richteri*, hybrid *S. invicta/richteri* and *S. geminata*, a member of a species complex different from that of the other taxa. Just as in *S. invicta*, virgin queens of *S. richteri* and the hybrid dealated and began to lay eggs within days of the removal of the queen. In addition, workers executed many of the reproductively active virgin queens, a phenomenon also found in *S. invicta*. In contrast, virgin queens of *S. geminata* did not dealate or quickly begin to lay eggs upon separation from the queen. Reasons for the variability in the response of virgin queens of the different species may be 1) higher probability of reproductive success for unmated dealated queens compared to normal claustral founding in *S. invicta* and *S. richteri* linked to relatively frequent loss of the mother queen; or 2) phylogenetic constraint.

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## Phenotypic plasticity in an ant with strong caste-genotype association

Kuhn, A; Darras, H; Aron, S. 2018

Caste determination in social Hymenoptera (whether a female egg develops into a reproductive queen or a sterile worker) is a remarkable example of phenotypic plasticity where females with highly similar genomes exhibit striking differences in morphology and behaviour. This phenotypic dichotomy is typically influenced by environmental factors. However, recent studies have revealed a strong caste-genotype association in hybridogenetic ants: workers are all interlineage hybrids while queens are all purebred, suggesting that female caste fate is genetically determined. Using the hybridogenetic ant *Cataglyphis mauritanica*, we show that under laboratory conditions, purebred offspring develop into reproductive queens but occasionally give rise to workers. Moreover, while hybrids typically become workers, juvenile hormone treatment can switch their developmental pathway to the reproductive caste. These results indicate that phenotypic plasticity has been retained in an ant with a strong caste-genotype association, despite its lack of expression in natural conditions.

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## Pollination of Rapeseed (Brassica napus) by Africanized Honeybees (Hymenoptera: Apidae) on Two Sowing Dates

Chambo, ED; De Oliveira, NTE; Garcia, RC; Duarte, JB; Ruvolo-Takasusuki, MCC; Toledo, VA. 2014

In this study, performed in the western part of the state of Parana, Brazil, two self-fertile hybrid commercial rapeseed genotypes were evaluated for yield components and physiological quality using three pollination tests and spanning two sowing dates. The treatments consisted of combinations of two rapeseed genotypes (Hyola 61 and Hyola 433), three pollination tests (uncovered area, covered area without insects and covered area containing a single colony of Africanized *Apis mellifera* honeybees) and two sowing dates (May 25th, 2011 and June 25th, 2011). The presence of Africanized honeybees during flowering time increased the productivity of the rapeseed. Losses in the productivity of the hybrids caused by weather conditions unfavorable for rapeseed development were mitigated through cross-pollination performed by the Africanized honeybees. Weather conditions may limit the foraging activity of Africanized honeybees, causing decreased cross-pollination by potential pollinators, especially the Africanized *A. mellifera* honeybee. The rapeseed hybrids respond differently depending on the sowing date, and the short-cycle Hyola 433 hybrid is the most suitable hybrid for sowing under less favorable weather conditions.

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## Extensive mitochondrial heteroplasmy in the neotropical ants of the Ectatomma ruidum complex (Formicidae: Ectatomminae)

Meza-Lazaro, RN; Poteaux, C; Bayona-Vasquez, NJ; Branstetter, MG; Zaldivar-Riveron, A. NA

We assembled mitogenomes from 21 ant workers assigned to four morphospecies (*E. ruidum* spp. 1-4) and putative hybrids of the *Ectatomma ruidum* complex (*E. ruidum* spp. 2x3), and to *E. tuberculatum* using NGS data. Mitogenomes from specimens of *E. ruidum* spp. 3, 4 and 2 x 3 had a high proportion of polymorphic sites. We investigated whether polymorphisms in mitogenomes are due to nuclear mt paralogues (numts) or due to the presence of more than one mitogenome within an individual (heteroplasmy). We did not find loss of function signals in polymorphic protein-coding genes, and observed strong evidence for purifying selection in two haplotype-phased genes, which indicate the presence of two functional mitochondria' genomes coexisting within individuals instead of numts. Heteroplasmy due to hybrid paternal leakage is not supported by phylogenetic analyses. Our results reveal the presence of a fast-evolving secondary mitochondrial lineage of uncertain origin in the *E. ruidum* complex.

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#### **An annotated list of the Ants (Hymenoptera : Formicidae) of Mississippi**

Macgown, JA; Hill, JG; Brown, RL; Lewis, JG. 2021

One hundred and ninety-three species of ants, plus the hybrid fire ant, *Solenopsis invicta* X *richteri*, are reported for Mississippi. Thirty-two species are considered to be exotic to Mississippi. County records are given for each species.

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#### **Field releases of the decapitating fly *Pseudacteon curvatus* (Diptera : Phoridae) for control of imported fire ants (Hymenoptera : Formicidae) in Alabama, Florida, and Tennessee**

Graham, LC; Porter, SD; Pereira, RM; Dorough, HD; Kelley, AT. 2003

The little decapitating fly, *Pseudacteon curvatus* Borgmeier, was released at 11 sites in Alabama, Florida, and Tennessee as a potential self-sustaining biocontrol agent of imported fire ants. We used a biotype from Buenos Aires Province, Argentina that parasitizes black fire ants (*Solenopsis richteri* Forel). Generally, several thousand flies were released as larvae in parasitized ant workers over a 1-2 week period. *Pseudacteon curvatus* flies were successfully established on hybrid fire ants (*Solenopsis invicta* x *Solenopsis richteri*) at a site near Talladega, Alabama where they have persisted more than two years and expanded out 5-20 km from the original release site. Flies failed to establish in Florida and Tennessee although a few 1st-generation field-reared flies were recovered at four sites in Florida. This fly is only the second parasitoid species to be successfully released against imported fire ants or any other pest ant species. Possible reasons for failures at the other sites include insufficient vegetation cover, competition with another *Pseudacteon* species in Florida, severe winter kill of ants at a site in Tennessee, and the possibility that the biotype of *R. curvatus* released was not a viable parasitoid of red imported fire ants.

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#### **Responses of Parasitoids to Volatiles Induced by *Chilo partellus* Oviposition on Teosinte, a Wild Ancestor of Maize**

Mutyambai, DM; Bruce, TJA; Midega, CAO; Woodcock, CM; Caulfield, JC; Van Den Berg, J; Pickett, JA; Khan, ZR. 2015

Maize, a genetically diverse crop, is the domesticated descendent of its wild ancestor, teosinte. Recently, we have shown that certain maize landraces possess a valuable indirect defense trait not present in commercial hybrids. Plants of these landraces release herbivore-induced plant volatiles (HIPVs) that attract both egg [*Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae)] and larval [*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)] parasitoids in response to stemborer egg deposition. In this study, we tested whether this trait also exists in the germplasm of wild *Zea* species. Headspace samples were collected from plants exposed to egg deposition by *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) moths and unexposed control plants. Four-arm olfactometer bioassays with parasitic wasps, *T. bournieri* and *C. sesamiae*, indicated that both egg and larval parasitoids preferred HIPVs from plants with eggs in four of the five teosinte species sampled. Headspace samples from oviposited plants released higher amounts of EAG-active compounds such as (E)-4,8-dimethyl-1,3,7-nonatriene. In oviposition choice bioassays, plants without eggs were significantly preferred for subsequent oviposition by moths compared to plants with prior oviposition. These results suggest that this induced indirect defence trait is not limited to landraces but occurs in wild *Zea* species and appears to be an ancestral trait. Hence, these species possess a valuable trait that could be introgressed into domesticated maize lines to provide indirect defense mechanisms against stemborers.

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#### **A 24 kDa parasitism-specific protein from the Caribbean fruit fly, *Anastrepha suspensa*: cDNA and deduced amino acid sequence**

Shi, XZ; Gomez, SP; Lawrence, PO. 1999

A 24 kDa parasitism-specific protein (PSP24) was previously reported from the hemolymph of the Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae) after parasitization by the wasp *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). This study was designed to sequence the open reading frame of PSP24 and to determine whether it is encoded by the wasp, fruit fly host or by the entomopoxvirus D1EPV which is normally injected into the host with the wasp's egg. Utilizing an existing partial amino acid sequence of PSP24, we obtained two cDNAs by reverse transcription-polymerase chain reaction, from the host hemolymph 48 h post parasitization. The smaller cDNA has an open reading frame (ORF) that encodes 85 amino acids (aa) with a molecular mass of 9711.33 Da and the larger encodes 203 aa with a molecular mass of 23 076 Da. Both cDNAs share a common N-terminus with a signal peptide predictive of secreted proteins, a characteristic that agrees with the observed nature of PSP24. The mature proteins have 39 and 157 aa with deduced molecular masses of 4286.86 Da and 17 651 Da, respectively. Western blots of host hemolymph probed with the anti-PSP24 serum reveal proteins of 0.10 and 0.24 kD, respectively. The discrepancy between the deduced and the observed molecular masses may be explained by their predicted O-linked glycosylation. The amino acid sequences are not homologous with any protein in the available databases. Southern blot hybridization experiments revealed that the proteins are encoded by both the host and the parasite. Furthermore, injection of D1EPV into healthy fruit fly puparia induces the two proteins. Thus, in surprising contrast to an earlier hypothesis that D1EPV encodes PSP24, these results clearly demonstrate that the PSP24 proteins are encoded by wasp and fruit fly but not D1EPV genes. However, their expression is D1EPV induced. (C) 1999 Elsevier Science Ltd. All rights reserved.

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#### **Eleven microsatellite markers in *Nasonia*, ASHMEAD 1904 (Hymenoptera; Pteromalidae)**

Pietsch, C; Rutten, K; Gadau, J. 2004

We designed primer sequences for 11 microsatellite markers in the jewel wasp *Nasonia vitripennis*. Most loci could be cross-amplified in *Nasonia longicornis* and *Nasonia giraulti*, which make them amenable for linkage analysis in hybrid crosses. Eight loci were assigned to specific chromosomes. Additionally, 10 loci showed allelic variation in a *Nasonia vitripennis* field population. The observed number of alleles in this population ranged from two to seven, with observed heterozygosities from 0.0750 to 0.4750.

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#### **Studies on the Gall Characteristics of *Dryocosmus kuriphilus* in Chestnut Genotypes in Yalova and Bursa Provinces of Turkey**

Gencer, NS; Mert, C. 2019

The Asian chestnut gall wasp *Dryocosmus kuriphilus* is a global pest of chestnut (*Castanea* spp.). It has been spreading in Turkey's forests and orchards since 2014. This pest imposes a big threat to the Turkish chestnut industry, which is among the top producers in the world. Its gall morphology has been related to pest pressure and host cultivar, thus eventually modulating plant damage with heavy impact on growth and fruit production. We compared gall characters (position on plant organ, ratios, dimensions, volumes, number of larval chambers) in wild *Castanea sativa*, two local cultivars and a Euro Japanese hybrid. Overall, leaf galls were more common (55.36%), followed by the stem (19.6%) and leaf stipule galls (15.29%). The mean number of chamber and volume value of gall types were 1.52-5.93 and 0.43-2.15 cm(3), respectively. The highest values were observed in 'stem gall'. The more gall formation was observed in the wild chestnut trees and 'Marigoule' than the other local varieties.

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## **The complete mitochondrial genome of the invasive Africanized Honey Bee, *Apis mellifera scutellata* (Insecta: Hymenoptera: Apidae)**

Gibson, JD; Hunt, GJ. 2016

The complete mitochondrial genome from an Africanized honey bee population (AHB, derived from *Apis mellifera scutellata*) was assembled and analyzed. The mitogenome is 16,411bp long and contains the same gene repertoire and gene order as the European honey bee (13 protein coding genes, 22 tRNA genes and 2 rRNA genes). ND4 appears to use an alternate start codon and the long rRNA gene is 48bp shorter in AHB due to a deletion in a terminal AT dinucleotide repeat. The dihydrouacil arm is missing from tRNA-Ser (AGN) and tRNA-Glu is missing the TV loop. The A+T content is comparable to the European honey bee (84.7%), which increases to 95% for the 3rd position in the protein coding genes.

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## **Hybrid Genome Assembly of a Neotropical Mutualistic Ant**

Hartke, J; Schell, T; Jongepier, E; Schmidt, H; Sprenger, PP; Paule, J; Bornberg-Bauer, E; Schmitt, T; Menzel, F; Pfenninger, M; Feldmeyer, B. 2019

The success of social insects is largely intertwined with their highly advanced chemical communication system that facilitates recognition and discrimination of species and nest-mates, recruitment, and division of labor. Hydrocarbons, which cover the cuticle of insects, not only serve as waterproofing agents but also constitute a major component of this communication system. Two cryptic *Crematogaster* species, which share their nest with *Camponotus* ants, show striking diversity in their cuticular hydrocarbon (CHC) profile. This mutualistic system therefore offers a great opportunity to study the genetic basis of CHC divergence between sister species. As a basis for further genome-wide studies high-quality genomes are needed. Here, we present the annotated draft genome for *Crematogaster levior* A. By combining the three most commonly used sequencing techniques-Illumina, PacBio, and Oxford Nanopore-we constructed a high-quality de novo ant genome. We show that even low coverage of long reads can add significantly to overall genome contiguity. Annotation of desaturase and elongase genes, which play a role in CHC biosynthesis revealed one of the largest repertoires in ants and a higher number of desaturases in general than in other Hymenoptera. This may provide a mechanistic explanation for the high diversity observed in *C. levior* CHC profiles.

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## **Comparative resistance of four honey bee (Hymenoptera : Apidae) stocks to infestation by *Acarapis woodi* (Acari : Tarsonemidae)**

De Guzman, LI; Rinderer, TE; Delatte, GT. 1998

The prevalence, mean intensity, and abundance of *Acarapis woodi* (Rennie) in 4 selected stocks of honey bees (*Apis mellifera* L.) were monitored under field conditions. The test stocks used were ARS-Y-C-1 (*A. mellifera carnica* imported from Yugoslavia), Hastings (*A. mellifera carnica* imported from northern Saskatchewan), F-1 hybrid between ARS-Y-C-1 and Hastings, and Louisiana stock available at the Baton Rouge laboratory apiaries. Two trials were conducted. The 1st trial used 20 colonies per stock and continued for 2 yr. The 2nd trial used 10 colonies per stock and was monitored for 1 yr. Results of both trials showed that ARS-Y-C-1 and F-1 hybrids consistently maintained approximately a 10% level of *A. woodi* infestation, which is below the level that causes economic damage to host colonies. These stocks also had significantly lower mean intensities of mites than the Hastings and Louisiana bees. *A. woodi* was least abundant in the F-1 hybrid. Similar numbers of mites per bee were found in ARS-Y-C-1, Hastings and Louisiana stocks. These observations suggest that both ARS-Y-C-1 and F-1 hybrids are resistant to *A. woodi*.

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## **Host-plant mediated effects of transgenic maize on the insect parasitoid *Campoletis sonorensis* (Hymenoptera : Ichneumonidae)**

Sanders, CJ; Pell, JK; Poppy, GM; Raybould, A; Garcia-Alonso, M; Schuler, TH. 2007

Determining the impact of genetically modified (GM) crops on beneficial organisms is an important aspect of the environmental risk assessment of GM crops. In the present study, the impact of Bt maize expressing Cry1Ab on the development and behaviour of the parasitoid *Campoletis sonorensis* was compared to individuals reared on hosts fed conventionally bred plants partially resistant to the European corn borer (*Ostrinia nubilalis* Hubner) and on susceptible maize hybrids. Adult parasitoids reared on Bt maize-fed *Spodoptera frugiperda* larvae were significantly smaller (15-30%) than those reared in hosts fed either of the conventional maize hybrids. The magnitude of this effect was dependent on the size of the host at oviposition and its subsequent growth rate. The development time of *C. sonorensis* was not affected by the maize treatment. In choice tests, female parasitoids displayed no preference for hosts fed a specific maize hybrid. No Cry1Ab was detected within adult parasitoids. (c) 2007 Elsevier Inc. All rights reserved.

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## **An Indirect Defence Trait Mediated through Egg-Induced Maize Volatiles from Neighbouring Plants**

Mutyambai, DM; Bruce, TJA; van den Berg, J; Midega, CAO; Pickett, JA; Khan, ZR. 2016

Attack of plants by herbivorous arthropods may result in considerable changes to the plant's chemical phenotype with respect to emission of herbivore-induced plant volatiles (HIPVs). These HIPVs have been shown to act as repellents to the attacking insects as well as attractants for the insects antagonistic to these herbivores. Plants can also respond to HIPV signals from other plants that warn them of impending attack. Recent investigations have shown that certain maize varieties are able to emit volatiles following stemborer egg deposition. These volatiles attract the herbivore's parasitoids and directly deter further oviposition. However, it was not known whether these oviposition-induced maize (*Zea mays*, L.) volatiles can mediate chemical phenotypic changes in neighbouring unattacked maize plants. Therefore, this study sought to investigate the effect of oviposition-induced maize volatiles on intact neighbouring maize plants in 'Nyamula', a landrace known to respond to oviposition, and a standard commercial hybrid, HB515, that did not. Headspace volatile samples were collected from maize plants exposed to *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) egg deposition and unoviposited neighbouring plants as well as from control plants kept away from the volatile emitting ones. Behavioural bioassays were carried out in a four-arm olfactometer using egg (*Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae)) and larval (*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)) parasitoids. Coupled Gas Chromatography-Mass Spectrometry (GC-MS) was used for volatile analysis. For the 'Nyamula' landrace, GC-MS analysis revealed HIPV production not only in the oviposited plants but also in neighbouring plants not exposed to insect eggs. Higher amounts of EAG-active biogenic volatiles such as (E)-4,8-dimethyl-1,3,7-nonatriene were emitted from these plants compared to control plants. Subsequent behavioural assays with female *T. bournieri* and *C. sesamiae* parasitic wasps indicated that these parasitoids preferred volatiles from oviposited and neighbouring landrace plants compared to those from the control plants. This effect was absent in the standard commercial hybrid we tested. There was no HIPV induction and no difference in parasitoid attraction in neighbouring and control hybrid maize plants. These results show plant-plant signalling: 'Nyamula' maize plants emitting oviposition-induced volatiles attractive to the herbivore's natural enemies can induce this indirect defence trait in conspecific neighbouring undamaged maize plants. Maize plants growing in a field may thus benefit from this indirect defence through airborne signalling which may enhance the fitness of the volatile-emitting plant by increasing predation pressure on herbivores.

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

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

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

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#### Division of labor in colonies of the eusocial wasp, *Mischocyttarus consimilis*

Torres, VO; Montagna, TS; Raizer, J; Antonialli, WF. 2012

The division of labor between castes and the division of labor in workers according to age (temporal polyethism) in social wasps are crucial for maintaining social organization. This study evaluated the division of labor between castes, and the temporal polyethism in workers of *Mischocyttarus consimilis* Zikan (Hymenoptera: Vespidae). To describe the behavioral repertory of this species, observations were made of 21 colonies, with 100 hours of observations. In order to observe temporal polyethism, each newly emerged wasp was marked with colored dots on the upper area of the thorax. This allowed the observation of behavioral acts performed by each worker from the time of emergence to its death. Through hybrid multidimensional scaling, a clear division between queens and workers could be identified, in which the behaviors of physical dominance and food solicitation characterized the queen caste; while behaviors such as adult-adult trophallaxis, destruction of cells, alarm, foraging for prey, foraging for nectar, and unsuccessful foraging characterized the worker caste. Hybrid multidimensional scaling characterized two groups, with intra-nest activities preferentially accomplished by younger workers, while extra-nest activities such as foraging were executed more frequently by older workers.

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#### Analysis of phylogenomic datasets reveals conflict, concordance, and gene duplications with examples from animals and plants

Smith, SA; Moore, MJ; Brown, JW; Yang, Y. 2015

Background: The use of transcriptomic and genomic datasets for phylogenetic reconstruction has become increasingly common as researchers attempt to resolve recalcitrant nodes with increasing amounts of data. The large size and complexity of these datasets introduce significant phylogenetic noise and conflict into subsequent analyses. The sources of conflict may include hybridization, incomplete lineage sorting, or horizontal gene transfer, and may vary across the phylogeny. For phylogenetic analysis, this noise and conflict has been accommodated in one of several ways: by binning gene regions into subsets to isolate consistent phylogenetic signal; by using gene-tree methods for reconstruction, where conflict is presumed to be explained by incomplete lineage sorting (ILS); or through concatenation, where noise is presumed to be the dominant source of conflict. The results provided herein emphasize that analysis of individual homologous gene regions can greatly improve our understanding of the underlying conflict within these datasets. Results: Here we examined two published transcriptomic datasets, the angiosperm group Caryophyllales and the aculeate Hymenoptera, for the presence of conflict, concordance, and gene duplications in individual homologs across the phylogeny. We found significant conflict throughout the phylogeny in both datasets and in particular along the backbone. While some nodes in each phylogeny showed patterns of conflict similar to what might be expected with ILS alone, the backbone nodes also exhibited low levels of phylogenetic signal. In addition, certain nodes, especially in the Caryophyllales, had highly elevated levels of strongly supported conflict that cannot be explained by ILS alone. Conclusion: This study demonstrates that phylogenetic signal is highly variable in phylogenomic data sampled across related species and poses challenges when conducting species tree analyses on large genomic and transcriptomic datasets. Further insight into the conflict and processes underlying these complex datasets is necessary to improve and develop adequate models for sequence analysis and downstream applications. To aid this effort, we developed the open source software phyparts (<https://bitbucket.org/blackrim/phyparts>), which calculates unique, conflicting, and concordant bipartitions, maps gene duplications, and outputs summary statistics such as internode certainty (ICA) scores and node-specific counts of gene duplications.

