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ABSTRACT BOOK



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On the evolutionary history of fig wasps

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Abstract Fig wasps belong to the superfamily Chalcidoidea which is among the most species rich, ecologically important, and biologically diverse groups of terrestrial organisms (estimated 500,000+ species provisionally placed in 25 families and 83 subfamilies). Only a phylogenetic tree for the whole Chalcidoidea will enable a better understanding of when and how the different lineages of fig wasps originated and evolved. We will begin our presentation by reporting on the first representative phylogenomic hypothesis for Chalcidoidea that we recently inferred from exons and Ultra-Conserved Elements (UCEs) (ca 2000 loci; 400+ taxa; all families; 95% of the subfamilies). This phylogeny lays the foundation for a revised classification of the superfamily and, together with divergence time analysis provides a first timeline for its origin, its worldwide colonization and the origin of fig wasp lineages. Then we will present a UCE phylogeny (ca 1000 loci) for 96% of the known genera of fig wasps (including 14 new genera) and will discuss the evolution of life history strategies in this group: conservatism versus convergence? We will discuss on the revision of the Agaonidae that we have just achieved based on the analysis of UCEs (ca 1000 loci) and morphological data (166 characters coded for all extant and fossil taxa). Finally, we will compare the backbones of our phylogenomic hypotheses of agaonids and figs (800 RAD markers, 177 species), and discuss their congruence and differences, as well as their pattern of co-diversification and the nature of the few ancient host shifts.

Keywords: Chalcidoidea, morphology, phylogeny, traits, Ultra-Conserved Elements

What goes in must come out? The metabolic profile of plants and caterpillars, frass, and adults of *Asota* (Erebidae: Aganainae) feeding on *Ficus* (Moraceae) in New Guinea

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Abstract Insect herbivores have evolved a broad spectrum of adaptations in response to the diversity of chemical defences employed by plants. Here we focus on two species of New Guinean *Asota* and determine how these specialist moths deal with the leaf alkaloids of their *Ficus* fig hosts. As each focal *Asota* species is restricted to one of three chemically distinct species of *Ficus*, we also test whether these specialised interactions lead to similar alkaloid profiles in both *Asota* species. We reared *Asota* caterpillars on their respective *Ficus* hosts in natural conditions and analysed the alkaloid profiles of leaf, frass, caterpillar, and adult moth samples using UHPLC–MS/MS analyses. We identified 43 alkaloids in our samples. Leaf alkaloids showed various fates. Some were excreted in frass or found in caterpillars and adult moths. We also found two apparently novel indole alkaloids—likely synthesised *de novo* by the moths or their microbiota—in both caterpillar and adult tissue but not in leaves or frass. Overall, alkaloids unique or largely restricted to insect tissue were shared across moth species despite feeding on different hosts. This indicates that a limited number of plant compounds have a direct ecological function that is conserved among the studied species. Our results provide evidence for the importance of phytochemistry and metabolic strategies in the formation of plant–insect interactions and food webs in general. Furthermore, we provide a new potential example of insects acquiring chemicals for their benefit in an ecologically relevant insect genus.

Keywords: alkaloids, plant–insect interactions, food-webs, biodiversity, host-specificity, herbivores

Chromosome-scale assembly of the fig wasp genome and evolution history of the pollinator-cheater fig wasp

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Abstract Fig/fig wasp interaction represented a unique system for studying the co-evolution of mutualisms because it is the only system in which active and passive pollination coexist. Molecular phylogenies show numerous transitions from active to passive pollination during the evolution of the fig wasps. However, how these transitions arise, persist, diversify, and affect the fig-fig wasp system remain a challenging question. Recently, genomic studies from multiple levels began to shed light on the genetic changes involved in the pollination mode evolving in this group.

Here, we first constructed a chromosome-scale assembly genome of the actively pollinating fig wasp (*Eupristina verticillate*). We performed whole-genome resequencing (WGS) on population levels of two pollinating-cheating systems (*Eupristina* spp.) associated with *Ficus altissima* and *F. microcarpa* from China to reveal the genetic diversity and population demography change during history. We found that pollinator and cheater wasps are highly genetic divergent owing to an old split during the early Pleistocene. There was no gene flow between the pollinator and cheater fig wasps even though they could coexist in the same fig syconium. Pollinator and cheater wasps associated with *F. altissima* showed higher genetic diversity and larger effective population size. In contrast, the pollinator in *F. microcarpa* showed significantly lower polymorphism than the cheater wasps and a considerably smaller effective population size, which has continuously declined throughout history. During and following the LGM, other species experienced population declines followed by subsequent rapid population expansions. We further searched for the concurrent selection of actively and passively pollinating fig wasp species, most of the candidate genes under positive selection are enriched in animal organ morphogenesis and neurogenesis and olfaction genes.

We further sequenced and assembled draft genomes of 25 fig wasp species across the fig wasp phylogeny to investigate genomic changes associated with convergent losses of active pollination. We performed kmer-GWAS and Forward selection methods to locate the selected genes under the different pollination modes. Our analyses reveal shared and lineage-specific

patterns of regulatory divergence affecting genes in multiple patterning networks and pathways. The signature of molecular convergence is generally rare, suggesting various mechanisms and evolutionary paths leading to loss of active pollination in fig wasps.

Keywords: fig wasp, active pollination, passive pollination, whole genome resequencing, demography history, positive selection

Nematode infection of male fig wasps: potential benefits for nematodes and consequences for fig-fig wasp communities

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Abstract All organisms are members of complex biological communities that are characterized by near-constant interactions among species. Such interactions range from obligate mutualism to severe antagonism and shape the evolutionary trajectories of individual species and the community. Interactions can occur between associates in unexpected ways, sometimes leading to profound community-level consequences. An excellent model system to examine these consequences is within the community comprising figs, fig wasps, and wasp-vectored entomopathogenic nematodes. Pollinating fig wasp females are definitive hosts for nematodes because they provide transportation to and nutrition within their reproductive environment (the interior of a new fig). Conversely, wasp males, which emerge as adults 3-5 days before their female counterparts, never leave a natal fig and therefore cannot provide nematodes access to a new fig. Thus, nematode infection of male wasps should be strongly selected against as a reproductive dead-end. Previous observations in Mexico and Panama indicated that such infective events do occur, but the frequency and ecological relevance of this interaction remained uncertain. In a survey of the *Ficus petiolaris* community in Baja California, Mexico, we found that nematode infection of male fig wasps is surprisingly common, present in 48% of sampled nematode-infested figs. Nematodes were observed consuming their male hosts and forming enormous mating aggregates on their bodies. This consumption appeared facilitated by a mouthpart polyphenism that differed substantially from nematodes emerging from female wasps. Overlap of nematode generations was also observed, suggesting the development of a second nematode generation within the fig, synchronous with definitive female wasp-host dispersal. This suggests that rates of evolution between nematodes and their hosts may be more dissimilar than previously assumed. Interestingly, infections of non-pollinating fig wasp males were especially common and may result in fewer non-pollinating females exiting figs inseminated, suggesting a novel role for infection on community modulation.

