IXth International Fig Symposium

List of Abstracts and Participants

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TALK: Human-*Ficus* Ecologies: biocultural interactions affecting universal concepts of *Fic*us species and their ecology across the planet

Yildiz AUMEERUDDY-THOMAS & Martine HOSSAERT-MCKEY

This presentation is the result of a joint reflection between an ethnobiologist and anthropologist, Yildiz Aumeeruddy-Thomas and a biologist and chemical ecologist regarding how the universal concepts and uses of human societies across the planet may have been partly influenced by *Ficus* biology. Inversely we shall also discuss how world views, practices and uses have affected *Ficus* ecologies and sometimes also their biology. The final scope of this paper is to discuss Human-*Ficus* mutualisms.

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and

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TALK: The *Ficus*-insect interactions: adaptations for mutualism

Anthony BAIN and Lien Siang CHOU

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Plant-insect interactions have been centered for long on the relations between the plants and antagonistic herbivores. For more than two decades, the bibliography of mutualistic interactions has greatly increased. The *Ficus* trees (Moraceae) are long known to be part of an obligate nursery pollination mutualism with their pollinating fig wasps (Hymenoptera: Agaonidae). This mutualism has attracted numerous parasites: the non-pollinating fig wasps that have coevolved tightly with the *Ficus* and the pollinating wasps. At local scale, the effects of the non-pollinating wasps are very negative and many researchers have attempted to understand how the mutualism between *Ficus* and agaonid pollinating wasps could withstand the parasitic pressure. Since the estimated appearance of the *Ficus*-agaonid wasp mutualism, more than 60 million years ago, numerous strategies from the fig trees have

been selected to ensure the survival of the pollinating figs wasps. Firstly, the appearance of dioecy in *Ficus* has permitted to decrease the number of nonpollinating wasp species. Whereas monoecious species have to rely on one type of figs, the dioecious fig species can invest more on the defense of the male figs only (where the pollinating wasps lay eggs). The difference in the defense investments can be seen in the fig wall, which is the structure separating the outside world from the closed inside part of the fig. Dioecious male figs and monoecious figs have developed protection structures in the fig wall against parasitic wasps. Secondly, biotic defenses, via ants, have been described as very effective in many plant species and rarely in *Ficus*. Until recently, the ant-*Ficus* relationship was very poorly known but it seems that the fig trees have developed many adaptations in order to attract ants and to be protected by them: nest sites, specific fig phenology and sugar secretion. This study aims to show the known strategies from the *Ficus* to decrease the effect of the non-pollinating fig wasps on the mutualism. This presentation will review the past studies and hope to shed light for the future study in this field emphasizing on the work done in Taiwan.

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Indian Institute of Science, Bangalore, India

TALK: Fast and slow fig wasps: How to move across microcosms in a fig wasp community

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Fig wasp communities are likely organised according to rules that involve life history traits and trophic levels of the wasps that in turn affect the dispersal abilities of wasps between the microcosms of fig syconia. We investigate the intrinsic dispersal propensities of fig wasps by determining flight abilities as well as the metabolic fuel required for flight for an entire fig wasp community. We examine the relationships between life history traits, opportunities for oviposition, and trophic levels with the intrinsic dispersal propensities of the wasps and suggest how fig wasp communities are structured based on a combination of these parameters.

BRUN-LUND Sam, sam.bruunlund@snm.ku.dk

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TALK: First plastid phylogenomic study reveals cyto-nuclear discordance suggesting repeated occurrence of introgressive hybridization associated with pollinator shifts in the evolutionary history of *Ficus* L. (Moraceae)