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#### Expression of immune-response genes in lepidopteran host is suppressed by venom from an endoparasitoid, *Pteromalus puparum*

Fang, Q; Wang, L; Zhu, JY; Li, YM; Song, QS; Stanley, DW; Akhtar, ZR; Ye, GY. 2010

Background: The relationships between parasitoids and their insect hosts have attracted attention at two levels. First, the basic biology of host-parasitoid interactions is of fundamental interest. Second, parasitoids are widely used as biological control agents in sustainable agricultural programs. Females of the gregarious endoparasitoid *Pteromalus puparum* (Hymenoptera: Pteromalidae) inject venom along with eggs into their hosts. *P. puparum* does not inject polydnaviruses during oviposition. For this reason, *P. puparum* and its pupal host, the small white butterfly *Pieris rapae* (Lepidoptera: Pieridae), comprise an excellent model system for studying the influence of an endoparasitoid venom on the biology of the pupal host. *P. puparum* venom suppresses the immunity of its host, although the suppressive mechanisms are not fully understood. In this study, we tested our hypothesis that *P. puparum* venom influences host gene expression in the two main immunity-conferring tissues, hemocytes and fat body. Results: At 1 h post-venom injection, we recorded significant decreases in transcript levels of 217 EST clones (revealing 113 genes identified in silico, including 62 unknown contigs) derived from forward subtractive libraries of host hemocytes and in transcript levels of 288 EST clones (221 genes identified in silico, including 123 unknown contigs) from libraries of host fat body. These genes are related to insect immune response, cytoskeleton, cell cycle and apoptosis, metabolism, transport, stress response and transcriptional and translational regulation. We verified the reliability of the suppression subtractive hybridization (SSH) data with semi-quantitative RT-PCR analysis of a set of randomly selected genes. This analysis showed that most of the selected genes were down-regulated after venom injection. Conclusions: Our findings support our hypothesis that *P. puparum* venom influences gene expression in host hemocytes and fat body. Specifically, the venom treatments led to reductions in expression of a large number of genes. Many of the down-regulated genes act in immunity, although others act in non-immune areas of host biology. We conclude that the actions of venom on host gene expression influence immunity as well as other aspects of host biology in ways that benefit the development and emergence of the next generation of parasitoids.

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#### Geographic variation in *Tetragonisca angustula* (Hymenoptera, Apidae, Meliponinae)

Castanheira, EB; Contel, EPB. 2005

*Tetragonisca angustula* is a small stingless bee occurring throughout Brazil as two subspecies. *Tetragonisca angustula fiebrigi* has a ferruginous coloured thoracic region (mesepisternum) and is found in south-western Brazil. In most parts of Latin America *T. a. angustula*, which has a black mesepisternum, is the common subspecies. A sample of *T. a. angustula* collected in south-east Brazil presented only the hexokinase Hk(100) allele, while a sample of *T. a. fiebrigi* displayed a high frequency of the Hk(88) allele. Samples from locations between these two regions had a ferruginous-and-black stained mesepisternum and intermediary Hk(88) allele frequencies suggesting a clinal distribution or racial admixture between the two subspecies. There was a highly significant correlation between the amount of ferruginous colour displayed in the mesepisternum and the Hk(88) allele frequency. Morphometric analysis of worker wings also suggests clinal distribution or racial admixture because the wings in the hybrid sample were of intermediate size.

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## Whole Genome Sequencing and Assembly of the Asian Honey Bee *Apis dorsata*

Oppenheim, S; Cao, XL; Rueppel, O; Krongdang, S; Phokasem, P; DeSalle, R; Goodwin, S; Xing, JC; Chantawannakul, P; Rosenfeld, JA. 2020

The Asian honey bee (*Apis dorsata*) is distinct from its more widely distributed cousin *Apis mellifera* by a few key characteristics. Most prominently, *A. dorsata* nest in the open by forming a colony clustered around the honeycomb, whereas *A. mellifera* nest in concealed cavities. Additionally, the worker and reproductive castes are all of the same size in *A. dorsata*. In order to investigate these differences, we performed whole genome sequencing of *A. dorsata* using a hybrid Oxford Nanopore and Illumina approach. The 223 Mb genome has an N50 of 35 kb with the largest scaffold of 302 kb. We have found that there are many genes in the *dorsata* genome that are distinct from other hymenoptera and also large amounts of transposable elements, and we suggest some candidate genes for *A. dorsata*'s exceptional level of defensive aggression.

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## BEES OF THE SUBGENUS SCAPHANDRENA (GENUS ANDRENA) IN COLORADO (HYMENOPTERA, APOIDEA)

LANHAM, UN. 1993

Colorado *Scaphandrena* in the strict sense are currently listed as members of a trihybrid cross, "*scurra* x *arabis* x *capricornis*" Ribble, under which 7 species are synonymized. Here *A. scurra* is considered a non-hybrid, and three species are resurrected from synonymy-*ellisiae* Cockerell, *montrosensis* Viereck and Cockerell and *sieverti* Cockerell. A key to species and data on distribution and composition of samples taken in a field survey are given. Trihybrid and dihybrid hypotheses explaining the Rocky Mountain *Scaphandrena* fauna are discussed.

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## Natural Biological Control of *Diaphania* spp. (Lepidoptera: Crambidae) by Social Wasps

Santana, PA; Gonring, AHR; Picanco, MC; Ramos, RS; Martins, JC; Ferreira, DD. 2012

The social wasps (Hymenoptera: Vespidae) are important agents of biological control for agricultural pests. *Diaphania hyalinata* L. and *Diaphania nitidalis* Cramer (Lepidoptera: Crambidae) are among the main pests of plants in the Cucurbitaceae family. Although the importance of social wasps is acknowledged, little is known about their activity as biological control agents in *Diaphania* spp. Thus, this work aimed to study the natural biological control of the caterpillars *D. hyalinata* and *D. nitidalis* by social wasps. We studied the natural biological control of caterpillars of *D. hyalinata* and *D. nitidalis* on cucumber hybrids Sprint 440 II and Vlasstar. The main predators of *Diaphania* caterpillars were the social wasps, followed by Diptera: Syrphidae; Hemiptera: Anthocoridae; Coleoptera: Coccinellidae, Anthicidae; Neuroptera: Chrysopidae and Arachnida: Araneae. Predation of *D. hyalinata* caterpillars by social wasps was high from the second to fifth instar. The predation of *D. nitidalis* caterpillars by social wasps was high from the second to fourth instar. There was no predation by social wasps on the first instar larvae of *Diaphania* spp. The cucumber hybrids did not influence the predation of *Diaphania* spp. by social wasps. The main social wasp predator of *Diaphania* spp. was *Polybia ignobilis* (Haliday). Also, we observed the social wasp *Polybia scutellaris* (White) preying on *D. hyalinata* but at low intensity.

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## Genetically manipulated Brassica genotypes affect demography and performance of *Diadegma semiclausum* parasitizing *Plutella xylostella*

Nikooei, M; Fathipour, Y; Javaran, MJ; Soufbaf, M. 2017

The performance of *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) reared on canola's progenitor (*Brassica rapa* L.), two cultivated canola cultivars (Opera and RGS(003)), one hybrid (Hyula(401)), one gamma-ray mutant-RGS(003) and one transgenic (PF) genotype was compared using the age-stage, two-sex life table parameters. All experiments were carried out in a growth chamber at 25 +/- 1 degrees C, 65 +/- 5% RH and a photoperiod of 16:8 (L:D) h. There were significant differences in duration of different life stages of *D. semiclausum* on its host larvae reared on different plant genotypes. The shortest (12.27days) and longest (15.21days) pre-adult developmental times were observed on cultivar-RGS(003) and hybrid-Hyula(401), respectively. The intrinsic rate of increase (*r*) in *D. semiclausum* ranged between 0.189/day (cultivar-Opera) and 0.141/day (transgenic-PF). Moreover, the highest (20.078 offspring) and lowest (12.027 offspring) net reproductive rates (*R*-0) were observed on cultivar-Opera and hybrid-Hyula(401). The mean generation time (*T*) of *D. semiclausum* was the highest (18.34days) and lowest (15.05days) on mutant-RGS(003) and cultivar-RGS(003). The maximum and minimum parasitism values of this parasitoid were observed on canola's progenitor (44.28%) and hybrid-Hyula(401) (37.09%). The heaviest pupae (3.82mg) and females (3.22mg) of the parasitoid were found on canola's progenitor and cultivar-Opera, respectively. The results showed that performance of this parasitoid was better on canola's progenitor and cultivated plants known to have higher levels of glucosinolates concentration than others.

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## A Survey of the Insects of the Southern High Plains (Llano Estacado) of Texas, with Particular Reference to Pollinators and Other Anthophiles

Begosh, A; Overall, LM; Smith, LM; McMurtry, ST. 2018

The economic value of insect pollination to the world economy is tremendous, and pollination service is critical for our food supply and persistence of native plants. Little data are currently available on pollinator occurrence in the Southern High Plains (SHP) of Texas. Past studies on hybrid cottonseed production in the region documented high abundance of six species of *Agapostemon* and lower abundance of *Diadasia* spp., *Melissodes* spp., and *Perdita* spp. Historical records indicated the presence of *Bombus fraternus* and *B. pensylvanicus* which now have Endangered and Vulnerable IUCN status respectively. In 2013 and 2014, we used blue vane traps and targeted netting to collect insect pollinators and anthophiles on the three dominant land uses in the region; cropland, Conservation Reserve Program (CRP), and native grasslands. With capture methods combined, we collected 74,215 insect specimens; 63% of the specimens were Hymenoptera (289 taxa), 28.61% were Coleoptera (150 taxa), 4.48% were Diptera (115 taxa), 2.34% were Lepidoptera (60 taxa), and 1.61% were of other orders (15 taxa). Our capture included all previously documented bee genera, but we did not detect *B. fraternus*. Studies support that blue vane traps are an effective method of trapping bees and our results demonstrated that they are also effective in attracting some families of anthophilous Coleoptera and Diptera. However, we captured several species of Hymenoptera, Diptera, and Lepidoptera with aerial nets that we did not capture in blue vane traps. This study documents pollinator and anthophile species present in a highly altered landscape and is the most comprehensive list of pollinators and anthophiles in this region to date.

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## Taxonomic revision proves *Trachusa pubescens* (Morawitz, 1872) *sensu lato* to be a complex of allopatric and sympatric species in South-Eastern Europe and Western Asia (Hymenoptera, Apoidea, Anthidiini)

Kasperek, M. 2018

*Trachusa pubescens* (Morawitz, 1872) s. 1. has a distribution extending from south-eastern Europe over Anatolia and the Caucasus to Iran and Turkmenistan, and was formerly regarded as a species with high intraspecific variation. By means of an examination of 208 specimens from all parts of the distribution area, covering structural features of the head (mandibles, dypeus), the apical terga and the genitalia, the colouration pattern as well as a morphometric analysis of 26 body measurements with multivariate statistical methods (Principal Component Analysis, Discriminant Analysis), it was possible to assign the material to five species of

which two are new to science (*Trachusa balcanica* sp. n. and *T. hakkariensis* sp. n.). Two taxa which had previously been described as "variations" or subspecies are elevated to species rank: *T. verhoeffi* (Mavromoustakis, 1955), stat. n. and *T. maxima* (Friese, 1931), stat. n. Additionally, some populations can be distinguished by their colouration pattern or by subtle differences in size or body shape, but these features are apparently of no taxonomic significance at the species level. *Trachusa balcanica* sp. n. and *T. verhoeffi* have distribution areas which do not overlap with any of the other members of the species group and can thus be characterised as allospecies. By contrast, the distribution areas of the other three species, *T. pubescens*, *T. maxima* and *T. hakkariensis* sp. n., overlap to a certain extent and they co-exist at least to some degree in sympatry. While they have been found in the same region, they have so far never been found together at exactly the same location and it is suggested that species divergence occurred in parallel with ecological differentiation. Niche partitioning such as flower preferences is a mechanism which may be invoked to explain this. Some specimens with intermediate characters were found, particularly in contact zones, and it is thought that some hybridisation may occur. A partly melanistic individual of *T. balcanica* sp. n. was found, which is probably the first described melanistic individual in the tribe Anthidiini.

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#### **Africanized honey bees (Hymenoptera : Apidae) have a greater fidelity to sunflowers than European bees**

Basualdo, M; Bedascarrasbure, E; De Jong, D. 2000

A study of sunflower, *Helianthus annuus* L., pollen collection by Africanized and European honey bees, *Apis mellifera* L., was conducted in a hybrid seed production field in Argentina. Africanized honey bees collected significantly larger proportions of sunflower pollen than did European honey bees. The result suggests that Africanized bees would be more efficient for commercial sunflower seed production.

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#### **A REVISION OF THE FIRE ANTS, SOLENOPSIS-GEININATA GROUP (HYMENOPTERA, FORMICIDAE, MYRMICINAE)**

TRAGER, JC. 1991

The subgenera and satellite genera of *Solenopsis* are reviewed. Synonymy under *Solenopsis* of all subgenera and of the genera *Bisolenopsis*, *Synsolenopsis*, *Paranamyra*, and *Labauchena* is confirmed. Certain placement of the genus *Lilidris*, known from a single alate female, will require study of additional material, but *Lilidris* appears to be distinct from *Solenopsis*. The fire ants and their close relatives, *S. substituta* and *S. tridens*, are collectively designated as the *S. geminata* species group, which together with the parasitic *S. daguerrei* group (*Labauchena*) form a monophyletic lineage. *S. virulens*, a species phenetically similar to minors of *S. saevissima*, is also included in this revision, though it probably does not belong in the *S. geminata* group. Four native species, 2 introduced species, and 2 hybrid forms occur in North America. Seventeen species are known from South America. A key to major workers and illustrations of all species are included. Notes on the identification of queens are provided where these are sufficiently distinctive. The native North American species are *S. aurea* Wheeler, *S. amblychila* Wheeler new status, *S. geminata* (Fabricius), and *S. xyloni* (MacCook), and the introduced species are *S. invicta* Buren and *S. richteri* Forel from South America. *S. xyloni* x *geminata* and *S. invicta* x *richteri* are the hybrid forms. *S. invicta* x *richteri* is abundant and highly fertile in parts of Alabama, Mississippi and northwestern Georgia in North America, but this hybrid has not been observed in South America, even though the parent species have overlapping distributions in at least Santa Fe Province, Argentina. In South America, only *S. bruesi* Creighton new status, *S. gayi* (Spinola), *S. geminata* and *S. weyrauchi* new species occur in or to the west of the Andes. The latter is unusual in that it occurs at elevations up to 3,500 m or higher in the Peruvian Andes. To the east of the Andes are found *S. electra* Forel new status, *S. geminata*, *S. interrupta* Santschi, *S. invicta*, *S. macdonaghi* Santschi new status, *S. megergates* new species, *S. pusillignis* new species, *S. pythia* Santschi, *S. quinquecupis* Forel, *S. richteri*, *S. saevissima* F. Smith, *S. substituta* Santschi new status, and *S. tridens* Forel. With the exception of *S. geminata*, *S. saevissima*, and *S. invicta* (at least part of whose geographic range is in the rain forest region) most of the South American species are endemic to the monsoon tropics or warm temperate regions of the southern part of the continent. *S. virulens* (F. Smith) new status, probably not a member of the *S. geminata* group but superficially resembling them, inhabits the forests of Amazonia.

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#### **Structure and composition of the assemblage of parasitoids associated to *Phyllocnistis citrella* pupae stainton (Lepidoptera : Gracillariidae) in citrus orchards in southern Brazil**

Jahnke, SM; Redaelli, LR; Diefenbach, LMG; Dal Soglio, FK. 2007

The structure and composition of the assemblage of pupal parasitoids of *Phyllocnistis citrella* Stainton, the citrus leafminer, were studied in two citrus orchards (*Citrus deliciosa* Tenore cv. Montenegrina and *Citrus sinensis* (L.) Osbeck x *Citrus reticulata* Blanco hybrid Murcott), in Montenegro County (29 degrees 68'S and 51 degrees 46'W), southern Brazil. At fortnightly samplings, from July 2001 to June 2003, all the new shoots from 24 randomly selected trees were inspected. The species richness reached five native species in the Murcott orchard, and six in Montenegrina. In Murcott, the presence of *Ageniaspis citricola* (Hymenoptera: Encyrtidae), an exotic species, was detected in the first year of sampling, probably migrating from the nearby areas where it had been released for the miner control. In Montenegrina, its presence was only registered in the second year. *A. citricola* in both areas was dominant and changed the community structure of parasitoid complex of *P. citrella* in both orchards.

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#### **INFLUENCE OF PHENOLGLUCOSIDES AND TRICHOME DENSITY ON THE DISTRIBUTION OF INSECTS HERBIVORES ON WILLOWS**

SOETENS, P; ROWELLRAHIER, M; PASTEELS, JM. 1991

The effects of both trichome density and phenolglucoside content of leaves of 76 willow hybrids (*Salix alba* x *fragilis*) were measured to estimate their influence on the distribution of *Phratora vitellinae* (L.), *Plagiodera versicolora* Baly (Coleoptera: Chrysomelidae) and *Pontania proxima* (Lepelletier 1823) (Hymenoptera: Tenthredinidae) in a nursery at Gramont, Belgium. The willows showed differences in their phenolglucoside content and pilosity of leaves and are classified on these basis into four groups by a clustering method. Correlations and multiple regressions showed that these chemical and physical characteristics are good predictors of the abundance of insects. First, the abundance of larvae of *Ph. vitellinae*, adults of *Pl. versicolora* and galls of *P. proxima* is correlated positively with a high phenolglucoside content and a low pilosity of the leaves. Secondly, the distribution of adults of *Ph. vitellinae* and of larvae of *Pl. versicolora* is influenced by neither the chemical nor the physical leaves characteristics studied.

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#### **Phenology and Development of the Yellow Rose Aphid, *Acythosiphon porosum* (Sanderson), in Northern Florida and Southern Georgia**

Mizell, RF; Hudson, WG. 2009

The yellow rose aphid, *Acythosiphon porosum* (Sanderson), is a cosmopolitan pest of roses and strawberries. *Acythosiphon porosum* is one of only a few arthropod pests of roses occurring in northern Florida and southern Georgia. Hybrid roses are widely planted around the city of Thomasville, GA, which is known as the Rose City. Three 'Grandiflora' rose cultivars 'Queen Elizabeth', 'Roundelay' and 'Spartan' were used to determine cultivar and location effects on *A. porosum* seasonal abundance and natural enemies. Developmental rate, longevity and nymph production of *A. porosum* were also investigated at 4 temperatures, 10.0, 15.5, 21.1, and 26.7 degrees C, in environmental chambers using cut leaf discs of 'Queen Elizabeth' on agar plates. *Acythosiphon porosum* occurred throughout much of the year except for the coldest winter and warmest summer months and reached highest populations during the cooler weather of spring and fall. Cultivar differences were statistically significant at all locations where 'Queen Elizabeth' averaged higher aphids per leaf than 'Roundelay', which averaged higher aphids than 'Spartan'. Aphid numbers did not significantly differ in comparisons of leaves sampled from the bottom, middle or top third of the plant's canopy height. Developmental rate calculated



as the time from nymph to nymph was most rapid ((x) over bar = 10.3 d) at 21.1 degrees C, but no aphid development was observed at 26.7 degrees C. Total egg production was highest ((x) over bar = 27.5 nymphs per aphid) at 10.0 degrees C as was longevity of 60.1 d, Highest nymph production (1.5 per d) was observed at 21.1 degrees C. Many generalist predators and two parasitoid species were found in low numbers attacking A. porosum: Syrphophagous aphidovorus (Mayr) (Hymenoptera: Encyrtidae) and Ephedius sp. (Hymenoptera: Aphididae).

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#### Genome wide association analysis of a stemborer egg induced "call-for-help" defence trait in maize

Tamiru, A; Paliwal, R; Manthi, SJ; Odeny, DA; Midega, CAO; Khan, ZR; Pickett, JA; Bruce, TJA. 2020

Tritrophic interactions allow plants to recruit natural enemies for protection against herbivory. Here we investigated genetic variability in induced responses to stemborer egg-laying in maize *Zea mays* (L.) (Poaceae). We conducted a genome wide association study (GWAS) of 146 maize genotypes comprising of landraces, inbred lines and commercial hybrids. Plants were phenotyped in bioassays measuring parasitic wasp *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) attraction to volatiles collected from plants exposed to stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) eggs. Genotyping-by-sequencing was used to generate maize germplasm SNP data for GWAS. The egg-induced parasitoid attraction trait was more common in landraces than in improved inbred lines and hybrids. GWAS identified 101 marker-trait associations (MTAs), some of which were adjacent to genes involved in the JA-defence pathway (*opr7*, *aos1*, 2, 3), terpene biosynthesis (*fps3*, *tps2*, 3, 4, 5, 7, 9, 10), benzoxazinone synthesis (*bx7*, 9) and known resistance genes (e.g. maize insect resistance 1, *mir1*). Intriguingly, there was also association with a transmembrane protein kinase that may function as a receptor for the egg elicitor and other genes implicated in early plant defence signalling. We report maize genomic regions associated with indirect defence and provide a valuable resource for future studies of tritrophic interactions in maize. The markers identified may facilitate selection of indirect defence by maize breeders.

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#### Inheritance of malathion resistance in the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae)

Baker, JE; PerezMendoza, J; Beeman, RW. 1997

The genetic basis of malathion resistance in the Bamberg (R) strain of *Anisopteromalus calandrae* (Howard) was determined with reference to a susceptible (S) laboratory strain of the parasitoid. Evidence from bioassays of male and female progeny from the parent R and S strains, F1 hybrids, backcrosses, and inter se (self) crosses indicates that the malathion resistance in this haplo-diploid wasp is inherited as an incompletely dominant trait controlled by a single gene. This inheritance pattern will slow the diluting effect of interbreeding of progeny from released resistant wasps with susceptible wasps present in any resident population within a particular grain storage facility. It may also allow either an inoculative or inundative release strategy, depending on the size of the resident parasitoid population, within an integrated chemical-biological management program for grain weevils in stored cereals.