Keywords: evolutionary ecology, host-parasite interactions, *Parasitodiplogaster*, *Pegoscopus*, *Idarnes*, community ecology

Fig types and wasp composition in *Ficus subpisocarpa*

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Abstract *Ficus* trees build an obligate mutualistic relationship with pollinating fig wasps by the enclosed flower structure. The non-pollinating fig wasps (NPFWs) also use the flower and even wasp resource, thus fig trees tend to display host sanction when the figs are over exploited. However, *F. subpisocarpa* produced three fig types, which had different wasp compositions. Except the common typical type, the rare pollinator type was also easily found in the nature environment, whereas the rare NPFW type could only be found on some trees which receives less direct sunlight. We conducted crop survey in Taipei, Taiwan for one year and collected figs and wasps for five crops, which contained three typical crops, one rare NPFW crop, and one rare pollinator crop. Typical type had the highest diversity, though the pollinator was parasitized by *Arachonia* sp., the abundance could reach 15-30% among all wasps to maintain the population. Diversity index of rare pollinator type was slightly lower than that of typical type, the figs produced fewer wasps and the wasp composition contained more large wasps, the figs were also significantly smaller and lighter. In rare NPFW type, the figs were significantly bigger and heavier, and the wasp composition was dominant by pollinator thus caused the lowest diversity. The figs in rare pollinator type tended to be in irregular shape after phase C, therefore some wasps oviposit during phase A might affect the fig development and prevent the tree display host sanction. The factors that affected this relationship still need further investigation.

Keywords: host sanction, NPFW, pollinator, wasp composition

The effects of seasonal changes on the dynamics of fig tree pollination

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Abstract Fig trees and their pollinating wasps depend on one another. Both partners' reproductive success is regulated by the wasps' capacity to get into receptive fig flowers appropriate for pollination or oviposition, which is usually dependent on successful female pollinator dispersal from one tree to another. Fig wasp pollinators are poor flyers and short-lived, but can still be carried long distances by the wind. The relative importance of local versus long-distance pollinator dispersal is nonetheless unclear, as is how this may vary with season. In the highly seasonal environment of the Makana Botanical Gardens, Grahamstown, South Africa, we recorded fruiting phenologies of all the trees in a population of monoecious *Ficus burtt-davyi* Hutchinson together with variation in the abundance of its pollinator *Elisabethiella baijnathi* Wiebes. By recording captures of fig wasps flying in the air with the numbers that emerged locally, we examined the independence of the fig tree population, which was separated from the nearest conspecifics by more than 1 km. Temperatures were correlated with the abundance of pollinators flying in the air and the number of wasps being released by figs, but during winter there were times when no pollinators were released locally, but they were still caught in the traps and figs were pollinated, showing that the wasps had dispersed from elsewhere and that the population was not totally independent. These results highlighted the ability of fig wasps to disperse between populations and likely significant impact of seasonal fluctuations on fig tree gene flow.

Keywords: dispersal, *Elisabethiella*, *Ficus*, fig wasps, gene flow, phenology

Pollinator diversification associated with *Ficus hispida*

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Abstract The obligate pollination mutualism of fig-fig wasp represented one of the most extreme cases of plant-insect codiversification. Usually, the biogeographic histories of the interacting species are tightly linked. However, the figs' generation time is much longer than pollinators, this may lead to different speciation rates, and cryptic pollinator species might be observed in one host fig species. Here we studied a widely distributed dioecious tree *Ficus hispida* and its associated pollinator *Ceratosolen solmsi*, covering the large nature distribution areas in Southeast Asia (SEA). Aim to reveal whether cryptic pollinator species exist and the phylogeographic pattern of the host fig tree and its associated pollinator fig wasp. We sampled 286 and 349 individuals for pollinators and trees, respectively, and compared the spatial genetic structures of plant and pollinator using both nuclear and cytoplasmic markers. For the host fig, nSSRs and cpDNA analysis showed an east-west separation into two large regional clusters and significant phylogeographic structure. While the combined results of k2P, Bayesian tree, jMOTU, and network analysis from *COI* gene support four cryptic pollinator species associated with *F. hispida*. Among them, *Sp2* and *Sp4* could be found in more locations covering large distribution areas, suggesting the long-distance dispersal. While, the *Sp1* and *Sp3* are only found in a few restricted sites like the most northern limit of the distribution, suggesting local adaptation and speciation in the pollinating wasps. Our research supplies one more example of more than one pollinator associated with a host fig species covered large distribution and suggests different diversification histories between plant and pollinator wasp.

Keywords: *Ficus hispida*, pollinator, codiversification, cryptic species

Fragmented *Ficus craterostoma* populations are genetically linked by their pollinator fig wasps

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Abstract *Ficus craterostoma* is a forest-limited fig tree that occurs in two groups of forests, one of which consists predominantly of high altitude Afromontane forest fragments along the Eastern escarpment of Africa (South Africa to Kenya). These forests became severely fragmented during the last glacial maximum and remain as many small fragments. For instance, 19 470 fragments with a median size of just 0.02 km² were mapped in South Africa. Fragmentation must have disturbed many ecological processes in the forests, including pollination services. To test the influence of fragmentation on pollen-flow we developed a program called HALFOTYPE. HALFOTYPE divides the genotypes of individuals that are heterozygous at x loci into all 2^x possible gametes that could have given the individual's genotype. It then calculates and compares all match probabilities of the potential gamete sets to allele frequencies of forest fragments and retains significantly more likely parental sets using likelihood ratio tests. Simulated data illustrates that this approach can determine populations that donated gametes when rates of gene flow are low. However, in *F. craterostoma*, the rates of between-fragment gene flow were so high that HALFOTYPE could not distinguish between migrant and local donors. This inability to distinguish seems to be the result of frequent long-distance dispersal by pollinator fig wasps.