Sam BRUNN-LUND, Wendy L. CLEMENT, Finn KJELLBERG, Nina RØNSTED

Standard chloroplast data provide limited information to resolve species level relationships within plants, in particular within large genera. Figs (*Ficus* L., Moraceae) compose one of the largest genera of angiosperms with ~750 species occurring in the tropics and subtropics worldwide. Figs, in addition to being a key food resource in rainforests, are well-known for the mutualistic interactions with their pollinating wasps. It is regarded as a model system for understanding co-evolution dating back more than 75 million years. However, despite significant taxon sampling, combinations of low copy nuclear, nuclear ribosomal and chloroplast regions have not been able to confidently resolve relationships among major groups of figs. Using a high throughput sequencing approach we attempted to resolve

the major lineages of *Ficus* based on plastome data. In this study, we show that the use of a de novo assembled plastome from within the genus provides less ambiguity and higher coverage across the 59 *Ficus* and 6 outgroup genome assemblies compared to using the nearest available reference genome resulting in improved resolution and higher support of the phylogenetic relationships within Ficus. Chloroplast genome data confidently resolved relationships among major groups of figs and largely support current understanding based on nuclear sequence data. However, cyto-nuclear discordance was detected concerning both individual species as well as relationships among some sections at deeper levels. We hypothesize that discordance between plastid and nuclear genomes may be caused by hybridization followed by introgression associated with pollinator shift several times throughout the evolutionary history of the genus.

CARDONA William, williamcardona@gmail.com

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POSTER: Non-pollinating fig wasps offset pollinator impact on seed production in *Ficus americana andicola.*

William CARDONA, Gustavo KATTAN

A hypothesis proposes that non-pollinating fig wasps (NPFW) may help to stabilize the fig-fig wasp mutualism. As they lay eggs in the syconium from the outside, they generate a differential probability of survival for the pollinator larvae which is higher in internal flowers and decreases toward the external wall. Some flowers could even be out of reach of NPFW, generating an enemy-free space. The presence of NPFW before or during the female phase of the syconium could change the oviposition behavior of pollinators, because they may avoid the occupied flowers and would prefer to lay eggs in those nearest to them. In syconia that have not been colonized by NPFW, the foundresses would only be limited by ovipositor size, egg load and the interaction with other foundresses; hence, they could lay eggs in a higher number of flowers, causing a decrease in the number of seeds produced in each syconium. To examine for differences in pollinator laying behavior, we analyzed data from syconia colonized and non-colonized by NPFW of *Ficus americana andicola* in the Colombian central Andes. We adjusted generalized linear models with negative binomial distribution and log-link function. When NPFW were not present, variation in the number of seeds was explained by syconium size and the number of pollinators; the latter had a negative impact. When we adjusted the model using data from syconia with NPFW and the complete data set, the number of seeds was explained by the number of one species of *Heterandrium* and the number of non-*Idarnes* NPFW. In this case, pollinators were actually positively correlated with number of seeds. The mean number of seeds that were produced in syconia with and without NPFW was not significantly different. However, the number of pollinators was lower in syconia colonized by NPFW. The presence of NPFW did not imply a higher cost for seed production compared with syconia that only had pollinators. Furthermore, the proportion of seeds and pollinators in the studied population was 46% and 43% respectively, and 11% were NPFW. The results suggest that non-pollinating wasps help avoid the over-exploitation of flowers by pollinators. This effect could help to stabilize the mutualism in the long term by establishing limits to the number of flowers that the pollinators can use.

CHANG Yen-Chen, yes30055@gmail.com

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TALK: Leaf structural traits of subgenus *Sycidium* (*Ficus*, Moraceae) and its significances in classification and ecology