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#### Africanized Honey Bee Sting-Induced Stress-Related Cardiomyopathy: A Bee or Octopus Trap

Seecheran, RV; Ramdin, R; Singh, S; Seecheran, V; Persad, S; Peram, L; Raza, SS; Seecheran, NA. 2021

The Africanized honey bee commonly referred to as the "killer bee," is a hybrid of the East African lowland honey bee with various European honey bees. These bees tend to be more hostile as compared to other bee species. Their stings may have devastating clinical sequelae, including cardiovascular, cerebrovascular events, and fatalities. We report the first-in-Caribbean case of a middle-aged woman who experienced stress-related, Takotsubo cardiomyopathy (also known as stress-related cardiomyopathy [SRC]) after being afflicted with innumerable Africanized honey bee stings.

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#### Asexuality cured: The relations and differences between sexual and asexual *Apoanagyrus diversicornis*

Pijls, JWAM; vanSteenbergen, HJ; vanAlphen, JJM. 1996

We investigated whether asexual (thelytokous) *Apoanagyrus diversicornis* and sexual (arrhenotokous) *A. diversicornis* (Hymenoptera: Encyrtidae) belong to the same biological species. The strains are allopatric and there are no morphological differences. The sexual *A. diversicornis* strain originates from south-western Brazil, It was introduced into Africa for the biological control of the cassava mealybug *Phenacoccus manihoti*. The asexual strain parasitizes *P. herreni* in Colombia. Feeding antibiotics to asexual *A. diversicornis* or rearing parasitized hosts at 33 instead of 25 degrees C led to male offspring production. These males could be crossed with virgin females of the sexual strain, leading to fertile female (i.e. hybrid) offspring. There were no indications of hybrid breakdown. Thus, asexuality in *A. diversicornis* is caused by microorganisms, can be cured, and the two strains belong to the same biological species. The microorganisms associated with thelytoky in *A. diversicornis* belong to the genus *Wolbachia*. There were differences between *A. diversicornis* strains and between host species in survival probability from egg to adult. In addition, some mortality was associated with the feeding of tetracycline and with the production of males, but not with rifampicin, sulphadiazine (which was not effective), or a temperature increase from 35 to 33 degrees C. Survival of hybrids was intermediate between the survival probabilities of the parental strains. Male behaviour and function were not different between the strains, but female behaviour clearly was. Females of the thelytokous strain were less often inseminated by males of either strain than were arrhenotokous females. This suggests a difference in the rate of decay between expressed traits (female sexual behaviour, which is possibly selected against) and traits that are not expressed (male sexual behaviour and function).

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#### Live oaks, new hosts for *Odontocynips nebulosa* Kieffer (Hymenoptera : Cynipidae) in North America

Wilson, AD; Lester, DG; Edmonson, RE. 2000

A study of root-feeding insects as potential vectors of the oak wilt fungus *Ceratocystis fagacearum* (T. W. Bretz) J. Hunt in live oaks, revealed root galls induced by the cynipid gall wasp *Odontocynips nebulosa* Kieffer The incidence of the wasp on roots of four oak species and natural live oak hybrids at 14 root excavation sites in 7 counties throughout the Hill Country of central Texas was surveyed. The study was limited to sites within and adjacent to oak wilt infection centers of the live oak-Ashe juniper ecotype where oak wilt infected live oaks were being uprooted and rogued for disease suppression by the Texas Oak Wilt Suppression Project. This is the first report of this root-galling wasp on live oaks, *Q. fusiformis* Small and *Quercus virginiana* Miller x *Quercus fusiformis* natural hybrids, in North America. The incidence of root-galling by the wasp occurred at relatively low levels among trees examined at excavation sites in each county, indicating a sporadic distribution throughout the region. However, examinations of root-colonization and gall induction by *O. nebulosa* in uprooted live oaks showed relatively high levels of root infestations in some trees, including trees exhibiting symptoms of oak wilt disease. This pattern suggests high population densities in small localized areas. Examinations of individual gall clusters formed by the wasp on live oak roots revealed new details of gall morphology and developmental stages of the insect within galls. The significance of this wasp as a potential vector of the oak wilt fungus is discussed.

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#### Ant (Hymenoptera: Formicidae) Communities of the Southern Cumberland Plateau

Davis, RA; Zigler, KS. 2012

The Cumberland Plateau supports diverse plant communities that vary greatly across cove and plateau habitats. Unfortunately, little is known about how terrestrial invertebrate communities vary across cove and plateau habitats and how disturbance affects these communities. To address this deficiency, we used the Ant Leaf Litter protocol to survey ant diversity on the southern Cumberland Plateau in Tennessee. We surveyed forested habitats on top of the plateau surface and on the cove slopes, as well as sites that were logged within the past 6 yr. After surveying 14 sites we found 1) 10,130 ants representing 55 species from 21 genera and six subfamilies, including five new records for Tennessee; 2) significant differences in ant communities by habitat type; 3) that south-facing cove slope ant communities more closely resembled the ant community of plateau surface native forest sites than the ant community of north-facing cove slopes; 4) that recently logged sites hosted a distinct ant community (including hybrid imported fire ants) not found in undisturbed habitats; and 5) by resampling three sites surveyed 5 yr earlier, that the ant leaf litter protocol can be used to track ant community change over time. Ants thus proved to be an excellent system for examining invertebrate diversity across habitats on the Cumberland Plateau.

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#### **CULTIVAR RESISTANCE AND ARTHROPOD PREDATION OF SUGARCANE BORER (LEPIDOPTERA, PYRALIDAE) AFFECTS INCIDENCE OF DEADHEARTS IN LOUISIANA SUGARCANE**

BESSIN, RT; REAGAN, TE. 1993

A 3-yr study on overwintering populations of the sugarcane borer, *Diatraea saccharalis* (F.), showed cultivar resistance provided significant reductions in numbers of sugarcane, a complex hybrid of *Saccharum* spp., deadhearts in the spring of 1986 and 1987. Although differences in infestation levels of *D. saccharalis* caused by arthropod predation were not detected in the fall, significantly greater numbers of deadhearts occurred in the spring where predators were suppressed. The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), was the dominant predator in these studies. Incorporating a covariable representing the number of larvae infesting sugarcane stalks in the fall did not adequately account for the variation in sugarcane shoots with deadhearts. Results from this study indicate that, in addition to the anticipated effect of cultivar resistance and predation on the stalk damage caused by *D. saccharalis* during the growing season, substantial reductions in the incidence of spring deadhearts can also be expected. The effect of cultivar resistance and arthropod predation of *D. saccharalis* on deadhearts is additive.

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#### **Differentiation of *Melipona quadrifasciata* L. (Hymenoptera, Apidae, Meliponini) subspecies using cytochrome b PCR-RFLP patterns**

Souza, RO; Moretto, G; Arias, MC; Del Lama, MA. 2008

*Melipona quadrifasciata quadrifasciata* and *M. quadrifasciata anthidioides* are subspecies of *M. quadrifasciata*, a stingless bee species common in coastal Brazil. These subspecies are discriminated by the yellow stripe pattern of the abdominal tergites. We found Vsp I restriction patterns in the cytochrome b region closely associated to each subspecies in 155 *M. quadrifasciata* colonies of different geographical origin. This mitochondrial DNA molecular marker facilitates diagnosis of *M. quadrifasciata* subspecies matrilineal and can be used to establish their natural distribution and identify hybrid colonies.

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#### **Honey bee visitation to sunflower: effects on pollination and plant genotype**

Chambo, ED; Garcia, RC; de Oliveira, NTE; Duarte, JB. 2011

Sunflower (*Helianthus annuus* L.) is an allogamic plant, which needs insects on flowering, especially the honeybees for seed production. Collecting nectar and pollen by honeybees in agricultural crops is essential to apiculture, as well as a better understanding of plant biology. The foraging behavior of Africanized *Apis mellifera* L. (Hymenoptera, Apidae) and its efficiency of pollination on seed yield of sunflower genotypes (open pollination and restricted pollination) were evaluated. There were peaks of visits by *A. mellifera* for nectar collection on the 2(nd) and 3(rd) flowering days between 7h00 and 8h30. The average density of *A. mellifera* during increased visitation ranged from 2.27 to 2.94 bees per capitulum. Nectar collecting bees were more frequent (2.28 bees per capitulum) than pollen collecting (0.40 bees per capitulum). On the 3(rd) flowering day, Helio 360 and Aguara hybrids had higher ( $p \leq 0.05$ ) number of bee visits per flower head than the other genotypes. Seed yield was 43 % higher ( $p \leq 0.05$ ) from sunflower plants that were visited by pollinator-insects compared with plants restricted to pollinators.

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#### **Interference of host plant morphology and phenology and their correlation with abundance patterns of the leaf galling sawfly *Pontania proxima***

Kehl, A; Rambold, G. 2011

The host taxon preference of *Pontania proxima* (Serville 1823) (Tenthredinidae, Hymenoptera) was investigated by observing densities of galls on previously genotypically and phenotypically characterized clones representing three taxa of a hybrid complex, i.e., *Salix alba*, *Salix x rubens*, and *Salix fragilis*. Gall densities among these three taxa were observed by using two experimental designs: (1) an indoor experiment in a greenhouse flight cage, and (2) an outdoor experiment on a plantation of cuttings. Subsequently, observed gall densities were related with selected, characteristic phenotypical properties of the host plants. In the indoor experiment, *S. fragilis* was clearly preferred by *P. proxima*, while in the outdoor experiment, *S. x rubens* revealed the highest gall densities. The factor "foliation start" was excluded in the indoor experiment, but best explained the preference of *S. x rubens* in the outdoor experiment. The results of a linear regression model affirmed on the one hand that morphological properties should be expected as relevant signals during the host plant selection process of *P. proxima*. On the other hand, it was shown that other phenotypical factors such as foliation start in spring contribute to an interference of factors being relevant for host plant selectivity. The earlier foliation start of the morphological intermediate *S. x rubens* in the outdoor experiment shifts resource availability towards that taxon at an early oviposition phase of *P. proxima* and therefore entails a different gall distribution pattern with a preference for the hybrid under natural conditions.

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#### **HYBRID SUNFLOWER POLLINATION BY A MANAGEABLE COMPOSITE SPECIALIST - THE SUNFLOWER LEAFCUTTER BEE (HYMENOPTERA, MEGACHILIDAE)**

PARKER, FD; FROHLICH, DR. 1983

#### **BETWEEN-SPECIES AND WITHIN-SPECIES RECOGNITION AMONG IMPORTED FIRE ANTS AND THEIR HYBRIDS (HYMENOPTERA, FORMICIDAE) - APPLICATION TO HYBRID ZONE DYNAMICS**

OBIN, MS; VANDERMEER, RK. 1989

#### **Hybrid capture data unravel a rapid radiation of pimpliform parasitoid wasps (Hymenoptera: Ichneumonidae: Pimpliformes)**

Klopfstein, S; Langille, B; Spasojevic, T; Broad, GR; Cooper, SJB; Austin, AD; Niehuis, O. 2019

The parasitoid wasp family Ichneumonidae is among the most diverse groups of organisms, with conservative estimates suggesting that it contains more species than all vertebrates together. However, ichneumonids are also among the most severely understudied groups, and our understanding of their evolution is hampered by the lack of a robust higher-level phylogeny of this group. Based on newly generated transcriptome sequence data, which were filtered according to several criteria of phylogenetic informativeness, we developed target DNA enrichment baits to capture 93 genes across species of Ichneumonidae. The baits were applied to DNA of 55 ichneumonids, with a focus on Pimpliformes, an informal group containing nine subfamilies. Phylogenetic trees were inferred under maximum likelihood and Bayesian approaches, at both the nucleotide and amino acid levels. We found maximum support for the monophyly of Pimpliformes but low resolution and very short branches close to its base, strongly suggesting a rapid radiation. Two genera and one genus-group were consistently recovered in unexpected parts of the tree, prompting changes in their higher-level classification: *Pseudorhyssa* Merrill, currently classified in the subfamily Poemeniinae, is transferred to the tribe Delomeristini within Pimplinae, and *Hemiphanes* Forster is moved from Orthocentrinae to Cryptinae. Likewise, the tribe Theroniini is resurrected for the Theronia group of genera (stat. rev.). Phylogenetic analyses, in which we gradually increased the numbers of genes, revealed that the initially steep increase in mean clade support slows down at around 40 genes, and consideration of up to 93 genes still left various nodes in the inferred phylogenetic tree poorly resolved. It remains to be shown whether more extensive gene or taxon sampling can resolve the early evolution of the pimpliform subfamilies.

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#### MORPHOMETRIC DIFFERENCES AMONG AFRICANIZED AND EUROPEAN HONEY-BEES AND THEIR F1 HYBRIDS (HYMENOPTERA, APIDAE)

RINDERER, TE; DALY, HV; SYLVESTER, HA; COLLINS, AM; BUCO, SM; HELLMICH, RL; DANKA, RG. 1990

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#### A homolog of the vaccinia virus D13L rifampicin resistance gene is in the entomopoxvirus of the parasitic wasp, *Diachasmimorpha longicaudata*

Lawrence, PO; Dillard, BE. 2008

The parasitic wasp, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), introduces an entomopoxvirus (DIEPV) into its Caribbean fruit fly host, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), during oviposition. DIEPV has a 250-300 kb unipartite dsDNA genome, that replicates in the cytoplasm of the host's hemocytes, and inhibits the host's encapsulation response. The putative proteins encoded by several DIEPV genes are highly homologous with those of poxviruses, while others appear to be DIEPV specific. Here, a 2.34 kb sequence containing a 1.64 kb DIEPV open reading frame within a cloned 4.5 kb EcoRI fragment (designated R1-1) is described from a DIEPV EcoRI genomic library. This open reading frame is a homolog of the vaccinia virus rifampicin resistance (rif) gene, D13L, and encodes a putative 546 amino acid protein. The DIEPV rif contains two EcoRV, two HindIII, one XbaI, and one DraIII restriction sites, and upstream of the open reading frame the fragment also contains EcoRV, HindII, SpEI, and Bsp106 sites. Early poxvirus transcription termination signals (TTTTTnT) occur 236 and 315 nucleotides upstream of the consensus poxvirus late translational start codon (TAAATG) and at 169 nucleotides downstream of the translational stop codon of the rif open reading frame. Southern blot hybridization of HindIII-, EcoRI-, and BamHI-restricted DIEPV genomic DNA probed with the labeled 4.5 kb insert confirmed the fidelity of the DNA and the expected number of fragments appropriate to the restriction endonucleases used. Pairwise comparisons between DIEPV amino acids and those of the *Amsacta moorei*, *Heliothis armigera*, and *Melanoplus sanguinipes* entomopoxviruses, revealed 46, 46, and 45 % similarity (identity + substitutions), respectively. Similar values (41-45%) were observed in comparisons with the chordopoxviruses. The mid portion of the DIEPV sequence contained two regions of highest conserved residues similar to those reported for *H. armigera* entomopoxvirus rifampicin resistance protein. Phylogenetic analysis of the amino acid sequences suggested that DIEPV arose from the same ancestral node as other entomopoxviruses but belongs to a separate clade from those of the grasshopper-infecting *M. sanguinipes* entomopoxvirus and from the Lepidoptera-infecting (Genus B or Betaentomopoxvirus) *A. moorei* entomopoxvirus and *H. armigera* entomopoxvirus. Interestingly, the DIEPV putative protein had only 3-26.4 % similarity with RIF-like homologs/orthologs found in other large DNA non-poxviruses, demonstrating its closer relationship to the Poxviridae. DIEPV remains an unassigned member of the Entomopoxvirinae (<http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm>) until its relationship to other diptera-infecting (Gammaentomopoxvirus or Genus C) entomopoxviruses can be verified. The GenBank accession number for the nucleotide sequence data reported in this paper is EF541029.

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#### MONITORING LABORATORY AND FIELD BIOTYPES OF THE WALNUT APHID PARASITE, *TRIOXYS PALLIDUS*, IN POPULATION CAGES USING RAPD-PCR

EDWARDS, OR; HOY, MA. 1995

A discriminate analysis, based on the frequencies of occurrence of six random amplified polymorphic DNA (RAPD) fragments, distinguished individuals of a pesticide-resistant laboratory biotype (SEL) of the walnut aphid parasite, *Trioxys pallidus* Haliday (Hymenoptera: Aphididae), from individuals of a wild orchard biotype (RB). In mixed populations in the laboratory, the SEL biotype was heavily favored, irrespective of pesticide treatment suggesting a high degree of laboratory adaptation. The resistance levels in untreated and pesticide-treated mixed (SEL + RB) populations were not different after eight generations. Analyses of RAPD-polymerase chain reaction (PCR) data suggested that the selective advantage of the SEL biotype may have been aided by a partial mating incompatibility between the biotypes. The data can be explained by either of two types of reproductive incompatibility: (1) RB females mating with SEL males produced only male offspring; or (2) the RB biotype could not successfully find mates under laboratory conditions. Successful discrimination of *T. pallidus* biotypes and their hybrid progeny in population cages suggests that RAPD-PCR offers a method to determine the fate of the pesticide-resistant SEL biotype after release into orchards populated by the RB biotype.

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#### Host specificity and colony impacts of the fire ant pathogen, *Solenopsis invicta* virus 3

Porter, SD; Valles, SM; Oi, DH. 2013

An understanding of host specificity is essential before pathogens can be used as biopesticides or self-sustaining biocontrol agents. In order to define the host range of the recently discovered *Solenopsis invicta* virus 3 (SINV-3), we exposed laboratory colonies of 19 species of ants in 14 genera and 4 subfamilies to this virus. Despite extreme exposure during these tests, active, replicating infections only occurred in *Solenopsis invicta* Buren and hybrid (*S. invicta* x *S. richteri*) fire ant colonies. The lack of infections in test *Solenopsis geminata* fire ants from the United States indicates that SINV-3 is restricted to the *saevissima* complex of South American fire ants, especially since replicating virus was also found in several field-collected samples of the black imported fire ant, *Solenopsis richteri* Forel. *S. invicta* colonies infected with SINV-3 declined dramatically with average brood reductions of 85% or more while colonies of other species exposed to virus remained uninfected and healthy. The combination of high virulence and high host specificity suggest that SINV-3 has the potential for use as either a biopesticide or a self-sustaining biocontrol agent. Published by Elsevier Inc.

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#### HONEY BEE POLLINATION AND VISITATION PATTERNS ON HYBRID OILSEED SUNFLOWERS IN CENTRAL WYOMING (HYMENOPTERA, APIDAE)

KRAUSE, GL; WILSON, WT. 1981

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#### The effects of transgenic Bt rice on arthropod community structure during storage

Wagan, TA; Cai, WL; Hua, HX; Yang, S; Wang, P; Gao, GL. 2017

Effects of transgenic *Bacillus thuringiensis* (Bt) rice and non-Bt rice on the arthropod community structure during storage were studied. Dried and cleaned grains (200 kg of F1 hybrid transgenic rice) from each rice variety were stored at unglazed ceramic pots (100 cm high, 80 cm diameter), and then stored in a 10-year-old barn. To investigate the species and number of arthropods using a needle-point sampler, each pot had 1 kg of grain sampled from both the upper layer (0-35 cm) and the lower layer (35-70 cm) every 15 days. The results showed 12 species of arthropods including Corrodentia, Acarina, Chelonethida, Lepidoptera, Coleoptera and Hymenoptera were found. No significant differences were observed in the community structure parameters of the arthropods between transgenic Bt rice and non-Bt rice. In both rice varieties, *Liposcelis bostrychophilus* exhibited the highest level of abundance of community structure, followed by *Rhizopertha dominica* and *Tyrophagus putrescentiae*. Overall, there was no significant difference in the temporal dominance curve profiles of the three dominant arthropods between Bt and non-Bt rice. Our results indicated that transgenic Bt rice generally exerts no remarkable negative effects on the arthropod community during storage.

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#### Phylogenomics of Ichneumonoidea (Hymenoptera) and implications for evolution of mode of parasitism and viral endogenization

Sharanowski, BJ; Ridenbaugh, RD; Piekarski, PK; Broad, GR; Burke, GR; Deans, AR; Lemmon, AR; Lemmon, ECM; Diehl, GJ; Whitfield, JB; Hines, HM. 2021

Ichneumonoidea is one of the most diverse lineages of animals on the planet with >48,000 described species and many more undescribed. Parasitoid wasps of this superfamily are mostly beneficial insects that attack and kill other arthropods and are important for understanding diversification and the evolution of life history strategies related to parasitoidism. Further, some lineages of parasitoids within Ichneumonoidea have acquired endogenous virus elements (EVEs) that are permanently a part of the wasp's genome and benefit the wasp through host immune disruption and behavioral control. Unfortunately, understanding the evolution of viral acquisition, parasitism strategies, diversification, and host immune disruption mechanisms, is deeply limited by the lack of a robust phylogenetic framework for Ichneumonoidea. Here we design probes targeting 541 genes across 91 taxa to test phylogenetic relationships, the evolution of parasitoid strategies, and the utility of probes to capture polydnavirus genes across a diverse array of taxa. Phylogenetic relationships among Ichneumonoidea were largely well resolved with most higher-level relationships maximally supported. We noted codon use biases between the outgroups, Braconidae, and Ichneumonidae and within Pimplinae, which were largely solved through analyses of amino acids rather than nucleotide data. These biases may impact phylogenetic reconstruction and caution for outgroup selection is recommended. Ancestral state reconstructions were variable for Braconidae across analyses, but consistent for reconstruction of idiobiosis/koinobiosis in Ichneumonidae. The data suggest many transitions between parasitoid life history traits across the whole superfamily. The two subfamilies within Ichneumonidae that have polydnaviruses are supported as distantly related, providing strong evidence for two independent acquisitions of ichnoviruses. Polydnavirus capture using our designed probes was only partially successful and suggests that more targeted approaches would be needed for this strategy to be effective for surveying taxa for these viral genes. In total, these data provide a robust framework for the evolution of Ichneumonoidea.