Keywords: gene flow, pollen flow, assignment, immigration, *Ficus craterostoma*

Foraging adaptation by predatory ants regulate the stability of fig-wasp system

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Abstract Previous studies showed that the top-down effect of predatory ants could avoid excessive utilization of fig flowers by non-pollinating fig wasps, which could benefit the maintenance of fig tree-fig wasp symbiotic system. However, former studies only explored the influence of ants on fig wasps, and quantitative effect of ants on the fitness of fig trees are still lack of research. Recent theoretical studies have investigated the stability of species coexistence of a food web consisted of “fig pollinators–non-pollinators–predatory ants”, but this theoretical work ignores the flexible variability of food web structure and the role of low trophic levels (fig tree) play in the food web. Therefore, in this study we build a new food web model in which fig tree is incorporated. In this modeling work, Michio Kondoh's adaptive food web theory was applied and foraging preference was introduced into the food web model as a new state variable. This variable could be regarded as a phenotypic trait of predatory ants. Therefore, a complete food web model was established consisting of “fig tree-fig wasp-ants”. At last, numerical simulation techniques were used to investigate the effects of top predators' foraging selection on species diversity, structural complexity and food web stability of this food web.

Keywords: fig wasp, predatory ants, food web model, species coexistence

Chemical communication in a highly specialized plant-pollinator interaction: kinetic of emission of plant VOCs and genes involved in their production

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Abstract A large number of studies have described the Volatile Organic Compounds (VOCs) emitted by flowers and involved in plant/pollinator interactions. Nevertheless, there is a lack of information linking these VOCs to the enzymes involved in their biosynthesis and in turn to the genes coding these enzymes. Using a model system for specialized pollination interactions, those between the dioecious Mediterranean fig tree, *Ficus carica* and its pollinator, *Blastophaga psenes*, we combined VOC and RNA-seq analyses of receptive and pollinated figs to uncover the genes underlying chemical signaling by the plant to its pollinator, and the pathways of VOC biosynthesis. Knowing that *B. psenes*, is attracted by the mixture of four VOCs (Benzyl alcohol, S-Linalool, trans-Linalool oxide and cis-Linalool oxide) we sought to characterize the function of some genes potentially responsible for the synthesis of these VOCs. PTR-ToF-MS and GC-MS analyses of the VOCs emitted by *F. carica* allowed us to characterize the rate of emission of these compounds throughout the day and to characterize the changes occurring after pollination. Comparative transcriptomics between receptive and pollinated figs allowed us to highlight some genes whose expression is down-regulated after pollination. Expression in *Escherichia coli* and *Nicotiana benthamiana* of two genes coding for terpene synthases (TPS) revealed that a monoterpene synthase belonging to the TPS-b clade, converts GPP to S-Linalool, whereas a sesquiterpene synthase of the TPS-a clade catalyzes the formation of Germacrene D. This study provides new insights into plant/pollinator interactions and understanding of the molecular mechanisms of terpene biosynthesis.

Keywords: VOCs, *Ficus carica*, plant/pollinator interactions, terpene synthases

Codiversification pattern and phylogenetic cascade in a tri-trophic plant-insect community

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Abstract Species interactions among plants, herbivores and parasitoids are common in nature and can present diverse coevolution patterns. However, fewer studies have focussed on tri-trophic interactions and little is known about the codiversification and cascade patterns over evolutionary timescales. Here we compared pairwise codiversification and phylogenetic cascade patterns in a tri-trophic plant-insect community of fig species and their associated fig wasps occupied two trophic levels. Using newly acquired and downloaded gene sequences, we first inferred the phylogenetic trees of figs and each function group wasps. Then we used a distance-based and an event-based approach to assess phylogenetic congruence and the relative frequencies of co-speciation, association duplication, switching, and loss. We also compared the phylogenetic cascade pattern between different set of tri-trophic interactions. The paired phylogeny of parasitoids with their host wasps were significantly congruent, but that is not significant with host figs. The macroevolutionary events of parasitoids with their direct host wasps involved more co-speciation, unlike that with their indirect host figs. This result suggested that the phylogeny of parasitoids is more directly related to that of the insect host than of the host figs. In addition, parasitoids involved more congruent phylogeny with their host galling wasps and presented more cospeciation both for pairwise interactions with galling wasps and tri-trophic interactions in figs–galling wasps–parasitoid wasps community. Our findings provide details for codiversification pattern and will improve our understanding of the interactions among plant, herbivores and parasitoids.

Keywords: codiversification, fig, fig parasitoid wasp, phylogenetic cascade, tri-trophic community

What do fig wasp sex ratios really show?

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Abstract Pollinating fig wasps have been the subject of sex ratio studies for over a century. What have we learnt in the intervening years? Several models argued that if females enter figs simultaneously and lay identical clutches, then the female-bias of sex ratios should decrease as the number of mothers increase. This general pattern is clear from data of the combined behaviour of groups of mothers and it was claimed that their sex ratios are prime examples of how exact adaptations can be. However, data on individual moms' behaviours are required rather than groups. Furthermore, the combined data of 25 species show that the decrease in female bias of their sex ratios is too little as was already pointed out in several studies. We propose to look at the number of sons and daughters rather than their ratio. This approach shows two groups of species where sex ratios are increased in different ways (still considering collective behaviour). In the first group, two mothers lay more sons per daughter than single mothers do and higher foundress numbers normally lay fewer daughters. In the other group, the number of daughters is reduced strongly as the number of mothers is increased, resulting in an increased sex ratio. In this second group the number of sons may also be reduced, but to a much smaller extend than daughters. A simple explanation for adjustments through female numbers would be if females lay most of their sons first (a ladies last strategy). To date, all studied species show a negative slope between the probability of an egg being male and its laying sequence (i.e. ladies last). These slopes must be quantified for more species and under higher foundress numbers. Next, we looked at the variation in single foundress sex ratios. If it is assumed that wasps normally enter figs sequentially, rather than simultaneously, then single mothers need to "pre-empt" the chance of a second foundress entering later by increasing her ratio of sons. Considering 36 species, sex ratios increase as the probability of a second female increases.

Keywords: Agaonidae, fig wasps, adaptation, sex ratio, sex allocation, ladies last, sequential

Community structure and interaction networks of fig wasps and Neotropical fig trees

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Abstract Fig trees and agaonid wasps are involved in an intricate and specialized pollination mutualistic interaction. Whilst agaonid wasps pollinate fig flowers while utilizing them as oviposition sites, other chalcid wasp species also explore fig inflorescences without performing pollination. The structure of fig wasp communities is complex, as a result of ecological interactions, phylogenetic factors and evolutionary convergence of morphological characters and life histories. We analyzed the community structure of fig wasps associated with neotropical fig trees, section *Americanae*. Our dataset encompasses 49 *Ficus* species sampled over 18 localities in the Neotropical region. We used multivariate (cluster and permanova) and interaction network analyzes approaches to assess the data. Our results showed that fig wasp communities are strongly host structured, with high levels of host specialization for most of the wasp species. Gallling species were, in general, more specialized in relation to the host than the kleptoparasite and parasitoid species. Confirming the species specialization, network analyses showed a modular structure between fig tree species and their associated wasps.