The relative stability of leaf structural traits (LSTs) among plant genera reflects plant adaptive strategies in different environments, and they are often used in taxonomy at generic or subgeneric levels to distinguish closely related taxa but also to highlight ecological differences between taxa. In this study, the leaf structural traits (dry weight, leaf area, specific leaf area (SLA), composition of the transverse section of the lamina and stomatal density) of six Taiwan *Ficus* taxa belonging to the subgenus *Sycidium* (three from the section *Sycidium* and three from the section *Palaeomorphe*) were analyzed. Our results indicate that LSTs permit to discriminate the subgenus *Sycidium* species and their ecology. For example, the leaves of *Ficus tinctoria* subsp. *swinhoei* were the thickest, and its stomatal density was the highest among the studied taxa. These features can be linked to the environmental characteristics of *Ficus tinctoria* subsp. *swinhoei* habitat in the South of Taiwan: the dry coastal uplifted coral reef remnants of the Hengchun Penisula where the plants live under strong solar radiation and frequent salty sea-sprays. The SLA of *Ficus heteropleura* var. *caudatifolia* was the largest. As an understory species, this characteristic might reflect the adaptation to the life under the canopy: the fig tree enlarging the leaf area in order to catch more solar energy. The result of principle component analysis (PCA) of LSTs showed clear segregation between sections *Sycidium* and *Palaeomorphe*. Thus section *Sycidium* taxa were more closed clustered than the *Palaeomorphe* taxa. This difference may be due to the greater range of habitats of section *Palaeomorphe* taxa. In summary, the LSTs permit to identify and classify the Taiwan *Ficus* species of the subgenus *Sycidium* displaying adaptive characteristics linked to the ecology of the species. This trend may be similar to other species from other subgenera in Taiwan and in Asia in general.

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TALK: The northern limits of figs and the expected impacts of climate change

Around 750 *Ficus* species have been described in the global tropics and subtropics. The environmental factors and mechanisms which determine the northern limits of figs are still uncertain and are essential in understanding the probable impacts of climate change. Climatic factors influence the phenology, physiology, distribution and interactions of species, and climate change is altering these processes. Previous studies have found fig abortions and prolonged B phases at northern sites due to lack of pollinators. Some pollinators arrive at host trees before syconia are receptive, suggesting phenological mismatch. We observed the seasonal pattern of fig and wasp phenology in three shared species (*Ficus altissima*, *F. racemosa* and *F. semicordata*) in northern and southern Yunnan, and related this to seasonal changes in climate. Our analysis suggests that the fig-wasp mutualism near the northern range limits shows decreased fitness and thus survivorship of both taxa. Figs show poor survival near the northern limits due to poor pollinator survival and slower syconium development which reduces reproductive fitness.

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TALK: Co-divergence of *Ficus tikoua* and its *Ceratosolen* pollinator? Does geneflow contribute?

It was predicted most coevolution dynamics were shaped by the geographic variation of reciprocal selection of interacted species and the geneflow between their populations. A lot of studies revealed high genflow of the pollinating fig wasps were quite common, which impeding the differentiation of both the *Ficus* hosts and their pollinators. However, significant divergence of key traits (the distribution pattern of male flowers and the anther-to-ovule ratios) presented in the natural populations of *Ficus tikoua*, with allopatric distribution in Southwest China. We wondered whether its *Ceratosolen* pollinator co-divergent with it, and how geneflow contributed.

Two genes (COI, ITS_2) were employed to assess the divergence of pollinator, and polymorphic microsatellite (SSR) loci were used to scan the genetic variations of both the host and pollinator.

69 COI haplotypes and 97 combined haplotypes (COI+ITS_2) were detected in 49 pollinator populations. But the genetic divergence of those genes were quite shallow, with two monophyletic clades and several ungrouped haplotypes. The genetic distances between clades were smaller than the mean distances of *Ceratosolen* sister species, and the average distance of Hymenoptera congeneric species.

Two SSR groups were detected in both host and pollinator populations, with corresponded distribution patterns between host and pollinator, suggesting co-divergence. The co-divergence was further verified by the significant correlation between the population-differentiation of host and pollinator. Obvious dispersal barriers were detected and shaped the geographic distribution of genetic groups in both host and pollinator populations. However genetic structure could not be explained by dispersal barriers totally. Isolation by distance was significant in both interacted species, which also contribute to the co-divergence dynamics.

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KEYNOTE TALK: Spatial genetic structure in figs and fig pollinating wasps, a perspective from China

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TALK: Species interactions in a complex fig wasp community associated with *Ficus subpisocarpa*

Agaonidae wasps build a species-specific mutualistic relationship with *Ficus* trees, playing the key role of pollination due to the enclosed flower structure. Meanwhile the non-pollinating fig wasps (NPFWs), including gall-maker, parasitoid and inquilines, are also important in the study of the community structure: species with diverse trophic levels are gathered around one fig, opening great opportunities for the understanding of community ecology. *Ficus subpisocarpa* had highly diverse wasp fauna with most of the wasp species still undescribed and little is known about interspecific interactions.