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#### BIOCHEMICAL PHENOTYPIC AND GENETIC STUDIES OF 2 INTRODUCED FIRE ANTS AND THEIR HYBRID (HYMENOPTERA, FORMICIDAE)

ROSS, KG; VANDERMEER, RK; FLETCHER, DJC; VARGO, EL. 1987

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#### SEASONAL CYCLES OF AGAPOSTEMON-ANGELICUS COCKERELL RELATIVE TO HYBRID COTTONSEED PRODUCTION IN TEXAS (HYMENOPTERA, HALICTIDAE)

BERGER, LA; MOFFETT, JO; RUMMEL, DR. 1985

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#### Apparent Acquired Resistance by a Weevil to Its Parasitoid Is Influenced by Host Plant

Goldson, SL; Tomasetto, F. 2016

Field parasitism rates of the Argentine stem weevil *Listronotus bonariensis* (Kuschel; Coleoptera: Curculionidae) by *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) are known to vary according to different host *Lolium* species that also differ in ploidy. To further investigate this, a laboratory study was conducted to examine parasitism rates on tetraploid Italian *Lolium multiflorum*, diploid *Lolium perenne* and diploid hybrid *L. perenne* x *L. multiflorum*; none of which were infected by *Epichloa* endophyte. At the same time, the opportunity was taken to compare the results of this study with observations made during extensive laboratory-based research and parasitoid-rearing in the 1990s using the same host plant species. This made it possible to determine whether there has been any change in weevil susceptibility to the parasitoid over a 20 year period when in the presence of the tetraploid Italian, diploid perennial and hybrid host grasses that were commonly in use in the 1990's. The incidence of parasitism in cages, in the presence of these three grasses mirrored what has recently been observed in the field. When caged, weevil parasitism rates in the presence of a tetraploid Italian ryegrass host were significantly higher (75%) than rates that occurred in the presence of either the diploid perennial (46%) or the diploid hybrid (52%) grass, which were not significantly different from each other. This is very different to laboratory parasitism rates in the 1990s when in the presence of both of the latter grasses high rates of parasitism (c. 75%) were recorded. These high rates are typical of those still found in weevils in the presence of both field and caged tetraploid Italian grasses. In contrast, the abrupt decline in weevil parasitism rates points to the possibility of evolved resistance by the weevil to the parasitoid in the diploid and hybrid grasses, but not so in the tetraploid. The orientation of plants in the laboratory cages had no significant effect on parasitism rates under any treatment conditions suggesting that plant architecture may not be contributing to the underlying mechanism resulting in different rates of parasitism. The evolutionary implications of what appears to be plant-mediated resistance of *L. bonariensis* to parasitism by *M. hyperodae* are discussed.

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#### Abundance of *Cardiochiles nigriceps* (Hymenoptera: Braconidae) on *Nicotiana* species (Solanaceae)

Jackson, DM; Nottingham, SF; Schlotzhauer, WS; Horvat, RJ; Sisson, VA; Stephenson, MG; Foard, T; McPherson, RM. 1996

The presence of adult *Cardiochiles nigriceps* Viereck, a nearly obligatory parasitoid of tobacco budworm larvae, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), was monitored in fields with up to 117 accessions of *Nicotiana* species (Solanaceae) during 1985-1994 at Oxford, NC, Tifton, GA, and Athens, GA. Large numbers of wasps were observed flying over or resting on several *Nicotiana* species, especially *N. noctiflora* Hooker and *N. sanderae* Hort. ex Watson (hybrid between *N. alata* Link & Otto and *N. forgetiana* Hort. ex Hemsley). During the 10-yr sampling period, an average of 60 times as many *C. nigriceps* adults were counted in *N. noctiflora* plots and 19 times as many wasps were counted in *N. sanderae* plots than were found in commercial tobacco, *N. tabacum* L. ('NC 2326'), even though *N. noctiflora* and *N. sanderae* are poor host plants for tobacco budworm larvae. Male wasps predominated in plots of *N. noctiflora*; whereas, female wasps predominated in cultivated tobacco fields. There were no significant differences in the sex ratios of wasps collected from *N. alata* or *N. sanderae*. *C. nigriceps* adults were associated primarily with the flowers of *N. sanderae*, *N. alata*, and *N. forgetiana*, but they were found equally on the leaves and flowers of *N. noctiflora*. *C. nigriceps* adults appeared to be attracted to volatile components from *N. noctiflora* leaves and flowers. Several kilograms of field-grown *N. noctiflora* flowers and leaves were extracted with methylene chloride, and the volatile components were isolated. Gas chromatography showed only 4 volatile peaks of interest in *N. noctiflora*, and they were identified as nitrogen-sulfur heterocycles.

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#### Tissue-specific glycosylation in the honeybee: Analysis of the N-glycomes of *Apis mellifera* larvae and venom

Hykollari, A; Malzl, D; Stanton, R; Eckmair, B; Paschinger, K. 2019

Background: Previous glycomylogenetic comparisons of dipteran and lepidopteran species revealed variations in the anionic and zwitterionic modifications of their N-glycans; therefore, we wished to explore whether species- and order-specific glycomic variations would extend to the hymenoptera, which include the honeybee *Apis mellifera*, an agriculturally- and allergologically-significant social species. Methods: In this study, we employed an off-line liquid chromatography/mass spectrometry approach, in combination with enzymatic and chemical treatments, to analyse the N-glycans of male honeybee larvae and honeybee venom in order to facilitate definition of isomeric structures. Results: The neutral larval N-glycome was dominated by oligomannosidic and paucimannosidic structures, while the neutral venom N-glycome displayed more processed hybrid and complex forms with antennal N-acetylgalactosamine, galactose and fucose residues including Lewis-like epitopes; the anionic pools from both larvae and venom contained a wide variety of glucuronylated, sulphated and phosphoethanolamine-modified N-glycans with up to three antennae. In comparison to honeybee royal jelly, there were more fucosylated and fewer Man(4/5)-based hybrid glycans in the larvae and venom samples as well as contrasting antennal lengths. Conclusions: Combining the current data on venom and larvae with that we previously published on royal jelly, a total honeybee N-glycomic repertoire of some 150 compositions can be proposed in addition to the 20 previously identified on specific venom glycoproteins. Significance: Our data are indicative of tissue-specific modification of the core and antennal regions of N-glycans in *Apis mellifera* and reinforce the concept that insects are capable of extensive processing to result in rather complex anionic oligosaccharide structures.

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#### **Africanized honeybees: Biological characteristics, urban nesting behavior and accidents caused in Brazilian cities (Hymenoptera : Apidae)**

Pereira, AM; Chaud-Netto, J. 2005

In 1956 African honeybee queens (*Apis mellifera scutellata*) were imported from South Africa and Tanzania to Brazil, as part of a government project to increase Brazilian honey production. The European honeybees existing in that country had not adapted well to the tropical conditions and consequently, had a low productivity. The newly introduced bee was known to produce substantially more honey than the other subspecies, but was also famous for its great aggressiveness and quicker attack of intruders with less disturbance. Hoping to create a new hybrid bee that would be both docile and productive, the scientist Warwick Estevam Kerr tried to cross the African and the European subspecies under controlled conditions. However, an accident resulted in the escape of 26 swarms into the Brazilian countryside, where their queens mated with drones of the European resident honeybees. The poly-hybrid bees resulting from these crossings expressed scutellata-like reproductive, foraging, and defensive behaviors and, for this reason, were called Africanized honeybees. They spread rapidly from the introduction area of the African honeybees (near Rio Claro, Sao Paulo state) to as far south as mid-Argentina and to the north of Texas, also settling in Arizona, New Mexico, California and Nevada, due to their high adaptability to variable ecological conditions. In spite of a few undesirable behaviors, these bees have been invoking larger economic interest because they produce much more honey, have good resistance to diseases and are excellent pollinators. In Brazil, because people frequently disturb the environment, the occupation of urban refuges by Africanized honeybees has been increasing in the last years. The concern with accidents is generally associated with the high swarming frequency recorded during the year and the variety of shelters available in urban areas. This paper deals with the biological characteristics of the Africanized honeybees, their nesting behavior in urban environments, and accidents caused by these bees in Brazilian cities.

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#### **THE FORAGING ACTIVITY OF AGAPOSTEMON-ANGELICUS COCKERELL (HYMENOPTERA, HALICTIDAE) RELATIVE TO HYBRID COTTONSEED PRODUCTION IN TEXAS**

BERGER, LA; MOFFETT, JO; RUMMEL, DR. 1988

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#### **Flower visitors of lettuce under field and enclosure conditions**

Goubara, M; Takasaki, T. 2003

Insects visiting lettuce flowers were investigated under field and enclosure conditions. In a 4-year field survey, visitors of 11 species belonging to 3 orders were observed. Most species visiting the flowers were sweat bees (Hymenoptera; Halictidae). The frequency (total days of visitation to lettuce flower/total days of flight by the insect) of visitations by the sweat bee *Lasioglossum villosulum trichopse* was obviously higher at 65.5% than those of other insects. Honeybees were not observed on the lettuce flowers. In a 5-year survey under enclosure conditions, 10 of 17 bee species reared and a hoverfly were observed to visit the lettuce flowers. The highest frequency of visitation was shown by *L. villosulum trichopse* (59.4%), followed by *Andrena knuthi* (19.2%) and *Osmia cornifrons* (8.6%). Most visitors were one-day foragers and classified as temporary nectar foragers and continual pollen (and nectar) gatherers. Continual nectar foraging was observed in only 3 species, *L. villosulum trichopse*, *An. knuthi* and *Ceratina boninensis*. Daily flight activities of *L. villosulum trichopse* and the *Lactuceae* oligolectic bee *An. knuthi* corresponded to the morning blooming time of lettuce. The sweat bee *L. villosulum trichopse* may be a pollinator for hybrid seed production of lettuce.

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#### **POLLINATION ECOLOGY OF 4 DALECHAMPIA SPECIES (EUPHORBIACEAE) IN NORTHERN NATAL, SOUTH-AFRICA**

ARMBRUSTER, WS; STEINER, KE. 1992

The pollination ecology of four *Dalechampia* species was studied in three areas in northern Natal, South Africa. All species were pollinated by resin- and/or pollen-collecting megachilid bees (Hymenoptera: Megachilidae). The most common of these at all study sites was *Heriades* sp. (Megachilini), which collected both pollen and resin. This bee was the primary pollinator of *D. galpinii* and *D. volubilis*, and a secondary pollinator of *D. aff. parvifolia* and *D. capensis*. The primary pollinators of these latter two *Dalechampia* were *Pachyanthidium* near *cucullatum* and *P. cordatum* (Anthidiini), respectively. These are larger bees that collected mainly resin. Other visitors varied among *Dalechampia* species and among sites. Pairs of *Dalechampia* species were often found in sympatry (within 30 m of each other) and shared pollinators to varying extents. "Transfer experiments," in which we placed inflorescences of two *Dalechampia* species together, reinforced observations of flower discrimination by bees foraging among naturally occurring sympatric pairs. *Heriades* sp. showed no obvious discrimination between *Dalechampia* species, but *Pachyanthidium* spp. "preferred" the *Dalechampia* species with the greater resin reward. Occasionally, however, *Pachyanthidium* would visit less rewarding species. Thus, although sympatric African *Dalechampia* species showed some pollinator partitioning, it was much weaker than found among New World species, and cannot alone explain the general absence of *Dalechampia* hybrids in northern Natal.

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#### **Nest defense behavior in colonies from crosses between africanized and European honeybees (Apis mellifera L) (Hymenoptera : Apidae)**

DeGrandi-Hoffman, G; Collins, A; Martin, JH; Schmidt, JO; Spangler, HG. 1998

Honey bee (*Apis mellifera* L.) colonies with either European or Africanized queens mated to European or Africanized drones alone or in combination were tested for defensive behavior using a breath test. The most defensive colonies were those with European or Africanized queens mated to Africanized drones. In colonies where both European and Africanized patriline existed, most of the workers participating in nest defense behavior for the first 30 s after a disturbance were of African patriline. Nest defense behavior appears to be genetically dominant in honey bees.



## Cell death and changes in primary metabolism: the onset of defence in *Eucalyptus* in the war against *Leptocybe invasa*

Pinto, ID; Sarmiento, MI; Martins, AO; Rocha, JPL; Pinto, G; Araujo, WL; Soares, AMVM; Sarmiento, RA. 2022

**BACKGROUND** Here, we investigated changes in primary metabolism and cell death around oviposition sites in two hybrid clones of *Eucalyptus* with different degrees of resistance to *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae), as well as tolerance to water deficiency. **RESULTS** We showed that apices of the resistant clone with oviposition had a higher content of amino acids, organic acids and the compound putrescine compared with those of the susceptible clone with oviposition. By contrast, apices of the resistant clone with oviposition had lower sugar and pyruvate organic acid content than those of the susceptible clone with oviposition. Small areas of necrosis were induced around the oviposition sites in the stem apices of *Eucalyptus* 24 h after infestation. The resistant clone developed larger necrotic areas that showed progressive increases 24-72 h after infestation compared with the susceptible clone, in which cell death was significantly lower and no changes were observed in necrotic area over time. Thus, the programmed death of cells around the egg, modulated by several amino acids, is likely the first defence response of *Eucalyptus* against *L. invasa*. **CONCLUSION** Our results serve as the basis for the early identification of key metabolites produced in plants in defence against galling insects.

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## Egg maturation, nest state, and sex ratios: a dynamic state variable model

Peterson, JH; Roitberg, BD. 2010

**Background:** Parents can invest in offspring through a variety of behaviours. Optimization models of these behaviours are usually based on determination of the single 'factor' parents optimize for a given set of conditions. Interactions between factors are rarely considered. Question: Do mothers optimize a single factor related to the investment in offspring (e.g. current nest state or mature egg state), or do mothers find an optimal balance between these two in relation to maximizing lifetime reproduction? Methods and key assumptions: A dynamic state variable model. We develop a 'hybrid' model that examines mothers' allocation decisions to offspring by considering mature egg and nest state as well as other environmental/ecological factors. We assume that mothers alter reproductive decisions based on their perception of costs and benefits of brood cell and nest construction. Some of these construction behaviours determine investment in one or a few offspring within a brood but others affect the entire brood. Egg maturation rate is a constant. Conclusions: Our results demonstrate that there is no single limiting factor; instead, there is some 'optimal balance' between mature egg and nest state that determines the optimal reproductive decision.

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## IDENTIFICATION OF FIRE ANTS (HYMENOPTERA: FORMICIDAE) FROM NORTHEASTERN MEXICO WITH MORPHOLOGY AND MOLECULAR MARKERS

Sanchez-Pena, SR; Chacon-Cardosa, MC; Resendez-Perez, D. 2009

The invasive red imported fire ant, *Solenopsis invicta* Buren, has successfully dispersed across many countries from its South American homeland and now has reached the US-Mexico border (e.g., Matamoros, state of Tamaulipas, Mexico), where it now coexists with native fire ants, *Solenopsis geminata*, *Solenopsis xyloni*, and others. The morphological identification of *Solenopsis* spp. workers is difficult, particularly small ones. We examined the sequence of the cytochrome oxidase I (COI) mitochondrial gene (mtDNA) as a marker for fire ants collected at several Mexican localities. PCR products from this locus yielded unique sequences and restriction patterns that allowed distinguishing between *S. invicta*, *S. geminata*, and specimens harboring *S. xyloni* sequences. The *S. invicta* sequences obtained were 99% identical to sequences reported from Florida and New Mexico specimens. The *S. xyloni* sequences obtained were 96% identical to New Mexico sequences. The *S. geminata* sequences were similar (93% identity) to those from Florida, and shared a Hinf I restriction site with some but not all Florida sequences. The *S. xyloni* sequences were detected in *S. geminata*/*S. xyloni* hybrids identified by morphology; along with other characters, the marker allows their characterization.

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## SUPERCOOLING STUDIES ON THE IMPORTED FIRE ANTS - SOLENOPSIS-INVICTA AND SOLENOPSIS-RICHTERI (HYMENOPTERA, FORMICIDAE) AND THEIR HYBRID

DIFFIE, SK; SHEPPARD, DC. 1989

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## DDT TOLERANCE OF AFRICANIZED BEES, ITALIAN BEES (APIS-MELLIFERA-LIGUSTICA) AND THEIR F-1-HYBRIDS (HYMENOPTERA, APIDAE)

MALASPINA, O; STORT, AC. 1983

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## Effect of nectar composition and nectar concentration on honey bee (Hymenoptera : Apidae) visitations to hybrid onion flowers

Silva, EM; Dean, BB. 2000

Floral nectar characteristics of nine inbred lines of onion (*Allium cepa* L.) were examined to determine their influence on the attractiveness of the onion flowers to honey bees (*Apis mellifera* L.). Potassium concentrations and sugar concentrations of the nectar did not significantly correlate with the number of bee visits received by an umbel. The average amount of nectar produced by both the umbels and the individual florets was significantly positively correlated with the number of bee visits. Our results suggest that selection for flowers with high nectar production may lead to a higher rate of pollination of the onion seed crop.

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## Fitness of a malathion-resistant strain of the parasitoid *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae)

Baker, JE; Perez-Mendoza, J; Beeman, RW; Throne, JE. 1998

Biological fitness of a malathion-resistant (R) and a malathion-susceptible (S) strain of the solitary parasitoid *Anisopteromalus calandrae* (Howard) was compared when the wasps were parasitizing immature rice weevils, *Sitophilus oryzae* (L.), in stored wheat. Despite having a >2,500-fold naturally occurring resistance, in the absence of insecticide the R strain was equal to the S strain in ability to parasitize hosts and in several developmental parameters. Development times of cohorts of male and female progeny produced over 7 d by R and S females at 25 degrees C and 75% RH were not significantly different. There was no significant strain effect on daily fecundity. Parasitization of hosts was not significantly affected by strain-host density interactions. There was no significant effect of strain on total progeny production at different host densities, but more female progeny were produced by the susceptible strain at high host densities. Otherwise, the proportion of females among progeny of the 2 strains was not significantly different. Frequency of the R allele in Hardy-Weinberg populations, set up with an initial R allele frequency of 0.5, was lower than expected but tended to stabilize after 4 generations. Frequency of the R allele in a population started with hybrid females was not significantly different from values expected under the hypothesis of no fitness costs. Failure to detect fitness costs associated with malathion resistance in *A. calandrae* could be caused by the lack of negative pleiotropic effects associated with the R allele.

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## Comparative life table statistics of *Apoanagyrus lopezi* reared on the cassava mealybug *Phenacoccus manihoti* fed on four host plants

Souissi, R; Le Ru, B. 1997

The demographic statistics of *Apoanagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) were studied in the laboratory on its host, the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), reared on four host plants characterized by different levels of antibiotic resistance to the mealybug: two cassava varieties, Incoza and Zanaga (*Manihot esculenta* Crantz: Euphorbiaceae), the faux-caoutchouc (hybrid of *M. esculenta* x *M. glaziovii* Muel. Arg.) and Talinum (*Talinum triangulare* Jack:Portulacaceae). Total and daily mean fecundities of female parasitoids were strongly influenced by the host plant but there was no link with antibiotic resistance. The mean duration of the oviposition period was also significantly modified by the host plant and was again not correlated to the level of antibiotic resistance. Net reproduction rates of female parasitoids were nearly 2 times higher on hosts fed on Zanaga and Talinum than on Incoza and Faux-caoutchouc, and were not related to the total fecundities. The generation time was significantly longer on Faux-caoutchouc than on the other three plants. The intrinsic rate of increase varied significantly with host plants and was higher on Talinum and Zanaga than on Incoza and Faux-caoutchouc. With the varieties of cassava, Incoza and Zanaga, it was observed that antibiosis had a significant negative effect on the survival of *A. lopezi*, which would influence the effectiveness of the parasitoid.

## Rearing the decapitating fly *Pseudacteon tricuspidis* (Diptera: Phoridae) in imported fire ants (Hymenoptera: Formicidae) from the United States

Porter, SD; Williams, DF; Patterson, RS. 1997

The South American phorid fly *Pseudacteon tricuspidis* Borgmeier was imported into quarantine facilities in Gainesville, FL, to study its life history and determine if it could be reared on imported fire ant workers from North America. The found that this fly developed successfully on *Solenopsis invicta* Buren workers from Florida and hybrid *Solenopsis richteri* Forel x *S. invicta* fire ants from Mississippi. It also was reared on *S. richteri* and *S. invicta* fire ants from Argentina. This fly, like its congener *Pseudacteon litoralis* Borgmeier, had the peculiar habit of decapitating its living host and using the ant's empty head capsule as its pupal case. We were able to rear this By through 1 complete generation in the laboratory, indicating that mass rearing for inoculative releases in the United States may be possible.

## Detection of mitochondrial DNA restriction site differences between the subspecies of *Melipona quadrifasciata* Lepeletier (Hymenoptera : Apidae : Meliponini)

Moretto, G; Arias, MC. 2005

The endemic range of the stingless bee *Melipona quadrifasciata* Lepeletier extends from the southern most state in Brazil, Rio Grande do Sul, up to the state of Paraíba, where it originally inhabited the Atlantic Rain Forest. Two subspecies have been morphologically recognized based on the tergal band pattern (yellow stripes across the abdomen), *M.q. quadrifasciata* and *V.q. anthidioides*. The objective of the present study was to characterize restriction site variation in the mitochondrial DNA of each subspecies. Four colonies of each subspecies were sampled. One individual per colony was used for DNA extraction and further analysis through amplification of nine fragments of the mitochondrial genome and digestion with 13 restriction enzymes (PCR+RFLP). Three enzymes showed restriction pattern differences between the subspecies. Those differences will be useful to determine the maternal origin of hybrids colonies, which may occur naturally or due to colony transportation among breeders.