Keywords: brood site pollination, community ecology, mutualism, network interactions

Chemoreceptor genes in fig wasps: evolutionary insights from comparative studies

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Abstract The mechanisms of chemoreception in fig wasps (Hymenoptera, Agaonidae) are of primary importance in their co-evolutionary relationship with the fig trees they pollinate. We used transcriptome sequences of 25 fig wasps in six genera that allowed a comparative approach to the evolution of key molecular components of fig wasp chemoreception: their odorant (OR), gustatory (GR) and Ionic (IR) receptor genes.

In total, we identified 311 ORs, 47 GRs, and 205 IRs with each species having from 5-30 OR genes, 1-4 GR genes and 4-12 IR genes. 304 OR genes clustered into 18 orthologous groups known to be sensitive to cuticular hydrocarbons (CHC), pheromones, acids, alcohols, and a variety of flora scents, such as cineole, Linalool, and Heptanone. 45 GR genes clustered into 4 orthologous groups that contain sweet, bitter, CO₂ and undocumented receptors. 189 IR genes clustered into 18 orthologous groups and can be divided into three types according to the homology with the other insects: IRco, antennal IRs and divergent IRs. Gene sequence in most orthologous groups for both ORs and GRs varied greatly among species, except for ORco (60.0% conserved) and sweet receptors (G1; 30.7% conserved), while IR genes are more conservative with 10 of 18 groups have more than 50% sequence similarities though two of groups with similarities as low as 12.8% and 14.5%. Strong purifying selection of ORs, GRs, and IRs was detected with low ω values of 0.047-0.312, 0.023 to 0.319 and 0.019-0.168, respectively. Signatures of positive selection were detected in loci from all three receptors orthologous groups. The proportions of the OR, GR and IR orthologous groups under positive selection were 73.3%, 25% and 22.2%, respectively.

Fig wasps have relatively few olfactory and especially gustatory receptors, reflecting their natural history of the system. While number of IRs is similar to some Hymenoptera insects, such as solitary wasps *Nasonia vitripennis* and eusociality honey bee *Apis mellifera*, though

lower than those of ant, mosquito and *Drosophila melanogaster*. Amino acid sequences of three receptors nonetheless vary significantly between species and are consistent with the phylogenetic relationships among fig wasps. The difference of ORs in same orthologous groups among the same species but different hosts and that of the closely related species within one host can reach as low as 49.3% and 9.8% respectively, implying the ORs of fig wasps can evolve rapidly to adapt ecological environments. Our results provide a starting point for understanding the molecular basis of the chemosensory system in fig wasps.

Keywords: adaptation, chemical communication, host specificity, *Ficus*, fig wasps, mutualism

Why are females of the pollinating fig wasp, *Platyscapa awekei*, polyandrous?

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Abstract In some species, females vary in the numbers of times they mate. This variation in matedness either reflects adaptive differences in females' requirements for mating or non-adaptive chance factors. Pollinating fig wasps have been considered to be mostly monandrous although polyandry has been confirmed in a number of species. Here we first show that the pollinating fig wasp, *Platyscapa awekei*, is polyandrous. Second, we show that intraspecific variation is not different from a simple model where receptive females will mate, but receptivity starts to shut down as soon as females have mated. While the variance in offspring number decreases with number of matings the average number of offspring is unaffected suggesting no direct fitness benefits from polyandry. Despite prior evidence for genotypic incompatibility, multiple mating is not combined with selective preference for more similar males.

Keywords: Agaonidae, polyandry, multiple mating, cryptic female choice, geometric mean fitness

Ficus floral scent diversity and co-phylogeny of sympatric fig-fig wasp mutualism

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Abstract Traits related to the interactions of coevolving taxa are critical in maintaining the relationships between them and may also play roles in their codiversification. A major challenge in evolutionary biology is understanding how complex traits of have diversified and codiversified across interacting lineages. Cophylogeny between fig (*Ficus*) and pollinating wasps are well documented. *Ficus* floral volatile organic compounds (VOCs) are crucial for pollinator to locate their host plants. Are floral VOCs diverse enough for pollinators to distinguish host in sympatric *Ficus* species is fundamental to understanding the species isolation and coevolution of fig-fig wasp mutualisms. We reconstructed the phylogeny of 18 sympatric *Ficus* species by chloroplast genomes and fig wasp phylogeny by the 13 mitochondria protein-coding genes. We collected floral VOCs from the 18 *Ficus* species and found high floral VOCs diversity. We identified 103 VOCs, of which 32 chemicals are unique for specific one *Ficus* species. Combined with the phylogeny of *Ficus* and their pollinators, we detected a strong phylogenetic signal of fig and pollinating fig wasps and congruence of VOCs with pollinator phylogeny. The results showed an extraordinary congruence between *Ficus* phylogeny and pollinator phylogeny, and that of *Ficus* VOCs cluster and pollinator phylogeny. Fig florals are enclosed in the syconium and produce limited visual signals (syconium) for wasps to locate the host. We propose that *Ficus* floral VOCs are the critical chemical signals to maintain the interaction of fig-fig wasp obligate mutualism and promote their codiversification.

Keywords: cophylogeny, codiversification, floral volatile, fig-fig wasp

Pollination strategy and variables relating to the reproductive success of *Ficus hispida* L. in subtropical region, Tripura, India

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Abstract Figs are pollinated by the tiny species-specific wasps and have great ecological importance as their fruits are consumed by many vertebrates almost throughout the year than other plants in the tropics. The present work reports on the details of fig reproductive phases, pollination strategy, and relationship among pollinating and nonpollinating fig wasps of *Ficus hispida* based on frequent observations and experiments in a subtropical region of Tripura, India. Female plants of *F. hispida* show 79.90 % seed production and carry 2-8 pollinators till female to post floral reproductive phases. Male plants hold 79.50 % pollinators, 17.79 % male wasps, and 2.71 % nonpollinators. Male plants are home to one pollinator, *Ceratosolen* sp. and two non-pollinators, *Apocrypta* sp. and *Philotrypesis* sp.. A close relationship were found between fig wasps production followed by number of pollinator entry to the female syconia and fecundation, seed production, and overall reproductive success. The fig species studied in the present work had a strong species-specific fig-fig wasp obligate mutual relationship as recognized in a number of other figs studied, which is important for the evolution of the fig species as well as the fig wasps.