Therefore, trees in NTU Campus and Daan Park, in northern Taiwan, were surveyed for studying the wasp fauna of *F. subpisocarpa*, and investigate the wasp interactions by describing the oviposition sequence and wasp exclusion experiments. Most of the NPFWs, which could affect the population of pollinators, laid eggs in C phase figs. Thus, when the figs were protected from the NPFWs, the abundance of pollinators increased by almost 50%. The results of exclusion experiments also showed that, the population of pollinators remain one-third of the wasp community. Therefore, even there were high diversity and density in NPFWs, the affection on pollinator abundance was small. The synchronous fig production of *F. subpisocarpa*, and the dispersive egg-laying strategy of NPFWs could also lower the threats on flower overused.

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TALK: Symbiosis between *Ficus pedunculosa* var. *mearnsii* and its associated fig wasps

The obligate mutualism between *Ficus* (Moraceae) and fig wasps (Hymenoptera: Agaonidae) is a spectacular example of coevolution and cospeciation. This study focuses on the interaction between a gynodioecious shrub fig, *Ficus pedunculosa* var. *mearnsii*, and its associated fig wasps living in Hengchun Peninsula, South Taiwan. The mutualistic partner of *Ficus pedunculosa* var. *mearnsii* is *Blastophaga pedunculosae* and only one non-pollinating fig wasp species from the genus *Apocrypta* has been observed ovipositing on the figs after the pollination. Fifty-five male figs at the insect emergence phase were collected during March to July in 2015. We counted the number of all the fig wasps, galls and flowers in each fig and measured them. We analyzed the data by Kendall's correlation coefficient tests. Several trends have been highlighted by the tests: (1) there is a positive correlation ($r = 0.569$) between fig size and the number of female flowers within the fig; (2) the number of *Apocrypta* wasps is negatively correlated ($r = -0.260$) with the number of pollinators; (3) the relationship between fig wall thickness and parasitism rate was negative but not significant ($r = -0.191$); (4) similarly the number of non-pollinating fig wasps was not significantly negatively impacting the sex ratio of pollinators ($r = 0.160$). Contrarily to many fig species, *Ficus pedunculosa* var. *mearnsii* is producing figs containing few hundreds of flowers. Therefore, the effects of one single non-pollinating wasp species can be relatively important on one single fig reducing greatly the production of pollinators. Moreover, *Ficus pedunculosa* var. *mearnsii* trees live in harsh and hazardous environments where full crops can be wiped out by meteorological events, it is then crucial to understand how the mutualism and its exploiter are surviving on the southern coast of Taiwan.

COMPTON Steve, s.g.a.compton@leeds.ac.uk

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TALK: Host-parasitoid relationships in figs of *Ficus microcarpa*: a fig wasp community structured by gall size.

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Figs often support complex communities of phytophagous and parasitoid fig wasp species. *Ficus microcarpa* is widely-grown and sometimes invasive outside its native range and elements of its diverse (>20 species) fig wasp community are also widely introduced. Disentangling their trophic relationships is notoriously difficult. Based on samples throughout their introduced and native ranges, we used path analysis and co-occurrences within figs, supplemented by within-fig spatial distributions and natal gall sizes, to reveal likely associations. Putative phytophagous species were far more abundant than parasitoids. Parasitoid host ranges mainly reflected the size of host galls, generating sub-units within the web, with limited host specificity within size classes. One phytophage with novel biology apparently lacks parasitoids. Both pollinators and seeds are potential targets for biological control via different members of the fig wasp community.