## Assemblies of the genomes of parasitic wasps using meta-assembly and scaffolding with genetic linkage

Wittmeyer, KT; Oppenheim, SJ; Hopper, KR. 2022

Safe, effective biological-control introductions against invasive pests depend on narrowly host-specific natural enemies with the ability to adapt to a changing environment. As part of a project on the genetic architectures of these traits, we assembled and annotated the genomes of two aphid parasitoids, *Aphelinus atriplicis* and *Aphelinus certus*. We report here several assemblies of *A. atriplicis* made with Illumina and PacBio data, which we combined into a meta-assembly. We scaffolded the meta-assembly with markers from a genetic map of hybrids between *A. atriplicis* and *A. certus*. We used this genetic-linkage scaffolded (GLS) assembly of *A. atriplicis* to scaffold a de novo assembly of *A. certus*. The de novo assemblies of *A. atriplicis* differed in contiguity, and the meta-assembly of these assemblies was more contiguous than the best de novo assembly. Scaffolding with genetic-linkage data allowed chromosomal-level assembly of the *A. atriplicis* genome and scaffolding a de novo assembly of *A. certus* with this GLS assembly, greatly increased the contiguity of the *A. certus* assembly to the point where it was also at the chromosomal-level. However, completeness of the *A. atriplicis* assembly, as measured by percent complete, single-copy BUSCO hymenopteran genes, varied little among de novo assemblies and was not increased by meta-assembly or genetic scaffolding. Furthermore, the greater contiguity of the meta-assembly and GLS assembly had little or no effect on the numbers of genes identified, the proportions with homologs or functional annotations. Increased contiguity of the *A. certus* assembly provided modest improvement in assembly completeness, as measured by percent complete, single-copy BUSCO hymenopteran genes. The total genetic sequence increased, and while the number of genes declined, gene length increased, which together suggest greater accuracy of gene models. More contiguous assemblies provide uses other than gene annotation, for example, identifying the genes associated with quantitative trait loci and understanding of chromosomal rearrangements associated with speciation.

## Detection of *Thelohania solenopsae* (Microsporidia : thelohaniidae) in *Solenopsis invicta* (Hymenoptera : formicidae) by multiplex PCR

Valles, SM; Oi, DH; Perera, OP; Williams, DF. 2002

Oligonucleotide primer pairs were designed to unique areas of the small subunit (16S) rRNA gene of *Thelohania solenopsae* and a region of the Gp-9 gene of *Solenopsis invicta*. Multiplex PCR resulted in sensitive and specific detection of *T. solenopsae* infection of *S. invicta*. The *T. solenopsae*-specific primer pair only amplified DNA from *T. solenopsae* and *T. solenopsae*-infected *S. invicta*. This primer pair did not produce any amplification products from DNA preparations from uninfected *S. invicta*, seven additional species of microsporidia (including *Vairimorpha invictae*), or *Mattesia* spp. The Gp-9-specific primers recognized and amplified DNA from *Solenopsis xyloni*, *Solenopsis richteri*, *Solenopsis geminata*, the *invicta/richteri* hybrid, and monogyne and polygyne *S. invicta*, but not from *T. solenopsae*, and, as such, served as a positive control verifying successful DNA preparation. Multiplex PCR detected *T. solenopsae* in worker fire ants infected with as few as 5000 spores. Furthermore, multiplex PCR detected *T. solenopsae* in all developmental stages of *S. invicta*. However, detection could be made more sensitive by using only the *T. solenopsae*-specific primer pair; ants infected with as few as 10 spores were able to be discerned. Multiplex PCR detection of *T. solenopsae* offers the advantages of a positive control, a single PCR amplification, detection of all developmental stages, and increased sensitivity and specificity compared with microscopy. (C) 2002 Elsevier Science (USA). All rights reserved.

## Influence of the host plant of the cassava mealybug *Phenacoccus manihoti* (Hemiptera : Pseudococcidae) on biological characteristics of its parasitoid *Apoanagyrus lopezi* (Hymenoptera : Encyrtidae)

Souissi, R; Le Ru, B. 1998

The influence of the host plant of the cassava mealybug, *Phenacoccus manihoti* Matile Ferrero on the encyrtid parasitoid *Apoanagyrus lopezi* De Santis was studied

in the laboratory. Four different host plants were used: two cultivars of cassava, *Manihot esculenta* (Euphorbiaceae), cv. Incoza and cv. MM79; Faux caoutchouc, a hybrid of *M. esculenta* x *M. glaziovii*; and talinum *Talinum triangulare* (Portulacaceae), a common weed in cassava fields. Plants were selected for different levels of antibiotic resistance to *P. manihoti*. Mealybug mortality due to host feeding by the adult parasitoid and the percentage of mealybugs parasitized were significantly lower when mealybugs were reared on the *Manihot* cultivars and hybrid than when reared on talinum. However, the encapsulation rate was significantly lower in *P. manihoti* reared on talinum. The highest percentage parasitism and the lowest rate of emergence were recorded on cv. Incoza, the most resistant *Manihot* cultivar. The sex ratio did not vary significantly with the host plant used. The total developmental time and size of male and female progeny of *A. lopezi* differed significantly between *P. manihoti* reared on different host plants. Among *Manihot* plants, parasitoid size was positively correlated with development time and negatively with plant resistance. Results suggest that the parasitoid, *A. lopezi*, might perform better if cassava cultivars were selected for their strong antixenosis but low antibiotic characteristics.

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#### **Biology of the galler *Diplolepis rosaefolii* (Hymenoptera : Cynipidae), its associated component community, and host shift to the shrub rose** Therese Bugnet

Shorthouse, JD; Brooks, SE. 1998

*Diplolepis rosaefolii* (Cockerell) induces a lenticular gall on the leaves of various roses across Canada. In Ontario and Quebec, *D. rosaefolii* is found only on *Rosa acicularis* Lindl., and its galls are inhabited by eight species of parasitoids, the most common being *Chrysocharis pentheus* (Walker), *Orthopelma luteolator* (Gravenhorst), and *Eupelmus dryorhizoxeni* Ashmead. Parasitoids and the inquiline *Periclistus* sp. make up 48-62% of gall inhabitants. Here we report a population : of *D. rosaefolii* on the hybrid rugosa shrub rose Therese Bugnet in a field of experimental roses in Quebec. Only two species of parasitoids and one specimen of *Periclistus* sp. were associated with these galls.

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#### **Variations in *Leptocybe invasa* (Hymenoptera: Eulophidae) population intensity and infestation on eucalyptus germplasms in Uganda and Kenya**

Nyeko, P; Mutitu, KE; Otieno, BO; Ngae, GN; Day, RK. 2010

*Leptocybe invasa*, an invasive gall-inducing wasp of Australian origin, recently emerged as a serious eucalyptus pest of global importance. We examined the spatial and temporal variations in *L. invasa* adult populations and evaluated eucalyptus germplasms for infestations by the wasp in Uganda and Kenya. There were significant differences in *L. invasa* abundance, gall incidence, severity and damage index between sites. Adults occurred throughout the year, indicating overlapping generations since the adults are known to live <7 d. There was no obvious peak in *L. invasa* population abundance although a general decline was observed in dry months. Out of 35 eucalyptus germplasms evaluated for *L. invasa* infestations, only *Eucalyptus henryi* and the clonal hybrids GC 578 and GC581 were resistant to the pest. Most germplasms were ranked as tolerant or moderately susceptible to wasp attack. Highly susceptible germplasms included *Eucalyptus camaldulensis*, GC540 and GC784 in Tororo, Uganda, and MAU1, GC14, GC15 and GC10 in Busia, Kenya. Implications of the year-round occurrence of *L. invasa* adult populations and gall infestations, and the potential for host resistance in managing the pest, are discussed.

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#### **Imported fire ant (Hymenoptera : Formicidae) mound shape characteristics along a north-south gradient**

Vogt, JT; Wallet, B; Freeland, TB. 2008

The nests of some mound-building ants are thought to serve an important function as passive solar collectors. To test this hypothesis, imported fire ant (*Solenopsis invicta* Buren, *S. richteri* Forel, and their hybrid) mound shape characteristics (south facing slope angle and area, mound height, and basal elongation in the plane of the ground) were quantified in 2005 and 2006 at a number of locations from approximate to 30 degrees 25' N (Long Beach, MS) to 35 degrees 3' N (Fayetteville, TN). Insolation ( $w \cdot h / m(2)$ ), maximum sun angle (sun elevation in degrees above the horizon at noon, dependent on date and latitude), cumulative rainfall (7 and 30 d before sampling), and mean ambient temperature (7 d before sampling) for each site X date combination were used as predictive variables to explain mound shape characteristics. Steepness of south-facing mound slopes was negatively associated with maximum sun angle at higher temperatures, with predicted values falling from approximate to 36 degrees at sun angle = 40 degrees to 26 degrees at sun angle = 70 degrees; at lower temperatures, slope remained relatively constant at 28 degrees. On average, mound height was negatively correlated with maximum sun angle. Rainfall had a net negative effect on mound height, but mound height increased slightly with maximum sun angle when rainfall was high. Mound elongation generally increased with increased mound building activity. Under favorable temperature conditions and average rainfall, imported fire ant mounds were tallest, most eccentric, and had the steepest south facing slopes during periods of low maximum sun angle. Mound shape characteristics are discussed with regard to season and their potential usefulness for remote sensing efforts.

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#### **Ground cover influence on microclimate and *Trichogramma* (Hymenoptera: Trichogrammatidae) augmentation in seed corn production**

Orr, DB; Landis, DA; Mutch, DR; Manley, GV; Stuby, SA; King, RL. 1997

As pest management practices include more biological control implementation, the effect of different cropping systems on microclimate and efficacy of natural enemies should be considered. In a 1993 experiment, physical and microclimatic differences between hybrid field corn, Zea mays L., and inbred seed corn were assessed. Seed corn fields had 4.2-fold less leaf surface area and higher maximum temperature at the soil surface than field corn fields. Emergence of *Trichogramma brassicae* Bezdenko from cardboard capsules was significantly lower in seed corn when capsules were placed at the soil surface, but was not significantly different when capsules were at canopy level. In 1994, a rye grass *Lolium multiflorum* Lambert intercrop planted with seed corn significantly reduced maximum soil surface temperature compared with seed corn plots in which soil was bare or was covered (62%) with corn residue. Soil surface temperature was significantly higher in corn residue plots than in bare soil plots. Minimum temperatures were not significantly affected by ground cover. The mean number of hours per day that temperatures were 35 degrees C or higher (lethal to *T. brassicae*) was significantly higher in residue and bare soil microhabitats than in rye grass plots or the corn canopy. When *T. brassicae* inside cardboard capsules were put into plots in the same manner as an augmentative release (i.e., 2 developmentally staggered cohorts), emergence from both cohorts was not significantly different in rye grass than in the corn canopy, but was significantly reduced in bare soil and corn residue plots.

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#### **INHIBITION OF *TELENOMUS-SPHINGIS* AN EGG PARASITOID OF *MANDUCA* SPP BY TRICHOME 2-TRIDECANONE-BASED HOST PLANT-RESISTANCE IN TOMATO**

FARRAR, RR; KENNEDY, G. 1991

The resistance of accession PI 134417 of the wild tomato *Lycopersicon hirsutum* f. *glabratum* C. H. Mull to *Manduca sexta* (L.) (Lepidoptera: Spingidae) and *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is conditioned by the high densities of 2-tridecanone-containing, glandular trichomes associated with the foliage. In laboratory experiments, rates of parasitism of *M. sexta* eggs by *Telenomus sphingis* (Ashmead) (Hymenoptera: Scelionidae) were lower among eggs on PI 134417 foliage than among eggs on foliage of the cultivated tomato *L. esculentum* Mill. (cv. Better Boy). The latter is characterized by a significantly lower density of type VI glandular trichomes than PI 134417 and an absence of 2-tridecanone. Parasitism by *T. sphingis* was also reduced among eggs on foliage of the F1 hybrid between PI 13447 and *L. esculentum*. The hybrid foliage lacks 2-tridecanone but has a density of type VI glandular trichomes that is intermediate between those of PI 134417 and *L. esculentum*, indicating that elevated densities of type VI glandular trichomes adversely affect *T. sphingis*. This conclusion was further substantiated by the finding that there were no differences among plant lines in the levels of parasitism of *M. sexta* eggs when the eggs were on foliage that had been divested of

glandular trichomes. In bioassays in which *T. sphingis* adults or immatures in host eggs were exposed to filter paper treated with 2-tridecanone at rates comparable to those associated with PI 134417 foliage, 2-tridecanone was acutely toxic and caused high levels of mortality. In addition, at high concentrations, 2-tridecanone vapors were repellent to *T. sphingis* adults. However, when exposed to PI 134417 foliage, few *T. sphingis* adults were killed. Parasitism of *M. sexta* eggs was unaffected when the eggs were deposited by moths reared as fifth instar larvae on diet containing 2-tridecanone and/or 2-undecanone at levels comparable to those associated with PI 134417 foliage.

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#### Flower-visiting insects of genus *Melastoma* (Myrtales: Melastomataceae) at the Fushan Botanical Garden, Taiwan

Huang, JCC; Hsieh, YC; Lu, SS; Yeh, WC; Liang, JY; Lin, CJ; Tung, GS. 2021

**Background** We investigated the diversity and behaviour of insects that visit flowers of four native *Melastoma* (Family Melastomataceae) species of Taiwan and a horticultural hybrid *Melastoma* species at the Fushan Botanical Garden, Taiwan biweekly from May to August 2020. Visits of flower-visiting insects were classified into seven behavioural categories, based on the insects' behaviour and positions on the flower. The data are further assigned into four insect-flower interactions, namely pollination, herbivory, commensalism and neutralism. Our goal is to provide baseline data of insect-plant interactions of *Melastoma*, which is a common, but understudied plant genus in the country. New information A total of 1,289 visits to flowers were recorded by at least 63 insect morphospecies belonging to seven orders. The number of insect species recorded per *Melastoma* species ranged from 9 to 39. Visiting, sonication and passing were the three most frequently recorded types of behaviour, collectively accounting for 90.2% (n = 1,240) of the total observations. Pollination was the most dominant insect-flower interaction, accounting for 70.2% of the total observations, followed by neutralism (20.0%), herbivory (6.3%) and commensalism (3.5%). Sweat bees of the genera *Lasioglossum* and *Maculonoma* (Hymenoptera: Halictidae) are considered key pollinators to *Melastoma* species in Fushan Botanical Garden, based on their high number of visits and sonication behaviour. Our study provides the first list of insects that visit the flowers of all Taiwan's known *Melastoma* species and description of their interactions with the plants.

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#### Global challenges faced by engineered *Bacillus thuringiensis* Cry genes in soybean (*Glycine max* L.) in the twenty-first century

Bengyella, L; Yekwa, EL; Iftikhar, S; Nawaz, K; Jose, RC; Fonmboh, DJ; Tambo, E; Roy, P. 2018

The most important insect pests causing severe economic damages to soybean (*Glycine max* L.) production worldwide are *Chrysodeixis includens* (Walker, Noctuidae), *Anticarsia gemmatilis* (Hubner, Erebidae), *Helicoverpa gelotopoeon* (Dyar, Noctuidae), *Crociosema aporema* (Walsingham; Tortricidae), *Spodoptera albula* (Walker, Noctuidae), *S. cosmiodes* (Walker, Noctuidae), *S. eridania* (Stoll, Noctuidae), *S. frugiperda* (Smith; Noctuidae), *Helicoverpa armigera* (Hubner, Noctuidae), *H. zea* (Boddie; Noctuidae) and *Telenomus podisi* (Hymenoptera, Platygastridae). Despite the success of biotech *Bacillus thuringiensis* (Bt)/herbicide tolerance (HT)-soybean in the past decade in terms of output, unforeseen mitigated performances have been observed due to changes in climatic events that favors the emergence of insect resistance. Thus, there is a need to develop hybrids with elaborated gene stacking to avert the upsurge in insect field tolerance to crystal (Cry) toxins in Bt-soybean. This study covers the performance of important commercial transgenic soybean developed to outwit destructive insects. New gene stacking soybean events such as Cry1Ac-, Cry1AF- and PAT-soybean (DAS-81419-2 (R), Conkasta technology), and MON-87751-7xMON-87701-2xMON 87708xMON 89788 (bearing Cry1A.105 [Cry1Ab, Cry1F, Cry1Ac], Cry2Ab, Cry1Ac) are being approved and deployed in fields. Following this deployment trend, we recommend herein that plant-mediated RNA interference into Bt-soybean, and the application of RNA-based pesticides that is complemented by other best agricultural practices such as refuge compliance, and periodic application of low-level insecticides could maximize trait durability in Bt-soybean production in the twenty-first century.

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#### MORPHOMETRIC TECHNIQUES DO NOT DETECT INTERMEDIATE AND LOW-LEVELS OF AFRICANIZATION IN HONEY-BEE (HYMENOPTERA, APIDAE) COLONIES

GUZMANNOVA, E; PAGE, RE; FONDRK, MK. 1994

We tested three different morphometric methods used to identify Africanized honey bee (*Apis mellifera* L.) colonies and determined the correlative relationships of their associated discriminant scores and colony defensive behavior. Workers within and between experimental colonies varied in the percentage of their genotype that was of African origin. Morphometric scores of colonies were compared with two defensive behavior traits: the time it took for the first worker in a colony to respond to, and sting, a moving leather-patch target, and the total number of strings received in the target during a 60-s interval following the first sting. All identification methods correctly classified all of the colonies that were presumed to be 100% Africanized or European. However, <45% of the hybrid samples were scored as Africanized. In all cases, as the level of Africanization decreased, so did the sensitivity and accuracy of the method. Correlations between morphometric scores and defensive behavior were significant when extreme genotypes were included in the analyses, but no method correlated with samples ranging in the interval >0 but <50% Africanized. Implications are discussed of using these and alternative identification methods in regulatory programs.

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#### Evaluation of the neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera : Apidae) as pollinator of greenhouse tomatoes

Del Sarto, MCL; Peruquetti, RC; Campos, LA. 2005

The Neotropical stingless bee *Melipona quadrifasciata* Lepeletier was evaluated for pollinating tomatoes (variety Rodas; long-life hybrid) in greenhouses under plastic and with a hydroponic system and "organic concepts" in Minas Gerais State, Brazil. Flowers not pollinated did not set any fruit. Pollination by bees plus manual pollination did not differ from either bee or manual pollination. Maximum fruit diameter, fruit height, and roundness (quotient between maximum fruit diameter and fruit height) were not significantly different between treatments, but fruit visited by *M. quadrifasciata* had 10.8% less seeds (dry mass) than manual pollination. This apparently low efficiency of *M. quadrifasciata* pollination was attributed to the overlap of only 30 min between highest bee foraging activity and highest flower stigma receptivity. Thus, it was concluded that *M. quadrifasciata* is a feasible pollinator of greenhouse tomatoes because of 1) the observed increase in fruit quality with lower mechanical injury than traditional manual pollination, 2) no significant decrease in fruit size, and 3) high price of such product in the market. Some considerations for sustainable use of *M. quadrifasciata* as greenhouse pollinator are presented. Although techniques for keeping captive colonies of *M. quadrifasciata* are currently available, the sole current method for acquiring new colonies is removing them from the forest, and if demand was created for large numbers of colonies for commercial use, techniques for captive rearing must be developed to prevent serious declines in wild populations.

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#### Curious case of the Kangaroo Island honeybee *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) sanctuary

Glatz, RV. 2015

Humans have had a long association with the honeybee *Apis mellifera* Linnaeus, 1758, which has been exploited for production of honey and for the crop pollination services it provides. This association facilitated movement of this species to such a degree that it is now virtually ubiquitous in all areas with flowering plants and available water. On Kangaroo Island (KI), a 'sanctuary' was created for the Ligurian bee subspecies *A. mellifera ligustica*, which is exotic to Australia and the entire New World. The Ligurian Bee Act was enacted in 1885 on the basis of perceived genetic purity and isolation of KI honeybee populations, and was updated in 1931 and 1997. It supports biosecurity protocols preventing importation of bees, bee-keeping equipment and bee-related products such as honey and wax. This represents a rare example of legislative protection for an invertebrate in Australia. This legislation and the apparent isolation of KI bees from mainland bees in the time since its enactment have led to the popular assertion that KI honeybee populations represent the last 'pure' genetic population of *A. mellifera ligustica*. However, historical

accounts of bee introductions to KI show that *A.mellifera mellifera*-like bees were present on KI prior to the introduction of *A.mellifera ligustica*, and that multiple *A.mellifera ligustica* introductions to KI occurred using bees of mixed heritage. Indeed, DNA sequence analyses of KI honeybees clearly indicate that while there is limited genetic diversity (supporting the assertions of limited introductions and recent geographic/genetic isolation), they are in fact hybrids and share more similarity with the *A.mellifera mellifera* subspecies. Therefore, the relevant state legislation should be updated to remove any mention of Ligurian or other honeybee strains. However, the biosecurity protocols relating to KI should continue due to the low incidence of some honeybee diseases and the threat posed by *Varroa mite*.