Keywords: *Ficus*, fig, wasp, pollination

A comprehensive study on how tropospheric ozone threatens the specific *Ficus*-fig wasp chemical communication in the mediterranean region

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Abstract In this study, we propose to investigate the effects of O₃ on the highly specialized interaction between the Mediterranean fig tree and its unique pollinator, the fig wasp by measuring (i) the plant's response, in terms of VOCs emission, to a high concentration of ozone over a short period of time, then (ii) the chemical reactions that occur between ozone and floral VOCs in the atmosphere, and finally, (iii) the response of the fig wasp to changes in the specific chemical signal it detects. To do this, we collected fig tree VOCs in a specially designed device, and then analyzed these VOCs by gas chromatography-mass spectrometry (GC-MS). In a second step, we characterized, in real time, the chemical reactions in air with an original instrument, the "Proton Transfer Reaction Time of Flight Mass Spectrometer" (PTR-ToF-MS). Finally, we coupled exposure to O₃ VOCs, emitted by fig tree branches bearing receptive figs (ready to be pollinated), with behavioral tests on the fig tree pollinator. Experiments showed that the emission of some VOCs by receptive figs is affected in response to exposure to high [O₃] over a short period of time, and stomatal conductance, characteristic of the stress generated, decreases. Then the concentration of linalool, a major compound in the emission of figs and the attractive bouquet for the pollinator, decreases during O₃ exposure whereas the concentrations of linalool oxides increase and two reaction products of linalool with O₃ appeared in the mixture when only fig VOCs were exposed to O₃. Finally, the observed variations in the concentrations of floral VOCs change the proportions of the attractive mixture for the pollinator, which is then no longer attracted by the odor exposed to O₃, and this, from 40 ppb. These results suggest that current O₃ episodes in the Mediterranean region are probably already affecting the chemical communication between the fig tree and its specific pollinator.

Keywords: VOCs, *Ficus*, chemical signal, flowers, ozone, pollution, Mediterranean ecosystems

Rethinking why there are so many fig and fig wasp

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Abstract Plant-pollinator coevolution has been considered as the main driver of species diversification in both insects and angiosperms. Nevertheless, the process and mechanism of coevolution remain elusive. *Ficus* (figs) is a diverse genus of angiosperms, including more than 800 species. It has a coevolution history with its pollinator fig wasps of more than 75 million years, which provides a model system for studying plant-pollinator coevolution and co-diversification. In this report, we will show the role of fig-fig wasp coevolution on sharpening the traits evolution and species diversification, and distinguish the main coevolution model facilitating codiversification. We argued that a diffuse coevolution model, characterized by pollinator host-switches and plant hybridization, is the main coevolution model facilitating the codiversification of this obligate pollination mutualism. Diffusive coevolution may buffer the influence of environmental changes, which will cause mismatches in obligate pollination mutualisms.

Keywords: *Ficus*, fig wasp, cospeciation, codiversification, plant-animal coevolution

Ozone pollution alters the olfaction and behavior of a specialized pollinator

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Abstract Volatile organic compounds (VOC) play a key role in the relationship between plants and their abiotic and biotic environments. For instance, pollinators usually rely on floral scents to locate their host plants. This chemical communication between plant and pollinator can be disturbed by exposure to pollutants such as ozone (O₃), whose levels have increased in the troposphere and are predicted to further increase over the coming decades. In the present study, we evaluated the impact of O₃ concentration on the behavior of the highly specific pollinating wasp *Blastophaga psenes* towards the chemical signal emitted by its host plant the dioecious Mediterranean fig, *Ficus carica*, and on its detection of plant VOC. Exposing individuals of *B. psenes* to different O₃ concentrations (0, 40, 80, 120, and 200 ppb for 60 min) and then testing them in ambient O₃ conditions we evaluated the direct effect of O₃ on the pollinator behavior and olfaction. Firstly, we observed that O₃ exposure, above a concentration of 120 ppb, affects immediately after the exposure the detection of VOC by antennae of *B. psenes*, as well as its attraction towards the attractive mixture of VOCs. Secondly, by monitoring exposed individuals hourly for 5 h after exposure, we found that ozone exposure induced major changes in insect behavior, which were already significant at 80 ppb with individuals displaying abnormal motility. The tracking over time clearly showed that exposed individuals might only have a reduced chance of recovery, with a decreasing proportion of active fig wasps despite the cessation of an O₃ exposure. These findings illustrate that short O₃ episodes can affect the detection of flower VOC by pollinators and pollinator behavior, which may have detrimental implications for pollination systems. It is, therefore, of importance to assess the effects of O₃ on insect behavior in order to predict how it could modify ecological interactions and species biodiversity in ecosystems.

Keywords: ozone, atmospheric pollution, plant-pollinator interactions, fig wasps, plant VOC perception, behavioral response

Patterns of genomic diversity among island populations of the fig wasp associated nematode, *Caenorhabditis inopinata*

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Abstract Achieving a full understanding of phenotypic diversity ultimately requires the integration of insights from evolutionary biology, ecology, and functional genetics. *Caenorhabditis inopinata* is a close relative of *C. elegans* that thrives in *Ficus septica* figs and disperses on its pollinating fig wasps. *C. inopinata* is huge compared to its close relatives, growing twice as long as other members in the genus, which proliferate on rotting plants as opposed to fresh figs. Additionally, the sophisticated experimental genetic tools of the *C. elegans* system (such as RNAi, CRISPR/Cas9, and transgenic reporters) are transferrable to *C. inopinata*, which can be maintained in laboratory conditions. As part of the initial steps toward harnessing the serendipitous co-occurrence of the *Caenorhabditis* nematode and fig/fig wasp systems, we used RADseq on individual worms isolated from the field to investigate genomic patterns of diversity and differentiation across three Okinawan island populations of *C. inopinata*. The male/female *C. inopinata* is about four times more diverse than the selfing *C. elegans*, although it does not approach the hyperdiversity of other male/female species in the *Caenorhabditis* genus. Variation is enriched on chromosome arms relative to chromosome centers, presumably due to the interaction of selection and recombination. F_{ST} is low among island population pairs, suggesting frequent migration of wasps between islands. Moreover, inbreeding coefficients are elevated in *C. inopinata*, consistent with field observations suggesting small *C. inopinata* founding populations in individual figs. These patterns in *C. inopinata* share similarities with those previously reported in *Ceratosolen bisulcatus* pollinating fig wasps and are consistent with the population dynamics of *C. inopinata* being driven by fig wasp dispersal. Ongoing comparative genomic and evo-devo studies are testing hypotheses regarding ecological and morphological divergence with the goal of creating an integrated research program that capitalizes upon the complex relationships among these organisms.