COOK James, James.Cook@westernsydney.edu.au

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KEYNOTE TALK: A geographic perspective on the diversity, ecology and evolution of an Australian fig wasp community

Ficus species host diverse multitrophic fig wasp communities inside their fruits. Each fig species has 1-5 pollinator species, but may host far more non-pollinating wasps, including gallers, parasitoids and hyperparasitoids. Many studies have focused on fig wasp community structure at a local level, but many *Ficus* sp. have wide distributions and geographic studies are necessary to reveal the total wasp diversity and explore variation in community structure. Combining such field sampling with DNA barcoding, population genetics and molecular phylogeny also allows exploration of how fig wasp diversity has evolved, and how key species interactions (e.g. host-parasitoid) may be influenced at the landscape scale by ecological and genetic factors.

We are addressing these issues using Australian fig species in the section *Malvanthera*, and especially in *Ficus rubiginosa*, which has a natural distribution of >2000 km running north to south along the eastern seaboard of Australia. We have detected about 25 wasp species on *F. rubiginosa,* some of which are cryptic species that require DNA barcodes to separate. Wide geographic sampling and DNA barcoding both contribute substantially to the revelation of diversity in this community. In addition, we have detected recurrent themes in wasp species distribution patterns that strongly suggest some underlying biotic and abiotic forces driving community composition and wasp speciation.

At a finer level, we have focused on the key interaction between the pollinator wasp (*Pleistodontes imperialis* "sp.1") and its most common parasitoid, *Sycoscapter* "long". We co-sampled the two species at sites in six regions across the range of *F. rubiginosa* for comparative population genetic studies. *P. imperialis*, like some other pollinators of monoecious figs, shows very little genetic differentiation over several hundred kilometres in the southern part of its range. Perhaps more surprisingly, the data support *Sycoscapter* dispersal that at least matches that of the pollinator, suggesting high connectance across the landscape in both species. However, a difference arises at the widest landscape scale. The parasitoid is found throughout the host plant range, but the pollinator is absent from a central part (where other pollinator species are found). *P. imperialis* has disjunct north and south populations and these are genetically distinct. Gene flow therefore differs between pollinator and parasitoid at the landscape scale and this may influence both coevolution and speciation.

Overall, we believe that integration of wide geographic sampling with field and molecular studies will bring much deeper understanding of ecology, diversity and evolution in fig wasp communities.

POSTER: Syco-killers: lethal mate competition in fighting fig wasps

In many fig wasp species, armoured wingless males engage in lethal fights for access to females inside fig fruits, which act as discrete mating patches. Kin selection generally opposes killing brothers, because their reproductive success provides indirect genetic benefits (inclusive fitness). However, siblicide may be avoided if a) brothers do not occur in the same figs, or b) males avoid fighting brothers. Alternatively, siblicide may occur because c) intense mate competition between brothers at the local scale overcomes kin selection effects, or d) males do not recognise kin. A fig may also contain wasps from other closely related species and it is not known if males also fight with these individuals.

We used nine microsatellite loci in the first genetic analysis of fighting behaviour in fig wasps. We assigned species and sibling identities to males and tested alternative fighting scenarios for three *Sycoscapter* wasp species (Chalcidoidea: Pteromalidae: Sycoryctinae) in 58 figs of *Ficus rubiginosa*. About 60% of figs contained males from more than one *Sycoscapter* species and, while 80% of fights were between conspecifics, a surprising 20% were between heterospecific males. Within species, few figs contained brothers, suggesting that females typically lay one son per fig. Overall, most males do not compete with brothers and all fights observed were between unrelated males.

We also analysed genetically all male and female *Sycoscapter* wasps from a complementary (co-sampled) set of 55 figs. This analysis revealed that individual adult female wasps typically lay eggs in several figs, but only a few eggs (1-10) per fig. Moreover, the typical clutch laid by a female in a given fig comprises one son plus two daughters, generating female-biased sex ratios overall. Our study illustrates the value of molecular markers for uncovering details of insect reproductive strategies in the field.