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#### **HONEY-BEE (HYMENOPTERA, APIDAE) VISITS AND POLLEN SOURCE EFFECTS ON FRUITING OF GULFCOAST SOUTHERN Highbush BLUEBERRY**

DANKA, RG; LANG, GA; GUPTON, CL. 1993

Bee visitation levels and pollen sources were varied in an effort to optimize fruit production (especially early ripening, fruit set, and berry weight) of southern highbush blueberry (low chill hybrids of *Vaccinium corymbosum* L.). Fruiting plants ('Gulfcoast') were enclosed in nylon-mesh cages with colonies of honey bees, *Apis mellifera* L., and pollinizer plants affording either intravarietal self-pollination, intervarietal crossing, or interspecific crossing with rabbiteye blueberries, *Vaccinium ashei* Reade. Newly opened blossoms were allowed 0, 1, 5, 10, or unlimited bee visits before being closed with a fine mesh bag. Significant improvements in fruiting characters were achieved between one and five visits and also usually between five and unlimited visits. Fruit set more than tripled between the fewest- and greatest-visit levels; set peaked near 70%. The pollination-to-harvest interval, a chief determinant of blueberry prices in the early season, was shortened by 5 d to 53 +/- 0.5 (SE) d. Berry weight increased 28% from the 0- and 1-visit groups to 1.77 +/- 0.05 g per berry with unlimited visits. Seed numbers increased 2.2-fold to 40 +/- 1 seeds per berry. Sugar concentration of juice ranged from 11.0 to 12.9% and was lower at greater levels of bee visitation. Pollen source did not have a significant effect on any fruiting character measured in 'Gulfcoast'.

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#### **Reproductive compatibility of several East and West African *Cotesia sesamiae* (Hymenoptera : Braconidae) populations and their crosses and backcrosses using *Sesamia calamistis* (Lepidoptera : Noctuidae) as the host**

Gounou, S; Chabi-Olaye, A; Poehling, HM; Schulthess, F. 2008

The relative importance of the braconid *Cotesia sesamiae*, a gregarious larval parasitoid of lepidopteran stemborers, varies greatly with region in Africa; while the most common parasitoid of noctuid stemborers in eastern Africa, it is rare in western Africa. Thus, several strains of *C. sesamiae* from Kenya are envisaged for introduction into western Africa. The present study investigates the reproductive compatibility between four populations of *C. sesamiae* from West Africa and Kenya with the noctuid *Sesamia calamistis* as the host using reciprocal crosses as well as backcrosses of hybrid females with males of the parental populations. Searching time of the male for the female and mating period varied significantly with couple and ranged between 0.78-1.9 min and 3.4-12.8 s, respectively. Crosses that involved females from inland Kenya (KI) did not yield any female offspring. However, backcrosses of hybrid female bearing a KI male genome with a KI male yielded both female and male offspring. Thus, there was a partial reproductive incompatibility between KI and West African populations which suggested that the latter were infected with *Wolbachia* sp. However, this should not affect the efficacy of a population introduced from East into West Africa, as there is a high degree of sib-mating in this gregarious parasitoid species. It was concluded that the regional differences in the relative importance of *C. sesamiae* was due to differences in the insect and plant host range of the different populations.

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#### **RAPID DETERIORATION OF SEARCHING BEHAVIOR, HOST DESTRUCTION, AND FECUNDITY OF THE PARASITOID MUSCIDIFURAX-RAPTOR (HYMENOPTERA, PTEROMALIDAE) IN CULTURE**

GEDEN, CJ; SMITH, L; LONG, SJ; RUTZ, DA. 1992

*Muscidifurax raptor* Girault & Sanders that had been in culture for seven generations (collected in 1987, strain MR87) were larger, killed more house fly pupae, produced more progeny, and had superior host-searching ability than parasitoids that had been in culture for 94 generations (collected in 1982, strain MR82). After 17 generations, attack rates of MR87 declined to equal those of MR82 and were substantially lower than those of MR82 after 42 generations. Heritability, based on mother-daughter regressions, was 0.52 for wing length and impossibly high ( $h^2$ , 1.56) for fecundity, indicating that fecundity is largely determined by nonchromosomal maternal influences. Single-pair reciprocal crosses between strains confirmed that fecundity was influenced by a maternal effect, although hybrids showed evidence of positive heterosis as well. Attack rates and progeny production by parasitoids collected from the field in 1990 declined by 50% after only two generations in culture. The results are best explained by a combination of a maternally transmitted pathogen that is amplified in the laboratory and, to a lesser extent, inbreeding depression. Examination of cultures after the conclusion of the experiments revealed infections of most colonies with an undetermined microsporidium.

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#### **Relationship between oviposition, virulence gene expression and parasitism success in *Cotesia typhae* nov sp parasitoid strains**

Benoist, R; Chantre, C; Capdevielle-Dulac, C; Bodet, M; Mougél, F; Calatayud, PA; Dupas, S; Huguet, E; Jeannette, R; Obonyo, J; Odorico, C; Silvain, JF; Le Ru, B; Kaiser, L. 2017

Studying mechanisms that drive host adaptation in parasitoids is crucial for the efficient use of parasitoids in biocontrol programs. *Cotesia typhae* nov. sp. (Fernandez-Triana) (Hymenoptera: Braconidae) is a newly described parasitoid of the Mediterranean corn borer *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae). Braconidae are known for their domesticated bracovirus, which is injected with eggs in the host larva to overcome its resistance. In this context, we compared reproductive success traits of four Kenyan strains of *C. typhae* on a French and a Kenyan populations of its host. Differences were found between the four strains and the two most contrasted ones were studied more thoroughly on the French host population. Parasitoid offspring size was correlated with parasitism success and the expression of bracovirus virulence genes (CrV1 and Cystatin) in the host larva after parasitism. Hybrids between these two parasitoid strains showed phenotype and gene expression profiles similar to the most successful parental strain, suggesting the involvement of dominant alleles in the reproductive traits. Ovary dissections revealed that the most successful strain injected more eggs in a single host larva than the less successful one, despite an equal initial ovocyte number in ovaries. It can be expected that the amount of viral particles increase with the number of eggs injected. The ability to bypass the resistance of the allopatric host may in consequence be related to the oviposition behaviour (eggs allocation). The influence of the number of injected eggs on parasitism success and on virulence gene expression was evaluated by oviposition interruption experiments.

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#### **GENETIC-VARIATION IN LOCOMOTOR-ACTIVITY RHYTHM AMONG POPULATIONS OF LEPTOPILINA-HETEROTOMA (HYMENOPTERA, EUCOILIDAE), A LARVAL PARASITOID OF DROSOPHILA SPECIES**

FLEURY, F; ALLEMAND, R; FOUILLET, P; BOULETREAU, M. 1995

The locomotor activity rhythm of *Leptopilina heterotoma*, a parasitoid insect of *Drosophila* larvae, was investigated under laboratory conditions. Under LD 12:12, the locomotor activity of females shows a clear rhythm which persists under continuous darkness (circadian rhythm). However, comparative study of five populations indicates that both the rate of activity and the profile of the rhythm vary according to the origin of females. The Mediterranean populations (Tunisia and Antibes) show two peaks of activity, at the beginning and at the end of the photophase, whereas more northern populations (Lyon and the Netherlands) are mostly active during the afternoon. Females originating from the area of Lyon have a very low level of activity. Reciprocal crosses (F-1 hybrids and backcrosses) between the



French and the Tunisian strains demonstrated the genetic basis of these variations and the biparental inheritance of the trait. This genetic variability is interpreted as a consequence of selective pressures and suggests a local adaptation of natural populations in host foraging behavior. The selective factors which could act on the daily organization of parasitoid behaviors are discussed.

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#### Dynamic thermal structure of imported fire ant mounds

Vogt, JT; Wallet, B; Coy, S. 2008

A study was undertaken to characterize surface temperatures of mounds of imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) and *S. richteri* Forel, and their hybrid, as it relates to sun position and shape of the mounds, to better understand factors that affect absorption of solar radiation by the nest mound and to test feasibility of using thermal infrared imagery to remotely sense mounds. Mean mound surface temperature peaked shortly after solar noon and exceeded mean surface temperature of the surrounding surface. Temperature range for mounds and their surroundings peaked near solar noon, and the temperature range of the mound surface exceeded that of the surrounding area. The temperature difference between mounds and their surroundings peaked around solar noon and ranged from about 2 to 10 C. Quadratic trends relating temperature measurements to time of day (expressed as percentage of daylight hours from apparent sunrise to apparent sunset) explained 77 to 88% of the variation in the data. Mounds were asymmetrical, with the apex offset on average 81.5 +/- 1.2 mm to the north of the average center. South facing aspects were about 20% larger than north facing aspects. Mound surface aspect and slope affected surface temperature; this affect was greatly influenced by time of day. Thermal infrared imagery was used to illustrate the effect of mound shape on surface temperature. These results indicate that the temperature differences between mounds and their surroundings are sufficient for detection using thermal infrared remote sensing, and predictable temporal changes in surface temperature may be useful for classifying mounds in images.

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#### Distribution of vespoid species in Europe

Fernandez, J. 2004

Purpose of review Knowledge of the different stinging vespids found in various parts of Europe and their venom cross-reactivity is important in order to improve the venoms available for diagnostic and therapeutic purposes. Recent findings In recent years the amino acid sequences of different vespoid venom allergens have been determined. Comparison of these sequences has led to an improved taxonomical classification of vespids compared with that based on morphological differences. However, the distribution of vespids in Europe is still based on a very good but somewhat old study carried out in the 1970s. Most recent epidemiological studies focus on the type of reaction produced rather than the insect responsible. Summary The genera *Vespula*, *Dolichovespula* and *Vespa* are found all over Europe, but the genus *Polistes*, although present in central Europe, is not found in the UK and only represents a specific clinical problem in areas around the Mediterranean sea. Although there are significant differences in the distribution of stings, the genus *Vespula* predominates over *Polistes* and *Vespa* throughout Europe, except in Mediterranean areas. The different species of *Polistes* in Europe show a great similarity in the sequences of their venom allergens, but the similarity to their American counterparts is less marked. *Vespula* allergens show up to 95% sequence identity and almost complete cross-reactivity. There is also great cross-reactivity among the genera *Vespula*, *Vespa* and *Dolichovespula*. This identity of amino acid sequences confirms the latest morphological taxonomy of Hymenoptera and opens the way for the use of recombinant hybrids of different species in venom immunotherapy.

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#### Resistance of maize landraces from Brazil to fall armyworm (Lepidoptera: Noctuidae) in the winter and summer seasons

Costa, EN; Fernandes, MG; Medeiros, PH; Evangelista, BMD. 2020

The fall armyworm (*Spodoptera frugiperda*) is a major maize (*Zea mays* L.) pest in Brazil, whose larvae cause losses from plant emergence to harvesting stage. This pest has been controlled almost exclusively with chemical insecticides and Bt plants (transgenics); however, resistance evolution has been detected to these two control tactics. Therefore, alternative control tactics are needed for management and control of the fall armyworm. Thus, this study aimed to evaluate the resistance of maize landraces from Brazil to larvae of this herbivore in the winter and summer seasons, studying also the occurrence of the predator *Doru luteipes* (Dermaptera: Forficulidae). The variety Perola had the highest resistance levels to fall armyworm in the summer season, with lower values of leaf injury, number of infested plants, and number of larvae. Conversely, this variety did not express resistance to the pest in winter. *Doru luteipes* was found abundantly in the summer season, showing no differences between varieties. This is the first study to evaluate the resistance of maize landraces from Brazil to fall armyworm in the winter and summer seasons. The next step is the molecular characterization of the variety Perola, as well as evaluating its effects on the most common natural enemies of the pest in Brazil, such as the predator *D. luteipes* and the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). These results may assist future maize breeding programs aimed at developing cultivars and hybrids resistant to the pest, and hence reducing agriculture's dependence on chemical insecticides and transgenic plants.

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#### Differential rates of invasion in three related alien oak gall wasps (Cynipidae : Hymenoptera)

Walker, P; Leather, SR; Crawley, MJ. 2002

Three related species of oak gall wasps, *Andricus corruptrix* (Schlechtendal), *A. kollari* (Hartig) and *A. lignicola* (Hartig) have entered Britain since the introduction of Turkey oak, *Quercus cerris* L. in 1735. Their lifecycles involve alternating generations between an agamic generation on the native oak species (*Q. petraea*, *Q. robur* and their hybrid *Q. x. rosacea*), and a smaller, sexual generation on the alien *Q. cerris*. In examining the distributions of these insects and *Q. cerris*, we hypothesized that: (1) the invasion will spread more rapidly in places where both host trees are equally abundant than through regions where one of the tree species is substantially less common than the other; (2) interspecific competition between these bud-galling species will lead to a negative correlation between their abundances at a particular site; (3) differential recruitment of natural enemies from the native hymenopteran fauna will slow the rate of spread in a species-specific manner. *A. kollari* arrived nearly 200 years ago and is now found throughout the British Isles, wherever Turkey oak is grown. *A. lignicola* and *A. corruptrix* have been here for 30 years, after establishing in S. E. England. *A. lignicola* is in its final rapid stages of range expansion across England, southern Scotland and N.E. Scotland. *A. corruptrix* is just beginning to spread through Central and S. W. England. It has occupied proportionally fewer sites behind its invasion front than have the other two species, but is no less abundant at these sites. Nevertheless, distance leaps of up to 50 km were identified in *A. lignicola* in N.E. Scotland, and the possibility of long-distance transport of infected trees through the horticulture and forestry trades remains. The co-occurrence of mature individuals of both host *Quercus* species does appear to have increased their rates of colonization in *A. lignicola* and *A. corruptrix*. There is no evidence, however, to suggest that interspecific competition between the three alien gall formers is an important factor in determining their distributions and abundance within their invaded ranges. All three species have recruited parasitoids and inquiline rapidly from the native fauna; attack rates were highly variable, but showed no evidence of density dependence across sites.

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#### Anchored phylogenomics and a revised classification of the planidial larva clade of jewel wasps (Hymenoptera: Chalcidoidea)

Zhang, JX; Heraty, JM; Darling, C; Kresslein, RL; Baker, AJ; Torrens, J; Rasplus, JY; Lemmon, A; Lemmon, EM. 2022

Planidia are free-living, mobile first-instar larvae that are notable in their ability to transition across different larval stages of their host, and for completing their development on the host prepupa as ectoparasitoids, effectively acting as larval-pupal external koinobionts. Chalcidoid taxa with a planidium form a monophyletic

group, the planidial larva clade (PLC). We conducted a phylogenomic study of the PLC using anchored hybrid enrichment data. Phylogenetic analyses support the backbone relationship of PLC as: (Eutrichosomatinae, ((Philomidinae, Chrysolampinae), (Perilampinae, Eucharitidae))). Although excluded from the main analyses, the genus *Jambiya*, based on only 11 loci recovered, was placed as the sister of Chrysolampinae + Philomidinae or Perilampinae + Eucharitidae. Our results demonstrate that Perilampidae (Philomidinae, Chrysolampinae and Perilampinae) are paraphyletic. Divergence dating based on four node calibrations based on fossils suggests that the PLC arose approximately 111 Ma and the evolution of ant parasitism at least 64 Ma. Host associations, direct versus indirect hyperparasitism, ability to attack a host within a cocoon, soft versus hard planidial forms and mobility of the planidium were explored using a likelihood-based ancestral state reconstruction method. A revised higher-level classification of the PLC is proposed, with Eutrichosomatinae elevated to Eutrichosomatidae (stat. rev.), Chrysolampinae and Philomidinae placed in Chrysolampidae (stat. rev.), Perilampidae (stat. rev.) restricted to what was referred to as Perilampinae, and Eucharitidae maintained with four subfamilies, with Akapalinae (unknown biology) as sister group to the core Eucharitidae, all of which are ant parasitoids. *Jambiya* is treated as an incertae sedis taxon within the planidial clade.

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#### **Parasitism of greenbugs (Homoptera : Aphididae) by *Lysiphlebus testaceipes* (Hymenoptera : Braconidae) in grain sorghum: Implications for augmentative biological control**

Fernandes, OA; Wright, RJ; Mayo, ZB. 1998

Field cage studies were conducted to describe the relationship between the percentage of *Lysiphlebus testaceipes* (Cresson) parasitism (as measured by aphid mummies) and densities of greenbug, *Schizaphis graminum* Rondani, on grain sorghum, *Sorghum bicolor* L. In 1993 and 1994, a biotype E-susceptible grain sorghum hybrid was grown in field cages and *L. testaceipes* adults were released after each plant was infested with 20 biotype E greenbugs. The release rates were 0, 0.5, 1.0, and 2.0 wasps per plant in 1993, and 0, 0.16, 0.33, and 0.5 wasps per plant in 1994. Greenbugs and mummies were counted 1-2 times a week on all leaves of 2-4 randomly selected plants per cage. A release rate of 0.33-0.5 wasps per plant infested with 20 greenbugs maximized the number of mummies produced and prevented the greenbugs from reaching an economic threshold of 1,000 greenbugs per plant. Peak numbers of mummies occurred approximate to 400-500 DD (10 degrees C base) after the initial wasp release. Regression analyses showed that the greenbug population started decreasing when the percentage of parasitism (as measured by mummies) reached 20-30%. Greenbugs in the absence of wasps significantly reduced yield in 1994, but not in 1993.

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#### **Fire ant decapitating fly cooperative release programs (1994-2008): Two *Pseudacteon* species, *P. tricuspidis* and *P. curvatus*, rapidly expand across imported fire ant populations in the southeastern United States**

Callcott, AMA; Porter, SD; Weeks, RD; Graham, LC; Johnson, SJ; Gilbert, LE. 2011

Natural enemies of the imported fire ants, *Solenopsis invicta* Buren, *S. richteri* Forel (Hymenoptera: Formicidae), and their hybrid, include a suite of more than 20 fire ant decapitating phorid flies from South America in the genus *Pseudacteon*. Over the past 12 years, many researchers and associates have cooperated in introducing several species as classical or self-sustaining biological control agents in the United States. As a result, two species of flies, *Pseudacteon tricuspidis* Borgmeier and *P. curvatus* Borgmeier (Diptera: Phoridae), are well established across large areas of the southeastern United States. Whereas many researchers have published local and state information about the establishment and spread of these flies, here distribution data from both published and unpublished sources has been compiled for the entire United States with the goal of presenting confirmed and probable distributions as of the fall of 2008. Documented rates of expansion were also used to predict the distribution of these flies three years later in the fall of 2011. In the fall of 2008, eleven years after the first successful release, we estimate that *P. tricuspidis* covered about 50% of the fire ant quarantined area and that it will occur in almost 65% of the quarantine area by 2011. Complete coverage of the fire ant quarantined area will be delayed or limited by this species' slow rate of spread and frequent failure to establish in more northerly portions of the fire ant range and also, perhaps, by its preference for red imported fire ants (*S. invicta*). Eight years after the first successful release of *P. curvatus*, two biotypes of this species (one biotype occurring predominantly in the black and hybrid imported fire ants and the other occurring in red imported fire ants) covered almost 60% of the fire ant quarantined area. We estimate these two biotypes will cover almost 90% of the quarantine area by 2011 and 100% by 2012 or 2013. Strategic selection of several distributional gaps for future releases will accelerate complete coverage of quarantine areas. However, some gaps may be best used for the release of additional species of decapitating flies because establishment rates may be higher in areas without competing species.

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#### **SOME COLORADO *ANDRENA* OF THE SUBGENUS *SCAPHANDRENA* OF PRESUMED HYBRID ORIGIN, WITH SPECIAL REFERENCE TO THE TARSAL CLAWS (HYMENOPTERA, APOIDEA)**

LANHAM, UN. 1981

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#### ***Wolbachia* (Rickettsiales) infections and bee (Apoidea) barcoding: a response to Gerth et al.**

Stahlhut, JK; Gibbs, J; Sheffield, CS; Smith, MA; Packer, L. 2012

In a recent Perspective, Gerth et al. (2011) expressed concern over how *Wolbachia* (*Wolbachia pipientis* Hertig) infections may affect the success of DNA barcoding efforts in bees. The potential and realized effects of endosymbiont-induced selective sweeps on host mitochondrial DNA diversity have been noted repeatedly and rightly so in the literature for some years. However, we are equally concerned with other misconceptions, including (a) presuming that a positive *Wolbachia* test indicates a stable infection, (b) presuming that *Wolbachia*-infected hosts cannot be identified with a single-locus barcode, and (c) inferring specific *Wolbachia*mtDNA interactions based only on incomplete genotyping of *Wolbachia* strains. We address these issues in the context of the Gerth et al. (2011) survey of *Wolbachia* prevalence among the German bee fauna. We also clarify some of the context-dependent strengths and limitations of DNA barcoding when it is used as a research tool by taxonomists and ecologists.

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#### **New fossil ephialtitids elucidating the origin and transformation of the propodeal-metasomal articulation in Apocrita (Hymenoptera)**

Li, LF; Shih, C; Rasnitsyn, AP; Ren, D. 2015

Background: Apocrita has a special structure that its first abdominal segment has been incorporated into the thorax as the propodeum. The remaining abdomen, metasoma, is connected to this hybrid region via a narrow propodeal-metasomal articulation forming a "wasp waist", which serves an important function of providing maneuverability, flexibility and posture for oviposition. However, the origin and transformation of the propodeal-metasomal articulation are still vague. Ephialtitidae, as the basal group of Apocrita from the Early Jurassic to the Early Cretaceous, have shown various types of propodeal-metasomal articulations. Results: This study describes and illustrates two new genera with three new species, *Acephialtitia colossa* gen. et sp. nov., *Proepialtitia acanthi* gen. et sp. nov. and *P. tenuata* sp. nov., collected respectively from the Early Cretaceous Yixian Formation at Liutiaogou and the Middle Jurassic Jiulongshan Formation at Daohugou, both in Inner Mongolia, China. These genera are assigned to the Ephialtitidae based on their complete wing venation, e.g. 2r-rs, 2r-m, 3r-m and 2 m-cu always present in the forewings and Rs, M and Cu in the hind wings. These new fossil ephialtitids have well-preserved propodeal-metasomal articulations indicating metasoma is broadly attached to propodeum. Conclusion: The broad articulation between the propodeum and metasoma in basal Ephialtitidae, likely passed on from a still more basal family Karatavidae, suggests three separate pathways of the transformation of the "wasp waist" in three different derived lineages leading from Ephialtitidae to: (i) Kuafuidae and further to the remaining Apocrita, (ii) Stephanidae, and (iii) Evaniidae. In addition, the demise of ephialtitid wasps lagging behind the flourishing of

angiosperms suggests that ephialtoid extinction might have been mainly driven by competition with numerous new taxa (eg. the abundant Cretaceous xylophilous Baissinae and Ichneumonoidea) appeared just before or/and soon after the J/K boundary.