Keywords: population genomics, dispersal, differentiation, *Caenorhabditis* nematodes

Poster session

Fitness losses inflicted by specialist fig-eating moths can be mitigated by ant bodyguards

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Abstract The fig-tree-centered communities contain many parasitic organisms that use the rich resources produced by the tree. This research focuses on an understudied group of fig-consuming moth larvae in central Panama. We found six different fig-consuming moth species and in this study, we describe their feeding habits and the interactions they form with different fig species present in the Barro Colorado Nature Monument. We also quantify their effect on tree fitness. Up to 80% of the crop was consumed by the larvae. We further found that the presence of ants had a mitigating effect; moth infestation rates were significantly lower in trees where ants were present. Our results suggest that these moths should be recognized as influential players in the fig-centered community.

Keywords: seed predation, Lepidoptera, ants, *Ficus*, fig wasps, interaction network.

Geographic variation in plant-insect signalling: the Achilles heel of the obligate fig-fig wasp mutualism when exposed to climatic change?

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Abstract Volatile organic compounds released at receptivity play a central role in attracting pollinators in plant-pollinator interactions. This is especially true for pollinators in brood-site pollination mutualisms. However, odours vary geographically even in the specialized mutualisms. This may affect pollinator attraction, which in turn reduces the resilience of the mutualistic associations to respond to climatic accidents. Fig and their associated pollinating fig wasps represent one of the most specialized examples of obligate pollination mutualism. In this study, we used the headspace technique to collect in situ receptive fig odours of a widely distributed Asian *Ficus* species-*Ficus hispida* in a series of locations in China and Thailand. Under controlled conditions, we tested the attraction of fig pollinating wasps from two locations (South China and South Western China) by local versus non-local receptive figs in Y tube experiments. Our results show that receptive fig odours varied geographically and were clustered into three groups, South China, South West China and South Thailand. This variation was not explained by pollinator species. Pollinating wasps were only attracted by local receptive fig odours. Our results suggest that despite a huge range as many other species involved in specialised pollination mutualisms, the association between *Ficus* and its pollinating wasps may be fragile as non-local wasps may fail to recognise receptive host plants and may be sensitive to the predicted increase in extreme climatic events.

Keywords: behaviour, co-evolution, *Ceratosolen marchali*, *Ceratosolen solmsi*, *Ficus*, *Ficus hispida*, floral scent, pollination

Capturing *Wolbachia* genomes from fig wasps using custom-designed baits

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Abstract Hymenoptera, and especially fig wasps, have a high *Wolbachia* infection rate. This endosymbiotic proteobacterium causes cytoplasmic incompatibility when host individuals with incompatible infection status mate (mating among hosts with different *Wolbachia* strains or mating between *Wolbachia*-free and *Wolbachia*-infected individuals). Recent evidence suggests that *Wolbachia* strain infections are not randomly distributed across fig wasp phylogenies, suggesting an ecological importance of the infections through evolutionary effects of the infection status on the wasp populations. In order to further test this hypothesis, it is necessary to have whole-genome data from the *Wolbachia* endosymbionts of different fig wasps to allow detailed comparisons of strain evolution and gene functionality in relation to the host phylogeny. The biggest challenge in getting access to whole-genome data from the prokaryote endosymbiont is the presence of eucaryotic host DNA during the extraction and library construction steps, with the target genome being shorter in relation to the host genome and with potentially lower number of copies. Here we present our approach for capturing the *Wolbachia* genome from 46 taxa of *Pleistodontes* wasps, a fig wasp genus for which the phylogeny has been constructed. Our method uses custom-designed biotin baits and streptavidin beads. The RNA baits were designed to be complementary to reference *Wolbachia* genomes, while additional baits were designed from a broader diversity of alleles for MLST, *wsp*, *cifA* and *cifB* loci available in additional databases, to ensure diversity of captured sequences and comparison with previous studies which have used these loci. A bait-hybridization step followed by a library enrichment step introduced in the laboratory protocol can increase the quantity of target genome compared to a usual library construction. Preliminary results of the library sequencing show that our method was successful in capturing *Wolbachia* genomes from infected individuals, with the potential of high genomic coverage of the target genome and high ratio of target/host sequences. Further evaluation of our method will be made after the completion of sequencing for all pooled libraries, for the total number of 48 taxa, including negative controls and positive control *Ceratosolen* wasps, whose *Wolbachia* genomes can serve as a potential outgroup for the *Pleistodontes* endosymbionts. Given the high prevalence of *Wolbachia* among fig wasps and its proposed evolutionary impacts, methods of

targeted capture, such as the one we present, will allow ecological and evolutionary conclusions in relation to fig wasps with more confidence and depth.

Keywords: *Wolbachia*, *Pleistodontes*, custom-designed baits, genomics, evolution, speciation

Biological and ecological aspects of *Sycobia hodites* Farache & Rasplus (Chalcidoidea: Pteromalidae), a species recently introduced in the Neotropical region

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Abstract Biological invasions are a challenge to species conservation. Several organisms are introduced around the world proposedly or accidentally. *Ficus benjamina* is an Asian native species introduced in the Neotropical region to urban afforestation. The presence of exotic fig trees involves a process of co-invasion of associated wasps, mutualistic Agaonidae pollinating wasps and other several non-pollinating wasps. *Sycobia hodites* is a non-pollinating wasp associated with *F. benjamina*, recently introduced in the Neotropical region. The occurrence of polymorphic males in *S. hodites* made this species a model to study different mate strategies associated with male morphology and other biological and ecological aspects related to (1) the fig development stage that the wasps lay their eggs, (2) the place that the eggs are laid, (3) the offspring size in different crops of figs and (4) the sex ratio concerning the offspring size. Through experiments and field observations it was possible to verify that *S. hodites* lay their eggs during the receptive phase of the fig. The ovipositor is inserted through the flower stigma and conducted to ovary through the style, mimetizing the oviposition mode of the pollinating wasp. The offspring size of *S. hodites* randomly varied between crops and time. In natural conditions, the proportion of females in the offspring did not correlate with offspring size, while the proportion of brachypterous and winged males showed positive and negative correlation, respectively. In figs colonized by only one female, the sex ratio was more female-biased than observed in natural conditions. Winged males were observed mating with females inside the figs, indicating that the morphology does not completely correlate with mating strategies. Our results helped us to understand the dynamics of a recent invasive species and open new questions about behavior and mechanisms of sex ratio control by non-pollinating fig wasps.