POSTER: One step ahead: parasitoids disperse farther and form larger populations than their fig wasp hosts

Tim L. SUTTON, Markus RIEGLER and James M. COOK

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The structure of populations across landscapes influences the dynamics of their interspecific interactions. Understanding the geographic structure of populations can thus shed light on the potential for coevolution and adaptive responses of interacting species. Parasitism is an important aspect of insect ecology in natural and agricultural settings, and also represents a significant force in the evolution of plant – insect interactions. However, there have been few comparisons of population structure in an insect host and its parasitoid. We used microsatellite markers to analyse the population genetic structure of *Pleistodontes imperialis* sp. 1 (Hymenoptera: Agaonidae), a pollinating wasp of *Ficus rubiginosa* (Moraceae), and its main parasitoid, *Sycoscapter* sp. A (Hymenoptera: Pteromalidae), in eastern Australia. Matched sampling was conducted at several sites in two regions separated by up to 2000 km. We found that pollinators occupying the two regions represent distinct populations, but there was limited genetic structure in the parasitoid across the sampled range. We observed weak patterns of isolation by distance for each species, suggesting that both pollinator and parasitoid are capable of long-distance dispersal. The contrasting geographic structure of host and parasitoid populations could have varying influences on the scale and direction of evolution in each species. Incongruent structure of host and parasitoid populations highlight the potential for geographic

mosaics in an ecologically important and evolutionarily significant system, supporting the significance of their role in the coevolution of interspecific interactions. Furthermore, we provide the first report on the population structure of a non-pollinating fig wasp species.

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CBGP, France

TALK: NGS methods, What for? How to? First results and perspectives

Astrid Cruaud, Sabine Nidelet, Gwenaelle Genson, P. Cruaud, Jean-Yves Rasplus

With NGS methods, routine sequencing of large numbers of markers on many samples is feasible, providing new opportunities to tackle questions of systematics, evolution and community ecology. To address such questions it is necessary to select genomic regions with appropriate signal and to enrich these regions before sequencing. During this talk we will report on our experiences with three types of genetic data: amplicon, Restriction site Associated DNA (RAD) and captured Ultra Conserved Elements (UCEs). We will describe the methods (wet lab / bioinformatics) we have used and the results that can be obtained using each. Briefly, providing that care is taken when analysing raw data,

i) Amplicon sequencing shows great promise to quickly increase the number of species / specimens in reference database and analyse species composition of fig wasp communities. Fast screening of microbiomes is also possible and may for example help to better assess barriers to gene flow that may be due to the presence of *Wolbachia*.

ii) RADseq allows analysing several thousands of loci and should help to resolve relationships within species complexes and at shallow levels of the fig wasp phylogeny (e.g. *Ceratosolen, Pegoscapus*).

iii) Finally, the capture and analysis of UCEs should shed a new light on the first events of divergence that took place during the evolution of the fig wasps.

The analysis of these huge data sets revealed that Sanger data sets might contain mistakes (coding pseudogenes, heteroplasms etc.) and reminded us that systematic errors should not be forgotten and accurate identification of fig wasps is a key starting point.

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KEYNOTE TALK: Is sycomore tree in the Mediterranean region a native or cultivated plant?

The sycomore tree (*Ficus sycomorus*) is one of the oldest edible fruit trees in human history. Remnants were found in Jericho excavations from the Neolithic period (8000-9000 B.C.), in ancient Egyptian tombs from more than 3000 years ago and in Pharaonic graves (2000-1000 B.C.), where these fruits were found seedless. The Sycomore tree was also mentioned in the Testament, in The Talmud, in the New Testament, and the Quran. Theophrastus (23-79 A.D) mentioned this plant in Cyprus, Rhodes and Crete. Evidently, this tree was very common and important all over the Mediterranean region.

The phytogeography of this plant lies from South Africa along East Africa, Egypt, Israel, and probably southern Lebanon and some countries in North Africa. Sycomore trees are bound to high ground water and can be found mainly along rivers.

As all other *Ficus* species, the sycomore flowers are concealed within an almost closed urn shaped inflorescence. As known since Aristoteles period (350 B.C), figs (*Ficus carica*) need to be pollinated by a small special wasp (*Blastophaga psenes*) in order to set edible fruits and viable seeds. Theophrastus described this phenomenon, caprification, in Italy.

Galil (1967) examined carbonized sycomore figs from Egyptian ancient tombs and found wasps of *Sycophaga sycomori* and its parasite *Apocrypta longitarsus* within the syconia.