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#### **HOST RECOGNITION AND ACCEPTANCE BEHAVIOR IN 2 APHID PARASITOID SPECIES - APHIDIUS-ERVI AND APHIDIUS-MICROLOPHII (HYMENOPTERA, BRACONIDAE)**

PENNACCHIO, F; DIGILIO, MC; TREMBLAY, E; TRANFAGLIA, A. 1994

The host preference and acceptance behaviour of populations of *Aphidius ervi* Haliday and *A. microlophii* Pennacchio & Tremblay from southern Italy was investigated. In no host-choice conditions, *A. ervi* females showed significantly higher attack and oviposition rates on the natural host *Acyrtosiphon pisum* (Harris) than on the non-host aphid *Microlophium carnosum* (Buckton) (Homoptera: Aphididae). In contrast, *A. microlophii*, which specifically parasitizes *M. carnosum* in the field, attacked both aphid species. However, dissections showed that oviposition of *A. microlophii* occurred only in a few of the attacked *Acyrtosiphon pisum* and was significantly less frequent than in *M. carnosum*. These results were confirmed in experimental host-choice conditions, suggesting that *Aphidius microlophii* oviposition is possibly regulated by a host haemolymphatic kairomone. Hybrids obtained by crossing *A. ervi* females with *A. microlophii* males attacked and oviposited in both aphid species, suggesting that these behavioural events have a strong genetic basis. The oviposition into host or non-host aphids did not elicit an immune defence reaction. The presence of the host's food-plant had no evident close-range effects on parasitoid attack and oviposition in non-host aphids. *Aphidius microlophii* reared on the non-host aphid *Acyrtosiphon pisum* produced a significant higher number of mummies after a few generations, suggesting a possible role of larval and early adult conditioning in the host selection process. These results, together with those from previous studies, suggest that *Aphidius ervi* is best considered as a complex of differentiated populations, characterized by a varying degree of genetic divergence.

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#### **Genetic variation and the performance of a mass-reared parasitoid, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), in laboratory trials**

Guzman-Laralde, A; Cerna-Chavez, E; Rodriguez-Campos, E; Loyola-Licea, JC; Stouthamer, R. 2014

During mass rearing, adaptation of biological control agents to the rearing environment is a potential problem. Using the parasitoid wasp *Trichogramma pretiosum*, the performance of 26 highly inbred lines, five composite 'populations' (created from the inbred lines) and one insectary-reared population was compared using fertility life tables. Of the composite populations, three were created with maximal and identical genetic variation as a mixture of all 26 inbred lines, but these were then reared for a different number of generations (2, 6 or 17) before their performance was measured. The remaining two composite populations were created based on the performance of the individual inbred lines: one was a combination of two inbred lines with a high intrinsic rate of natural increase (rm), 'high rm'; and the other was a combination of two lines with a 'low rm'. High and low rm populations were reared for two generations prior to testing. Parameters measured were fertility, longevity and sex ratio. We found no difference between the maximally variable population reared for two generations and the 'high rm' population (rm=0.285 and 0.282, respectively). 'Low rm' was the population with the lower performance (rm=0.255). Genetically variable population reared for two generations for 48h produced significantly more offspring than the populations reared for 6 and 17 generations. Hybrid population derived from the high-rm lines did significantly better than that derived from the low-rm lines. Low-performance populations become more male based than high performance at 48h. The potential benefits to improve population's performance using inbred lines for mass rearing are discussed.

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#### **Timing bait applications for control of imported fire ants (Hymenoptera : Formicidae) in Mississippi: Efficacy and effects on non-target ants**

Vogt, JT; Reed, JT; Brown, RL. 2005

An experiment was conducted to assess the efficacy of mid-day (11:00-13:00 h) and late evening (18:00-20:00 h) broadcast bait (Seige Pro(R)), 0.73% hydramethylnon) applications against black and hybrid imported fire ants (*Solenopsis richteri* Forel and *S. richteri* x *invicta*, respectively) and their impact on native ant species. It was hypothesized that evening bait applications would have less impact on native ant species that slow or cease foraging at night relative to mid-day applications. Bait was applied to a series of plots in northeastern Mississippi, USA, in summer of 2002 and 2003. Population densities and foraging activity of imported fire ants and native ants were compared between treated and control plots. Population density and foraging activity of imported fire ants were equally suppressed in plots receiving mid-day and evening broadcast bait applications. Population density of *Monomorium minimum* (Buckley), the little black ant, approached zero in treated plots during 2003 but remained relatively high in untreated control plots. Species richness declined in treated plots with no difference between mid-day and evening bait application. These data indicate that evening bait application offers no advantage over mid-day application in terms of preserving some native ant species.

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#### **Effects of selected midwestern larval host plants on performance by two strains of the gypsy moth (Lepidoptera: Lymantriidae) parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae)**

Kruse, JJ; Raffa, KF. 1997

The effects of gypsy moth, *Lymantria dispar* (L.), larval diet on parasitism rates and performance by 2 strains of the larval parasitoid *Cotesia melanoscela* Ratzeburg were studied in laboratory trials. Gypsy moth larvae that were parasitized by *C. melanoscela* strains from France and South Korea, and an unparasitized control group, were allowed to develop on foliage from 5 tree species (red oak, tamarack, sugar maple, hybrid poplar clone NC5271, and white pine) and synthetic diet. Food plant had a significant effect on gypsy moth relative growth, development time, and pupal weight. Gypsy moth food plant affected *C. melanoscela* development time and size. Host plant influenced percentage of parasitism, but this effect varied between blocks. Parasitoid strain was a major factor in development time, size, and host selection, but was of marginal influence on parasitism rates and sex ratio. Plant suitability to gypsy moth, both in host species and phenology, was the strongest underlying factor in *C. melanoscela* parasitoid success, development time, and size. Plant and strain effects on *C. melanoscela* may be important factors in guiding biological control efforts against gypsy moth.

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#### **The genetic basis of the interspecific differences in wing size in *Nasonia* (Hymenoptera; Pteromalidae): Major quantitative trait loci and epistasis**

Gadau, J; Page, RE; Werren, JH. 2002

There is a 2.5-fold difference in male wing size between two haplodiploid insect species, *Nasonia vitripennis* and *N. giraulti*. The haploidy of males facilitated a full genomic screen for quantitative trait loci (QTL) affecting wing size and the detection of epistatic interactions. A QTL, analysis of the interspecific wing-size difference revealed QTL with major effects and epistatic interactions among loci affecting the trait. We analyzed 178 hybrid males and initially found two major QTL for wing length, one for wing width, three for a normalized wing-size variable, and five for wing seta density. One QTL for wing width explains 38.1%, of the phenotypic variance, and the same QTL explains 221 of the phenotypic variance in normalized wing size. This corresponds to a region previously introgressed from *N. giraulti* into *N. vitripennis* that accounts for 44% of the normalized wing-size difference between the species significant epistatic interactions were also found that affect wing size and density of setae on the wing. Screening for pairwise epistatic interactions between loci on different linkage groups revealed four additional loci for will, length and four loci for normalized wing size that were not detected in the original QTL, analysis. We propose that the evolution of smaller wings in *X. vitripennis* males is primarily the result of major mutations at few genomic regions and involves epistatic interactions among some loci.

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## Development of a lateral flow immunoassay for rapid field detection of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae)

Valles, SM; Strong, CA; Callcott, AMA. 2016

The red imported fire ant, *Solenopsis invicta*, is an aggressive, highly invasive pest ant species from South America that has been introduced into North America, Asia, and Australia. Quarantine efforts have been imposed in the USA to minimize further spread of the ant. To aid the quarantine efforts, there remains an acute need for a rapid, field portable method for the identification of these ants. In this report, we describe two novel monoclonal antibodies that specifically bind the *S. invicta* venom protein 2 produced by *S. invicta*. Using these monoclonal antibodies we developed a lateral flow immunoassay that provides a rapid and portable method for the identification of *S. invicta* ants. The lateral flow immunoassay was validated against purified *S. invicta* venom protein 2 and 33 unique ant species (representing 15 % of the total species and 42 % of the Myrmicinae genera found in Florida), and only *S. invicta* and the *S. invicta/richteri* hybrid produced a positive result. These monoclonal antibodies were selective to *S. invicta* venom protein 2 and did not bind to proteins from congeners (i.e., *S. geminata* or *S. richteri*) known to produce a *S. invicta* venom protein 2 ortholog. This *S. invicta* lateral flow immunoassay provides a new tool for regulatory agencies in the USA to enforce quarantine protocols and limit the spread of this invasive ant.

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## *Cotesia cassina* sp. nov. from southwestern Colombia: a new gregarious microgastrine wasp (Hymenoptera, Braconidae) reared from the pest species *Opsiphanes cassina* Felder & Felder (Lepidoptera, Nymphalidae) feeding on *Elaeis* oil palm trees (Arecaceae)

Salgado-Neto, G; Vasquez, CAN; Max, DS; Whitfield, JB. 2021

A new species of microgastrine wasp, *Cotesia cassina* Salgado-Neto, Vasquez & Whitfield, sp. nov., is described from southwestern Colombia in Tumaco, Narino. This species is a koinobiont gregarious larval endoparasitoid, and spins a common mass of cocoons underneath the host caterpillars of *Opsiphanes cassina* (Felder & Felder) (Lepidoptera, Nymphalidae), feeding on oil palm trees (interspecific hybrid *Elaeis oleifera* x *E. guineensis*) (Arecaceae). While superficially similar, both morphologically and biologically, to *C. invirae* Salgado-Neto & Whitfield from southern Brazil, the two species are distinct based on DNA barcodes, host species, geographical range and morphological characters.

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## A female attractant for the blue gum chalcid, *Leptocybe invasa* (Hymenoptera: Eulophidae), from host plant(DH 201-2:*Eucalyptus grandis*x*Eucalyptus tereticornis*)

Ma, T; Lan, LJ; Lin, N; Zheng, LF; Sun, ZH; Li, YZ; Wen, XJ. 2019

The blue gum chalcid, *Leptocybe invasa*, is a severe insect pest of *Eucalyptus* trees leading to gall formation, stunting, leaf deformation, and death in cases, China. In the previous studies, we found that the *Eucalyptus* hybrid, DH 201-2 (*Eucalyptus grandis*x*Eucalyptus tereticornis*), is highly preferred to *L. invasa* larvae and adults, but its mechanism has not been fully understood. In the present study, we investigated the volatile organic compounds (VOCs) from DH 201-2 that attract *L. invasa*. Volatiles were collected from fresh young leaves and identified with gas chromatography-mass spectrometry (GC-MS) and gas chromatography-electroantennogram detection (GC-EAD). The attraction of identified volatiles to *L. invasa* was then measured under laboratory and field conditions. Two EAD-active compounds, gamma-terpinene and Limonene, were identified. In the dose-response experiments, we found that the EAG response to *L. invasa* was enhanced with the increased concentration of EAD-active compounds, and gamma-terpinene showed the stronger EAG response than Limonene. Both four-arm olfactometer bioassays and field studies showed that the blend of gamma-terpinene and Limonene with a ratio of 8:2 significantly attracted more *L. invasa* as compared to other ratios. The potential use of these compounds in the control of *L. invasa* is ultimately discussed.

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## The impact of GC bias on phylogenetic accuracy using targeted enrichment phylogenomic data

Bossert, S; Murray, EA; Blaimer, BB; Danforth, BN. 2017

The field of sequence based phylogenetic analyses is currently being transformed by novel hybrid-based targeted enrichment methods, such as the use of ultraconserved elements (UCEs). Rather than analyzing relationships among organisms using a small number of genes, these methods now allow us to evaluate relationships with many hundreds to thousands of individual gene loci. However, the inclusion of thousands of loci does not necessarily overcome the long-standing challenge of incongruence among phylogenetic trees derived from different genes or gene regions. One factor that impacts the level of incongruence in phylogenomic data sets is the level of GC bias. GC rich gene regions are prone to higher recombination rates than AT rich regions, driven by a process referred to as "GC biased gene conversion". As a result, high GC content can be negatively associated with phylogenetic accuracy, but the extent to which this impacts incongruence among UCEs is currently unstudied. We investigated the impact of GC content on phylogeny reconstruction using in silico captured UCE data for the corbiculate bees (Hymenoptera: Apidae). The phylogeny of this group has been the subject of extensive study, and incongruence among gene trees is thought to be a source of phylogenetic error. We conducted coalescent- and concatenation-based analyses of 810 individual gene loci from all 13 currently available bee genomes, including 8 corbiculate taxa. Both coalescent- and concatenation-based methods converged on a single topology for the corbiculate tribes. In contrast to concatenation, the coalescent-based methods revealed significant topological conflict at nodes involving the orchid bees (*Euglossini*) and honeybees (*Apini*). Partitioning the loci by GC content reveals decreasing support for the inferred topology with increasing GC bias. Based on the results of this study, we report the first evidence that GC biased gene conversion may contribute to topological incongruence in studies based on ultraconserved elements. (C) 2017 Elsevier Inc. All rights reserved.

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## Disentangling a Holobiont - Recent Advances and Perspectives in *Nasonia* Wasps

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

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

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**New Distribution Record and Behavioral Observations for *Pseudacteon curvatus* (Diptera: Phoridae), a Parasitoid of Imported Fire Ants and Their Hybrid (Hymenoptera: Formicidae)**

Vogt, JT; Rashid, T; Chen, J. 2008

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**THE HYBRID SWARM OF *ANDRENA* (HYMENOPTERA, APOIDEA) IN WESTERN NORTH-AMERICA - A POSSIBLE SOURCE FOR THE EVOLUTIONARY ORIGIN OF A NEW SPECIES**

LANHAM, UN. 1984

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**Presence after three decades of red wood ants (*Formica rufa* group; Hymenoptera: Formicidae) in forests in an agricultural landscape**

Van Buggenum, HJM. 2022

Conducting a repeat study of the presence of mound-building red wood ants (*Formica rufa* group) after a period of 30 years has rarely been done in Europe. From 1990 to 2020 such a study was done in an intensively used agricultural landscape with fragments of forest in the South-Eastern part of The Netherlands. In 1990, 280 nest mounds of three species of red wood ants and a hybrid were found in the forests and along forest edges. The highest occupancy was in forests of > 25 ha. The connectivity between the forest fragments mainly determined their presence. In 2020, only 160 nest mounds were found. The development of the colonies differed for *Formica polyctena* Forster, 1850 (slight decrease), *F. rufa* Linnaeus, 1761 (substantial increase) and *F. pratensis* Retzius, 1783 (strong decrease). The differences between the species in this highly fragmented landscape may be due to differences in their colonization strategies of either nest splitting or producing swarms of young queens. Furthermore, the analyses show that the type of environment (in a forest or along a forest edge) determines the probability of extinction or settlement. There is a need to undertake management measures to prevent the extinction of species of red wood ants in the area studied. These measures include creating small clearings in dense forests, reducing shading due to overgrowing shrubs or herbaceous plants, and creating buffer zones around intensively managed fields. More long-term monitoring is needed in Europe to compare the circumstances in different regions, detect trends and evaluate the effects of protection measures.

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**Pre- and Postzygotic Barriers to Reproduction Between Two Host-Foodplant Complex Sources of the Parasitic Wasp, *Cotesia congregata* (Hymenoptera: Braconidae)**

Bredlau, JP; Kester, KM. 2015

Parasitic wasps are among the most speciose of all animals yet the drivers and mechanisms underlying their remarkable diversity are not well understood. *Cotesia congregata* (Say) and host, *Manduca sexta* (L.), have long-served as a model system for host-parasite and tritrophic interactions at multiple levels, as well as the evolution of symbiotic coevolved bracoviruses that suppress host defenses to parasitism. We investigated potential barriers to reproduction between two genetically differentiated host-foodplant complex sources of *C. congregata* originating from caterpillar hosts, *M. sexta* on tobacco ("MsT wasps") or *Ceratomia catalpae* (Boisduval) on catalpa ("CcC wasps"). Both MsT and CcC wasps displayed similar to 30% lower responses to cuticular washes of females from the alternate complex. Male courtship songs differed with respect to frequency, absolute amplitude, and duration of song elements. Despite differences in courtship behaviors, MsT and CcC wasps mated and produced offspring in the laboratory. However, mating success of F-1 MsTa (TM), x CcCa (TM) Euro wasps was lower and fitness of F-1 CcCa (TM), aEuro parts per thousand x MsTa (TM) Euro hybrids was greatly reduced due to encapsulation and melanization of wasp eggs in *M. sexta*. Heritable differences in brood size and secondary sex ratios of emergent adults indicate host-specific adaptations. Overall, results demonstrate the evolution and accumulation of both pre- and postzygotic barriers to reproduction, two of which are asymmetrical, and offer additional support that MsT and CcC wasp are incipient species.

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**Pollinators as isolation mechanisms: field observations and field experiments regarding specificity of pollinator attraction in the genus *Ophrys* (Orchidaceae und Insecta, Hymenoptera, Apoidea)**

Paulus, HF. 2018

It is demonstrated that the biological species concept of the genus *Ophrys* corresponds to the general concepts of Ernst Mayr. On the example of two closely related forms of the fusca group in Andalusia, it is shown how to handle the different criteria of the biospecies concept. One is the high specificity of attraction of a male bee followed by a complex pseudocopulation behaviour which enables the correct pollen transfer within the species. The high specificity is caused by a highly remarkable imitation of the sexual pheromones of the mimicked female of the pollinator. Sexual pheromones of animals act as intraspecific signals which result in attractions of only males of the same species. Exactly this specific behaviour is triggered by the *Ophrys* flower, too. Thus the pollinator male is attracted only to those flowers which have the correct mixture of the pheromone molecules. To further ensure success the pollinating males also select other characters of the *Ophrys* flower as labellum size, colour, labellum hair characters, phenology and possibly habitat selections etc. In the sexual reproduction behaviour of the male insect acts as pregamic isolation mechanisms for the given *Ophrys* like for their own female. We were successful in confirming the high specificity of the pollination mechanisms in numerous field experiments, mainly choice experiments, this in contrast to the statements of Hennecke & Munzinger (2013). They had argued that there were many different pollinator species listed in the literature for a given *Ophrys* species. However, they did not critically assess the reliability of these citations. For this, they would have had to critically read the primary literature and not only the secondary quotations like in Delforge (2005, 2016) or others. Therefore, they reshaped many of the old mistakes concerning wrong identifications of the bees or even the *Ophrys* taxons which had long been corrected. Especially a mixture of old and actual names of the same species in different combinations is annoying and seems to demonstrate that the authors are not really fit in the nomenclature of *Ophrys* and their pollinators. This is demonstrated by some examples, which are very confusing without knowledge of the bees. For example, these authors did not realize that a 3-4 mm bee (*Andrena hesperia*) could not serve as a pollinator of a 20 mm labellum of a large *Ophrys omegaifera* species. However, there are some cases with more than one pollinator. Besides the main pollinator the others I call them "secondary pollinators" which in those cases we could check their pollination contribution in the population is very weak. In many cases, these secondary visitors are mainly pollinaria thieves because they are only attracted and try to copulate. However, they seem learn very quickly that *Ophrys* is not a true female and will never visit another flower of this kind. In some quantitative field experiments (with *Eucera nigrescens/longicornis* on *Ophrys holosericea*) we could confirm this hypothesis. The conclusion of the two authors that *Ophrys* species are not species-specific regarding sexual attractivity does not correspond to our field experiments regarding olfactory compound investigations in biotests, and does not agree with the molecular data of population biology and with the genetic analyses. The other proof the authors used in their argumentations are the supposed frequency of hybrids. But this is also a spurious argument. Hybrids are only frequent in literature and not in nature. In places frequently visited by orchid enthusiasts, the number of observed "hybrids" is conspicuously high, which may indicate "hand pollination". The aim of the two authors has been to reduce the many species within *Ophrys* because they cannot be discriminated. Consequently, they try to establish a simplified classification system without any biological background. A complete anthropomorphic typological systematic like the "Index of accepted plant names" is issued by Kew Gardens. However, this is in the 21st century, 150 years after Darwin, a kind of middle age taxonomy. They use a quite typological morphospecies concept like in the book of Pederson & Faurhold (2007). This does not fit with nature. If you pay attention to the different criteria of the biospecies concept within the genus *Ophrys* you will recognise the many species which the genus makes so interesting. A critical list of the known specific pollinators of the genus *Ophrys* is given as an appendix.

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**Insights into phylogenetic relationships between *Trioxys* Haliday, 1833 and *Binodoxys* Mackauer, 1960 (Hymenoptera, Braconidae, Aphidiinae), with a description of a new species of the genus *Trioxys***



Ckrkic, J; Petrovic, A; Kocic, K; Tomanovic, Z. 2021

Despite extensive research on the taxonomy and phylogeny of the subfamily Aphidiinae Haliday, 1833, certain questions about the relationships between genera remain unresolved. Genera *Trioxys* Haliday, 1833 and *Binodoxys* Mackauer, 1960 are considered closely related, based on morphological and molecular analyses. However, recent studies suggest there is a need for a taxonomic revision of the two genera, since molecular data does not support monophyly of the two groups when a larger number of species is used in the analysis. We examine those relationships using molecular data and including a new species we describe in the present study. *Trioxys ulmi* Ckrkic & Tomanovic, n. sp. is a parasitoid of the Japanese elm aphid (*Tinocallis takachihoensis* Higuchi, 1972) on elm hybrids (*Ulmus* x *hollandica* Mill.). Despite its probable Asian origin, this species has gone undescribed until its accidental introduction to Europe, highlighting the importance of continued research efforts.