Keywords: Agaonidae, invasive species, plant-insect interaction, mutualism

Effect of climatic oscillations on the distribution of fig tree species in the semiarid region, Brazil, during the last glacial cycle

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Abstract The Quaternary glaciations shaped the phylogeographic patterns of species in general, especially for South American biota. In some regions of South America (e.g. Cerrado and Caatinga domains) structural change was more pronounced. Seasonally Dry Tropical Forests and Tropical and Subtropical grasslands, savannas, and shrublands biomes predominated, whereas in more Tropical rainforests (Amazon and Atlantic Forest) changes were less pronounced, forming areas of forest refuges. Species distribution modeling (or niche-modeling) has been extensively used on population and conservation studies. The niche-modeling helps to estimate the potential areas of the species distribution and to estimate changes on the distribution patterns over time given a specific scenario for environmental change. Fig trees, *Ficus* spp., Moraceae, represented by some wide distributed and endemic/sub-endemic species do occur in Brazilian semiarid regions. In the present study we modelled the present and past potential distribution of four *Ficus* L. species that occur in the semiarid regions: *Ficus bonijesulapensis* and *F. caatingae* (endemic to the Caatinga); *F. holosericea* and *F. calyptroceras* (not exclusive of Caatinga). We generated models for the past (interglacial 140,000 years ago and Glacial 21,000 years ago) and present for the four study species using Maxent 3.4.4 program. Our results showed that, for all analyzed fig tree species, the annual precipitation was the most important environmental variable. The models designed for the interglacial stage showed areas of environmental suitability similar to the current scenario, for the four species. In the glacial period the potential distribution of all species expanded to north and northeast regions, encompassing regions considered species refuges. The models helped us to understand the environmental requirements for the occurrence of the four study species and to understand the pattern of colonization and distribution of the species over the time.

Keywords: climatic oscillations, maxent, predictive modeling, Pleistocene, semiarid, *Ficus*

How do pollinating fig wasp mothers adjust their offspring sex ratios?

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Abstract In line with optimal predictions, fig wasp mothers are believed to reduce their offspring sex ratios as fewer mothers oviposit in the same fig. The general understanding was that mothers enter almost simultaneously, "perceive" the number of mothers, and based on this number, lay a sex ratio that is optimal for that specific number. Exactly how the adjustment is achieved, is important to understand the adaptive significance of the sex ratio changes. If it requires a change in behaviour, then selection may well have fixed the trait and it should be considered an adaptation. On the other hand, if it happens automatically, then at most selection could have tweaked the trait to get it as close to optimal as possible. Then, only the tweaks can then be considered adaptations because selection did not fix the trait. Hao-Yuan Hu and colleagues argued that wasps use a dual mechanism where mothers change their behaviour when oviposition sites are not limited (an adaptation) but where they do not when oviposition sites are limited (not an adaptation). We test this in a species of *Ceratosolen* fig wasp where oviposition sites are not limited when two mothers oviposit, but is limited once three mothers oviposit. Our data is similar to that recorded by Hu and colleagues with two mothers showing a distinct increase in the number of sons they lay, a clear adaptation. On the other hand, while three mothers lay more sons per daughter than two mothers, this is achieved by laying fewer daughters. Laying fewer daughters could be an altered behaviour that would then suggest an adaptation. However, fig wasp mothers predominantly lay their daughter eggs last, and laying fewer eggs implies fewer daughters automatically. The reduction in female number should thus not be considered an adaptation. We also show that mothers ovipositing with another species increase their number of sons. This will reduce fitness and is a maladaptation.

Keywords: adaptation, exaptation, maladaptation, fig wasps, pollinator, sex ratio, sex allocation, ladies last

Trophic relationships in the wasp community associated with *Ficus citrifolia* Mill. (Moraceae)

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Abstract Interaction between fig and wasps is one of the most specialized and studied coevolutionary relationships. Plants of the genus *Ficus* Tourn ex Linn. (Moraceae) have a mutualistic relationship with small pollinating Agaonidae wasps, which use the pistillate flowers ovaries of the plant to lay their eggs. Plants are also exploited by non-pollinating wasps, usually from the Chalcidoidea superfamily. Wasp communities associated with neotropical fig trees section *Americanae* are quite taxonomically and ecologically diverse. These communities include gallmakers, kleptoparasites and parasitoids. However, trophic relationships between neotropical fig wasps are known for few species. Given this scenario, the aim of this study was to evaluate the trophic relationships in the wasp community associated with *Ficus citrifolia* in Brazil. Data were generated by (1) observing species colonization sequence, (2) performing experiments of wasp guild exclusions, and (3) examining gall content to determine direct parasite-host associations. Eighteen wasp species were observed colonizing *F. citrifolia* figs. Gall content analysis was very informative, complementing the data from field observations and manipulative experiments. We have shown that *Ficicola* sp.2 and *Idarnes dimorphicus* are early gallers, that colonize figs at the initial development phase. *Pegoscapus aerumnosus* (pollinator) and *Idarnes* group *flavicollis* sp.3 are flower ovary gallmakers and colonized figs during the receptive phase. *Aepocerus* sp.1a and sp.1b are *Idarnes dimorphicus* kleptoparasites and colonize figs during the receptive phase and part of the interfloral phase. *Idarnes* group *carme* is kleptoparasite of pollinating species and colonizes figs during the first half of interfloral phase. Other species observed are parasitoids and colonize the figs later. All trophic relationships are discussed at work. In general, each galling species associated with *F. citrifolia* is associated with a particular group of kleptoparasite/parasitoid wasps, resulting in a possibly modular community structure. At least five parasitoid species are associated with Cecidomyiidae larvae in *F. citrifolia*. Our results elucidated the trophic relationship in a Neotropical community of fig wasps, evidencing even patterns of generalism and specialization between the groups. Thus, our results open possibilities for a new understanding perspective of fig wasp communities.

Keywords: Agaonidae, plant-insect interaction, mutualism, tritrophic relationships, parasitism

Beta diversity of fig wasp communities associated with section *Americanae* fig trees

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Abstract Biological communities can be structured as a result of biogeography processes, phylogenetic relationship and ecological interactions. The biological community diversity can be measured in different spatial scale: (1) the alpha diversity which refers to the numbers of species from the homogeneous habitat; (2) the gamma diversity which refers to the numbers of species from the geographically wider and heterogeneous area; and (3) the beta diversity that represents the numbers of species that differ between areas. Fig wasp communities, constitute an interesting model to understand the diversification processes and patterns of species distribution. This study analyzed the beta diversity of fig wasp communities associated with three widely distributed Neotropical *Ficus* species: *F. citrifolia*, *F. obtusifolia* and *F. pertusa*. For each fig tree species, the beta diversity of the total wasp community and the beta diversity of galling, kleptoparasites and parasitoids guilds were estimated. Beta diversity partitioning metrics were used, based on three dissimilarity coefficients: Sørensen (total beta diversity), Simpson (spatial turnover in species composition) and nestedness (variation in species composition due to nestedness). Our results showed that the fig wasp communities exhibit high beta diversity, mainly due to turnover component along the host geography distribution, no significant differences were observed in the beta diversity and their components, between the *Ficus* species and between fig wasp guilds.