Today, as in the past, all sycomore trees in the Mediterranean basin as examined in Israel, Egypt, Cyprus, and Crete, are inhabited by *Sycophaga sycomori* and are seedless. As we have already proved, *Sycophaga* is not the legitimate pollinator of *Ficus sycomorus*, but is a parasite that partially mimics the pollinator which exists in Africa.

Sycophaga sycomori prevents the syconia from being dropped. It uses the ovules as nursery site for its progeny. In many aspects this wasp behaves similarly to the original pollinator, but refrains of pollen collection, does not convey pollen toward the stigmas, and above all, the relatively long ovipositor enables the *Sycophaga* to dominate all flowers and turn all ovules to become galls. Thus, the absence of the original pollinator in the Mediterranean region including Egypt does not enable seed set.

Propagation of Sycomore totally depends on human activity. We therefore came to the conclusion that sycomore tree is not native but a cultivated plant.

ELIAS Larissa, larissagelias@yahoo.com

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TALK: Chemical evidence shed light into species interaction in the fig-fig wasp mutualism

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Gall induction is considered one of the most sophisticated and complex events related to insect-plant interactions. However, insect-produced compounds that could trigger gall induction remain largely unknown. A better understanding of gall induction would help elucidate issues related to conservation, community ecology and evolution of insect-plant interactions, such as the fig wasp-*Ficus* mutualism. In this study, we use fig wasps as a model of a complex insect-plant community with representatives of different life histories (gallers, cleptoparasites and parasitoids). Gall induction may be triggered by the deposition of the wasp's venom gland secretion during oviposition in a *Ficus* flower. After oviposition, each flower ovary becomes a gall, which can be exploited by non-galling fig wasp species (cleptoparasites and parasitoids). These fig wasps use the plant tissue or the developing larvae as resources for their offspring. Thus, the secretion injected by the female wasp during oviposition might have different functions, *e.g.* gall induction or host manipulation. We used a venomic fingerprinting approach as to better understand the relationship between venom composition and function in the study species, according to each life history. We studied three Neotropical and four Indo-australasian species including gallers, cleptoparasites and parasitoids. Our results show that venom gland secretions are mainly composed of peptides, as no low molecular weight compounds were detected. Venomic profiles of gallers are more similar to each other than to those of non-gallers. Venom from female gallers induces modifications in plant tissue as soon as four days after oviposition, involving

morphological and chemical changes, especially regarding tannins. Phenolic compounds and condensed tannins are generally related to gall formation and larval protection. In *Ficus,* the localization of condensed tannins in the gall cortex as well as in ungalled flowers, along with their lower degree of polymerization and ion intensity in internal gall tissues suggests that traits beneficial to fig wasps were selected in fig trees along the codiversification history of these plants and insects. Regarding cleptoparasitic species, venom profiles differ from those of gallers and parasitoid. In this case, venom may be involved in gall induction enhancement or in developmental arrest of the host larva. The parasitoid species is the only one with multiple high molecular weight proteins in its venom, in accordance with data from other parasitoid species. Our results bring new perspectives to the understanding of species interactions and the role their secretions may play (Financial support: FAPESP 2013/01918-1)

POSTER: Evolution of ovipositor morphology and life history in Agaonidae fig wasps