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#### Consumption of Supplemental Spring Protein Feeds by Western Honey Bee (Hymenoptera: Apidae) Colonies: Effects on Colony Growth and Pollination Potential

Hoover, SE; Ovinge, LP; Kearns, JD. NA

Adequate nutrition is required to support productive honey bee colonies, therefore beekeepers supplement colonies with additional protein at targeted time points. We tested the effects of commercially available protein feeds in spring, in advance of colonies being used for hybrid canola pollination. The feed treatments across the three-year study included the following patty types: Global 15% pollen, Global 0% pollen, Bee Pollen-Ate, FeedBee, and Healthy Bees, as well as an unsupplemented control in year two of the study only. The amount of feed consumed varied among colonies, treatments, date, and year. Similarly, there were also differences in feed efficiency (bees reared per gram of feed consumed), likely due to the relative availability of external forage sources to supplement the feed provided. Unsupplemented colonies were able to rear less brood, and subsequently had fewer adult bees than supplemented colonies, in an apiary where pollen was not abundant. Differences in consumption among treatments often failed to translate in to differences in amount of brood reared or subsequent adult population. All the protein feed treatments contained all ten amino acids essential to honey bees, however lysine and arginine were below the optimal proportion required for growth in all patties except the FeedBee patty. The amount of protein and amount and types of sugars and fats in the products also varied among product type and batch. The results of this study demonstrate a benefit to supplementary spring protein feeding to increase honey bee colony populations in advance of a summer pollination market.

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#### RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS TO MONITOR LABORATORY-SELECTED, PESTICIDE-RESISTANT *TRIOXYS-PALLIDUS* (HYMENOPTERA, APHIDIIDAE) AFTER RELEASE INTO 3 CALIFORNIA WALNUT ORCHARDS

EDWARDS, OR; HOY, MA. 1995

A nearest-neighbor discriminant analysis based on the frequencies of occurrence of six random amplified polymorphic DNA markers was used to identify individuals from an insecticide-resistant laboratory biotype (SEL) of the walnut aphid parasite *Trioxys pallidus* after release into three California walnut orchards. Releases of SEL individuals were made in a Hanford, CA, orchard in 1988 and in a Gridley, CA, orchard in 1989. Nearest-neighbor analysis of postrelease collections from both the Hanford and Gridley orchards showed a continuing influence of the SEL biotype up to 3 yr after the release. SEL individuals also were released in a Red Bluff, CA, walnut orchard in 1991. At this orchard, SEL males were recovered from the release area throughout the season of release, but a June 1992 sample indicated that no SEL individuals remained in the orchard. Comparison of observed data at Hanford and Gridley to simulated hybrid data provided evidence that there was interbreeding between the SEL and the native field biotypes. Bioassays indicated that the level of azinphosmethyl resistance in these two orchards was intermediate between the level in the orchards before release and the level in the pure SEL biotype. Therefore, the release of pesticide-resistant *T. pallidus* in two California walnut orchards significantly increased the level of resistance, but did not completely displace the native populations, despite multiple treatments of postrelease populations with azinphosmethyl.

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#### MIXED PLANTINGS OF UPLAND MALE STERILE AND PIMA RESTORER FOR INCREASED BEE (HYMENOPTERA, APIDAE) POLLINATION IN PRODUCTION OF F1 INTERSPECIFIC HYBRID COTTON SEED

DAVIS, DD; CARTER, FL; JAYCOX, EL. 1988

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#### Conserved numts mask a highly divergent mitochondrial-COI gene in a species complex of Australian stingless bees *Tetragonula* (Hymenoptera: Apidae)

Francoso, E; Zuntini, AR; Ricardo, PC; Silva, JPN; Brito, R; Oldroyd, BP; Arias, MC. 2019

*Tetragonula carbonaria*, *Tetragonula davenporti*, *Tetragonula hockingsi* and *Tetragonula mellipes* comprise a species complex of Australian stingless bee species known as the 'Carbonaria' group. The species are difficult to distinguish morphologically and the major species-defining characters relate to comb architecture and nest entrance ornamentation. The taxonomy of the group is further complicated by likely nuclear mitochondrial pseudogenes (numts) and inter-specific hybrids. Here we demonstrate the existence of COI numts and isolate and characterize the 'true' mt-COI gene in *T. carbonaria* and *T. hockingsi*. Numts were isolated from enriched-nuclear DNA extraction followed by PCR amplification and Sanger sequencing, and were recognized by the presence of deletions and/or premature stop codons in the translated sequences. The mt-COI sequences were obtained from NGS sequencing using purified mtDNA. In *T. carbonaria*, two numts (numt1 and numt2) were identified and a third (numt3) was identified in *T. hockingsi*. Numt2 and numt3 are similar (1.2% sequence divergence), indicating a recent common origin. The genetic distance between the mt-COI of the two *Tetragonula* species was higher than might be expected for closely related species, 16.5%, corroborating previous studies in which *T. carbonaria* and *T. hockingsi* were regarded as separate species. The three numts are more similar to the COI of other stingless bee species, including Australian *Austroplebia australis* and South American *Melipona bicolor* (81.7-83.9%) than to the mt-COI of their own species (70-71.4%). This is because the mt-COI of *T. carbonaria* and *T. hockingsi* differ greatly from other Meliponinae. Our findings explain some formerly puzzling aspects of Carbonaria biogeography, and misinterpreted amplifications.

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#### CHEMICALS INVOLVED IN HONEYBEE-SUNFLOWER RELATIONSHIP

PHAMDELEGUE, MH; ETIEVANT, P; GUICHARD, E; MARILLEAU, R; DOUAULT, P; CHAUFFAILLE, J; MASSON, C. 1990

We present a review of work on the plant chemicals involved in the honeybee-sunflower model system. Combined behavioral and chemical analyses were conducted under natural and controlled conditions. First the distribution of forager bees' visits on two pairs of sunflower genotypes producing a different level of hybrid seed yield was recorded under pollen-proof tunnels. Mirasol parental lines producing high seed yields were visited at random, whereas forager bees visited preferentially the female parental line of Marianne, resulting in low seed yield. Nectar samples collected on the genotypes were analyzed by gas chromatography. Fructose, glucose, and sucrose were identified. Parental lines of Mirasol showed similar sugar profiles, whereas the female line of Marianne contained higher amounts of sucrose than the male line. We assume that the bees' preferences between genotypes might rely on differences in the sugar composition of floral nectars, especially in the amount of sucrose. Aromas from headspace collection were compared between pairs and periodically during the flowering period. Of the 144 components indexed for Marianne lines and 136 components for Mirasol lines, 17 of the components for Marianne lines and 18 for Mirasol lines differed significantly according to flowering stage. Significant differences appeared in eight of the 134 components of Marianne lines and in 20 of the 250 components for Mirasol lines. Such differences, even

restricted to a few components, might account for honeybees' discrimination between genotypes or flowering stage. Experiments then were conducted in a flight room using an artificial flower device. A total volatile extract was used as a conditioning scent previous to the test where the total extract was successively compared to several of its subfractions. Fractions significantly less visible than the total extract were discarded, whereas fractions confused with the total extract were kept. From step to step, a restricted fraction of 28 polar components, among which 15 were identified, was shown to be as active as the initial conditioning extract. These data emphasized honeybees' abilities to generalize from simplified to more complex chemical information. Finally, this work considers the possible use of such plant chemicals, from nectars of aromas, either as targets for genetic modification of crop plants or as direct attractants when sprayed on the crop, for the improvement of entomophilous cross pollination.

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#### SOIL FAUNA CHARACTERIZATION IN *Eucalyptus* spp. PLANTATIONS

Garlet, J; Costa, EC; Boscardin, J. 2013

Forest soils provide good conditions for the development and the establishment of soil fauna, mainly by the deposition of litter. However, monoculture systems conducted in a single food providing substrate, can promote the development of certain fauna groups over others, causing outbreaks of pest species. The aim of this study was to characterize the soil fauna and its relationship with meteorological variables, in plantations of *Eucalyptus* spp. This study was conducted in six stands of three species of *Eucalyptus*: *Eucalyptus dunni* Maiden, *Eucalyptus grandis* Maiden and *Eucalyptus grandis* x *Eucalyptus urophylla* S. T. Blake (clone hybrid) and two ages (planted in 2006 and 2007). The stands were located at Cabanha da Prata and Chica Barbosa farms in Alegrete, and at Taquari farm in São Francisco de Assis, Rio Grande do Sul state. For sampling soil insects, pitfall traps were used. Eight traps were installed ten meters apart from each other, distributed in two rows in the center of each stand, totaling 48 traps. Samples were collected fortnightly from September 2008 to August 2009. After counting and identifying the taxonomic groups, the parameters of richness (total number of taxonomic groups), abundance and Shannon diversity index were determined. The results were analyzed using a 3x2 factorial model (three species and two ages) in a randomized design with Test F. During this study, 8,811 specimens representing 12 taxonomic groups were collected. The groups Coleoptera, Hymenoptera, and Diptera had the highest percentages of individuals collected: 31.1, 23.2, and 19.6%, respectively. The plantation with three years of age had a higher number of individuals compared to the stands of two years of age. The species *Eucalyptus grandis* and *Eucalyptus grandis* x *Eucalyptus urophylla* had a higher number of individuals collected when compared with the species *Eucalyptus dunni* at both ages. Thus, the species and age of the planting exerted influence on the organisms collected.

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#### GREENHOUSE TRIALS OF *APHIDIUS COLEMANI* (HYMENOPTERA: BRACONIDAE) BANKER PLANTS FOR CONTROL OF APHIDS (HEMIPTERA: APHIDIDAE) IN GREENHOUSE SPRING FLORAL CROPS

Van Driesche, RG; Lyon, S; Sanderson, JP; Bennett, KC; Stanek, EJ; Zhang, RT. 2008

Banker plants with *Aphidius colemani* Viereck were tested in greenhouses in Massachusetts and New York for control of cotton aphid *Aphis gossypii* Glover, and green peach aphid *Myzus persicae* (Sulzer) on 2 spring flower crops, pansies (*Viola tricolor hortensis*) and Marguerite daisies (*Argyranthemum* hybrid). Banker plants consisted of pots of barley plants infested with the bird cherry-oat aphid *Rhopalosiphum padi* (L.), inoculated at the start of the crop with adults of *A. colemani* purchased from a commercial insectary. Initial trials were conducted in University of Massachusetts greenhouses containing flats of the crop plants. Sentinel plants in flats were infested uniformly with aphids, and particular greenhouses were subjected to the presence of banker plants or left as controls. Prior to University trials, a survey was conducted in commercial greenhouses in Massachusetts and New York to determine the frequency and species of aphid infestation in spring flower crops. After University trials, the efficacy of banker plants was tested in commercial greenhouses in both states. In surveys of commercial greenhouses, *M. persicae* was the most frequently detected species, accounting for 53% of all infestations. In University greenhouse trials, in absence of parasitism, *A. gossypii* increased fastest on daisy, followed by *M. persicae* on daisy, *M. persicae* on pansy, and *A. gossypii* on pansy. Parasitoid suppression of population increase was strongest for *A. gossypii* on daisy and poorest for *M. persicae* on pansy. The presence of 2 aphid species in the same greenhouse did not alter the level of biological control in our trial. In commercial greenhouses, banker plants failed to control *M. persicae* deployed on infested pansies as sentinel hosts. In the laboratory, a 12-h exposure to dried residues of pyriproxyfen or pymetrozine, insecticides commonly used to control aphids, reduced survival of *A. colemani* adults, compared to a water control (82% survival), to 71% and 53%, respectively. Adult parasitoid emergence from pesticide-treated aphid mummies was reduced from 68% for the controls to 56% for pyriproxyfen and 62% for pymetrozine.

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#### Zig-zagging across Central Europe: recent range extension, dispersal speed and larval hosts of *Aproceros leucopoda* (Hymenoptera, Argidae) in Germany

Blank, SM; Kohler, T; Pfannenstiel, T; Neuenfelde, N; Zimmer, B; Jansen, E; Taeger, A; Liston, AD. 2014

*Aproceros leucopoda*, the zig-zag sawfly, an invasive pest of elms (*Ulmus* spp.), was found in two separate areas of Germany through July 2014, i.e., a northern area including the states of Berlin, Brandenburg, Mecklenburg-West Pomerania, Saxony and Saxony-Anhalt, and a southern area in Bavaria. A speed of self-dispersal of 45-90 km/yr has been calculated from earlier and present records. Observations of *A. leucopoda* in Belgium and the Netherlands during 2013, which are 360-610 km distant from records in Germany of that year, are interpreted as resulting from human-mediated jump dispersal. Larvae, feeding traces and cocoons were frequently found on the native elm species *U. minor* and *U. glabra*, whereas none could be detected on *U. laevis*. Other occurrences were often on *Resista* (circle dot) elms, causing severe defoliation in a recent planting. New host plant records for *A. leucopoda* are: *U. minor* 'Webbiana', *U. minor* var. *suberosa*, and the *Resista* (circle dot) cultivars *U. 'New Horizon'*, *U. 'Regal'* and *U. 'Rebona'*. The future dispersal of *A. leucopoda* throughout most of Germany is expected, because at least *U. glabra* and *U. minor* are widespread in this country.

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#### A collection of *Poecilcrypticus formicophilus* Gebien (Coleoptera : Tenebrionidae) from a mound of the imported fire ant hybrid, *Solenopsis invicta* x *richteri* (Hymenoptera : Formicidae) from Mississippi, USA

MacGown, JA. 2005

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

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

Four polymorphic enzymes (PEP, PGI, PGM and IDH) were separated from adult individuals of five aphid parasitoid species of the genus *Aphidius* Nees (*A. ervi* Haliday, *A. microlophii* Pennacchio & Tremblay, *A. eadyi* Stary, Gonzalez & Hall, *A. picipes* Nees and *A. urticae* Haliday) using horizontal cellulose acetate plate electrophoresis. These markers were used to investigate the genetic relationships, including reproductive isolation and host adaptation/specificity, in laboratory and field populations. Samples were collected from the pea aphid, *Acyrtosiphon pisum* (Harris) and/or the nettle aphid, *Microlophium carnosum* (Buckton) in the UK and Bulgaria between 1991 and 1994. Whilst all loci discriminated between some species, PGM discriminated all five species, one species (*A. eadyi*) bearing two unique alleles (PGM(a) and PGM(c)). *Aphidius microlophii* (from nettle aphid) and *A. ervi* (from pea aphid), which are difficult to separate morphologically, possessed unique PGM alleles - PGM(b) and PGM(e), respectively. Both parasitoids occur sympatrically, and whilst hybrids heterozygous for PGM were produced in the laboratory (pGM(b,e)), such genotypes were not observed in the field populations sampled. Hence, the species appear to be reproductively isolated. Most parasitoid populations

studied showed mean heterozygote deficiencies per locus (homozygote excess) compared with Hardy-Weinberg expectations. In particular, *A. eadyi* bearing PGM(a) alleles were always homozygous whilst additionally, many were homozygous for another allele, PGI(b). This is evidence for the existence of one or more morphologically-indistinguishable 'cryptic' species occurring sympatrically within European field populations. A dendrogram of relatedness was produced following calculation of Nei's genetic identity coefficient, I from the parasitoid population allele frequency data. All species showed very high similarity between populations at the intraspecific level (>0.9), but fewer interspecific similarities (0.23-0.63). These values compare well with previously published values for Aphidius populations and for other insects.

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**Species associations of gall-formers on willows (*Salix* spp) at the floodplains of the Rhine River (Hymenoptera : Tenthredinidae : *Euura*, Phyllocolpa, *Pontania*; Diptera : Cecidomyiidae : *Dasineura*, *Iteomyia*, *Rabdophaga*)**

Kopelke, JP; Amendt, J. 2002

The gall-former communities of willows (*Salix* spp) have been investigated in 1999 and 2000 at nine natural field sites along the floodplains of the Rhine River. 15 *Salix* species and two hybrids occurred at the willow stands studied, revealing great differences between the sites with regard to composition and abundances of the willow species. A total number of 1,277 willow individuals were mapped, checking the presence and densities of gall-forming species. A total of 43 gall-forming species have been registered, consisting of sawflies (Tenthredinidae: 29 spp) and gall midges (Cecidomyiidae: 14 spp). These gall-formers represented about 67% of the total of 64 potential gall-formers which may occur on the particular willow species studied. The distribution pattern of the gall-formers varied noticeable within a site as well as between the different sites. The maximum number of 7-8 gall-forming species has been recorded from *S. alba* (subgenus *Salix*), *S. caprea*, and *S. purpurea* (subgenus *Vetrix*), respectively. A complete utilization of the host plant resource by its particular gall-formers was uncommon at all sites. The gall-former densities varied between the individuals and sexes of the host plants as well as between the sites studied. Among the gall-former associations within a host plant population those consisting of only two species were most frequent. The relationship of densities of the associated gall-formers were analyzed at the level of shoots among their host plant species. The results indicate that species pairs of gall-formers co-occurring on the same host plant species tended to attack different willow specimens or different shoot length classes within the same willow individual.

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**Relation between the size of the acid gland and the quantity of venom produced in africanized bee, *Apis mellifera* L. (Hymenoptera : apidae), in the region of Dourados, MS, Brazil**

Brizola-Bonacina, AK; Alves, VV; De Moraes, MMB. 2006

Crosses between African and European honeybees in Brazil resulted in a highly detensive hybrid bee. The acid gland is important in the expression of this characteristic, being responsible for venom production. Morphological variations in this gland could influence the quantity of venom. Glandular morphology was analyzed, along with the quantity of venom produced and the bees' genetic characteristics. The gland and the venom reservoir were removed from workers. The gland was placed on a histological frame for measurement and the contents of the reservoir were weighed. The results were submitted to an analysis of regression and submitted to Test Z, to evaluate the differences between the averages. The phenotypes were evaluated according to the standard found in literature. Gland length varied from 7.42 mm to 20.33 mm, the quantity of venom from 0.19 mg to 0.34 mg, and as far as the genetic characteristics are concerned, 63.3% of the colonies had workers with large glands. In 53.3% of the colonies, 90% of individuals had simple glands, suggesting the evolutionary process leading to the loss of branching, since the presence of branching indicates primitiveness. The production of venom is associated with the length of the gland and branching does not influence the quantity of venom. There was no statistical difference between the size of the branched and simple glands or in the quantity of venom produced, therefore the large glands can favor commercial exploration of venom, producing larger quantities.

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**ANT HOMOPTERAN INTERACTIONS IN A TROPICAL ECOSYSTEM - DESCRIPTION OF AN EXPERIMENT ON COCOA IN GHANA**

BIGGER, M. 1993

Over a period of six years, the presence or absence of 23 species of arboreal insects (13 Homoptera, 1 Heteroptera, 9 ants (Hymenoptera: Formicidae)) was recorded on each of 866 young Amazon and Amazon hybrid cocoa trees at the Cocoa Research Institute, Tafo, Ghana. The general form of the time-series, derived from the proportion of trees occupied by each species, is described. Analysis of 18 of these series, taken either singly or jointly, was carried out using analysis of variance and principal components analysis and the degree of spatial association between 16 of the species was also investigated. For the latter, the number of joint occurrences of pairs of species was compared with the expected number by means of a chi squared test. An index of association was derived from the proportion of occasions on which the difference was significant. For all but four species, there were highly significant differences between annual means in the analysis of variance and, in many, the overall annual trend was downward with time. For those species where significant differences were not shown between years, trends were often apparent on individual plots but were negated because of inconsistencies between plots in the direction of the trend. In the joint analysis of all 18 species by principal components, the first component accounted for approximately 33% of the total variance and could be identified as representing to a large extent the long-term upward or downward trends shown up by the analysis of variance. The second principal component, accounting for a further 13% of total variance, was interpreted as being a further manifestation of the long-term trend, representing a tendency for deviation from the trend line shown up by the first component to become less with time and to approach the trend line in a damped oscillation. This is thought to be partly caused by a drought at the start of the experiment which affected certain species adversely. The third principal component accounted for 11% of total variance and the scores followed an irregular annual cycle which was related to relative humidity. The scores on this axis were high during dry periods of the year and low during wet periods. In the analysis of variance it was found that 13 species showed significant differences in spatial distribution between seasons and, for most, there was evidence for an annual cycle. There was also some evidence of correlation between the seasonal cycles of some insects with seasonal rainfall level or morning or afternoon relative humidity. These same species are important in contributing to the third principal component scores. Spatial association analysis showed eight species to be strongly associated. These fell into three groups with positive associations between members of the same group and negative association between members of other groups. Each group featured an ant-homopteran association, *Crematogaster* spp. with *Planococcoides njalensis* (Laing) in group 1, *Oecophylla longinoda* (Latreille) with *Stictococcus* spp. in group 2, and *Camponotus* spp. and *Polyrhachis laboriosa* F. Smith with *Planococcus citri* (Risso) and *Toxoptera aurantii* Boyer de Fonscolombe in group 3. Three other coccids were loosely linked to these groups through particular members, *Waxiella* sp. nr *zonatus* (Newstead) to group 1 through *Planococcoides njalensis* and to group 2 through *Camponotus* spp. and *Phenacoccus hargreavesi* (Laing) and *Steatococcus* spp. to group 2 through *Planococcus citri*. Changes taking place with time in the relative abundance of members of these groups could be discerned in the trajectories of the first two principal components. The spatial re-arrangements taking place with time of the territories of the major ant species are followed in some detail, and the effect of these changes on Homoptera is discussed.

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