Keywords: beta diversity, community ecology, insect-plant interactions, mutualism

Fine-scale genetic structure and gene flow in closely related sympatric fig trees

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Abstract Species coexistence and reproductive isolation are the foundation to understand the origination and mechanism of the species diversity. The interactions between closely related species after the speciation event are a key stage to explore these two biological process. Therefore, the revelation of genetic structure and gene flow among closely related sympatric taxa can provide the new insights into species diversity. *Ficus*, as a species-rich genus, harbors vast species complexes in which the members are often sympatric. In this study, we focused on *F. sarmentosa* species complex to detect its genetic background and evolutionary history. To avoid the spatial scale of sampling over the effective pollinated distance in *Ficus*, we confined all the samples in 15 km × 15 km region in Nanling National Natural Reserve in China. In total, 409 individuals representing six taxa from *F. sarmentosa* complex, including *F. sarmentosa* var. *henryi*, var. *impressa*, var. *luducca*, var. *nipponica*, *F. guizhouensis* and *F. pumila*, as well as eleven microsatellite makers were used to population genetic analysis.

In six taxa in *F. sarmentosa* complex, *F. pumila* and *F. guizhouensis* are mainly distributed at low elevations with least Shannon Information Index, whereas four variants of *F. sarmentosa* have a larger distributed range at elevation with the higher Shannon Information Index, indicating that genetic diversity may be related to the distributed range at elevation. Isolation by distance can't not be detected in the whole complex and all single taxon, which suggests the mating are free within the taxon under the current sampling scale. *Ficus pumila* and *F. sarmentosa* var. *luducca* have larger Nei's genetic distances to other taxa. The analysis of contemporary gene flow used by BayesAss discovered an obvious asymmetric results that *F. sarmentosa* var. *nipponica* and *F. sarmentosa* var. *impressa* are "sink" taxa, i.e., the gene flow from them to the other taxa is only ca. one fifth of that from the other taxa to themselves. The Bayesian cluster analysis by STRUCTURE demonstrated that all six taxa monopolized an own genetic cluster but mixed vast hybrid individuals. Under the small K values (4 or 5), *F. pumila* and *F. sarmentosa* var. *luducca* shared a cluster, whereas *F. sarmentosa* var. *impressa* have two clusters when when a larger K values (7 or 8).

As a result, six closely related sympatric taxa in *F. sarmentosa* complex have a clear genetic differentiation, although vast hybrid individuals and asymmetric gene flow existed. Compared to other similar works in section *Sycocarpus*, *F. auriculata* complex, or *F. erecta* complex, our study builds on a strict scenario in closely related sympatric taxa. The study provided a new coexistent case and will be meaningful to explore accumulated mechanism of species diversity in *Ficus*.

Keywords: *Ficus sarmentosa*, fig tree, genetic cluster, genetic differentiation, historical dynamics

Interactions within a small and a large fig: a glance on the community of fig wasps in the Brazilian savanna

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Abstract A diverse community of arthropods inhabit the fig microcosm and establish a multitude of interactions that may influence biological aspects of figs. Whilst agaonid wasps pollinate fig flowers while utilizing fig flowers as oviposition sites, several chalcid wasps also oviposit within fig inflorescences and may influence on the reproductive success of both figs and pollinators. The effect of non-pollinating fig wasps (NPFW) may depend on their abundance, and since the availability of oviposition sites (female flowers) within a fig vary according with fig sizes, the effect may also differ in host species with different fig sizes. In this study, we analyzed the community of wasps associated with *Ficus obtusifolia* and *Ficus pertusa* in the vicinities of Rio Verde/GO and evaluated the effect of NPFW on the male (production of wasps) and female (production of seeds) reproductive components of figs. The fig wasp community was composed by ten and nine respectively for *F. obtusifolia* and *F. pertusa*. Early gall makers were absent in *F. pertusa*, and overall, the most abundant NPFW belonged to *Idarnes carme* sp. group. Statistical models separating NPFW in different life histories (early gall-inducer, receptive phase gall-inducer, and parasites) were not significantly better than the ones considering the sum of NPFW as predictor variable, suggesting that the natural history of NPFWs did not influence on the effect in seed and pollinator production in this study. We observed no effect of NPFW over the seed production in both fig species, whilst pollinator production was negatively affected by NPFW abundance in *Ficus pertusa*. This suggests that the effect of NPFW may be neglectable in seeds, at least when NPFW are in low abundances. The effect on pollinating wasps may be accentuated in smaller fig species, where the smaller availability oviposition sites may intensify the effect on NPFW.

Keywords: Agaonidae, non-pollinating fig wasps, interaction, parasitism, mutualism

Comparative chloroplast genome analysis of *Ficus* (Moraceae): insight into adaptative evolution and mutational hotspot regions

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Abstract *Ficus* is the largest genus of Moraceae, is widely distributed across the tropical and subtropical regions, and is represented by a high degree of adaptability to different environments. *Ficus* is also well known for its 75 million years of coevolutionary history with its obligate pollinator, fig wasps. Previous studies have shown that the phylogenetic relationships of this genus are not well resolved, and the evolution of chloroplasts in *Ficus* remains poorly understood. Here, we newly sequenced, assembled, and annotated the chloroplast genomes of ten species of *Ficus*, downloaded and assembled 13 additional species based on next-generation sequencing data, and further compared them with the other six previously published chloroplast genomes. We found a highly conserved genomic structure across the genus, with a plastid genome size ranging from 159,929 bp (*Ficus langkokensis*) to 160,657 bp (*Ficus religiosa*). Most of the chloroplasts encoded 113 unique genes, including a set of 78 protein-coding genes, 30 transfer RNA (tRNA) genes, four ribosomal RNA (rRNA) genes, and one pseudogene (*infA*). The number of SSRs ranged from 67 (*Ficus sagittat*) to 89 (*Ficus microdictya*) and generally increased linearly with plastid size. Among plastomes, comparative analysis revealed eight intergenic spacers that were hotspot regions for divergence. Additionally, we found that the genes *clpP*, *rbcL*, and *ccsA* were subjected to positive selection. Phylogenetic analysis showed that none of the six traditionally recognized subgenera of *Ficus* was monophyletic. Divergence time analyses based on the complete chloroplast genome sequences showed that species of *Ficus* rapidly diverged in Miocene. These findings provide the basic resources for evolutionary studies of *Ficus*.

Keywords: *Ficus*, chloroplast genome, nucleotide diversity hotspots, phylogenetic relationship, divergence time estimation, adaptive evolution