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Oviposition behaviour and ovipositor structure have an important adaptive role in insects, allowing exploitation of diverse oviposition sites. In this scenario, evolutionary changes in life history and oviposition sites can be associated with changes in ovipositor structure, allowing use of different resources for oviposition. Agaonidae fig wasps present great diversity regarding life histories and resource use, which represent different constraints for oviposition. Therefore, Agaonidae sensu Heraty et al. (2013) (Agaoninae + Sycophaginae) wasps represent a model group for investigating such functional diversity regarding ovipositors in an evolutionary framework. We investigated the correlation between ovipositor morphology and life history among 24 Agaonidae species using a phylogenetic approach. In order to characterise ovipositor morphology, we used characters related to ovipositor teeth for their potential role in ovipositor movement through the substrate (drilling and/or anchoring the ovipositor.) Our results showed that ovipositor morphology is generally correlated with life history in agaonid fig wasps. None of the receptacle gallers present teeth in their ovipositors. Among ovary gallers, pollinating wasps present one or two teeth in the distal extremity of their ovipositors and NPFW species present multiple homogeneously sized and spaced teeth. On the other hand, non-galling fig wasps present multiple teeth with heterogeneous size and spacing. Such diverse ovipositor morphologies seem to be related to constraints imposed by features of the oviposition sites, as ovipositor morphology has experienced convergent evolution at least 4 times in Sycophaginae according to the resource used (*i.e.*, fig receptacle, flower ovary, galls). Phylogenetic regressions showed that spacing among ovipositor teeth was correlated to life history, *i.e.,*. ovipositors with short mean teeth distance and low variation in teeth distance seem to be adapted to oviposition in receptive figs (B phase), and are characteristic of ovary-galling fig wasps. The ancestral condition in Agaonidae for ovipositor morphology was probably uniform multiple teeth or one or two apical teeth. Regarding life history, ovary gallers probably represent the ancestral state. Our analyses suggest that the non-galling habit evolved three times independently in Agaonidae , and ovipositor morphology also changed independently in the *Idarnes* sp. 9 + *Idarnes punctata* + *Idarnes* sp.1 clade and in *Eukoebelea*. Furthermore, evolution of receptacle galling life history occurred at least two times and was always correlated with loss of ovipositor teeth. Our results bring new data to help understanding the origin and maintenance of the fig-fig wasp mutualism. Therefore, the ancestral of Agaonidae probably used flower ovaries as oviposition sites due to its high nutritional value. The use of other resources (fig receptacle or galls) emerged later in the evolution of the group in what seems to be a case of adaptive radiation related to the occupancy of new niches. Paradoxally, the greatest species diversification within Sycophaginae has occurred in non-galler wasps, due probably to their larger niche amplitudes.

However, the evolution of this life history, as well as receptacle galler life history, were conditional on the previous existence of the mutualism, highlighting the role of mutualistic interactions as sources of biological diversification (Financial support: FAPESP 2007/59059-3, 2013/01918-1).

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TALK: Diversification and host specificity in non-pollinating fig wasps: insights from taxonomy and phylogenetics of Sycophaginae (Agaonidae: Chalcidoidea).

Fernando H A FARACHE, Astrid CRUAUD, Jean-Yves RASPLUS, Rodrigo A S PEREIRA

Fig inflorescences host a chalcid-rich community, that include pollinating fig wasps (Agaoninae) and a multitude of non-pollinating fig wasps that explore the mutualistic relationship established between figs and pollinators. The Sycophaginae are retrieved as the sister group of the pollinating agaonines, forming Agaonidae, and show a considerable diversity of feeding habits and life histories. Although highly diversified and conspicuous in certain biogeographic regions, the taxonomy of Sycophaginae is still poorly known. Here we present the taxonomic revision of the genera of early gall-inducing Sycophaginae, which include 39 new species belonging to *Anidarnes* Bouček (9 n. spp.), *Conidarnes* (7 n. spp.) Farache & Rasplus, *Pseudidarnes* Girault (6 n. spp.), and *Idarnes incertus* species group (17 n. spp.). The new species described represent 75% of what is known for Sycophaginae to date. The early gall-inducing Sycophaginae were highly host specific, although some incongruences between their phylogenetic relationships and their host *Ficus* taxonomy may indicate that host shift patterns occurred in their evolutionary history. The specificity observed in early gall-inducers contrasts from cleptoparasite *Idarnes carme* sp. g and receptive-phase gallers *I. flavicollis* sp. g. in which, although mostly specialist species occur, polyphagous species are widespread (Financial support: FAPESP – 2015/06430-2; 2010/51158-5).

POSTER: Insights into the determinants of plant-insect community structure: specialism and generalism in a regional set of non-pollinating fig wasp communities.

Fernando H A FARACHE, Astrid CRUAUD, Jean-Yves RASPLUS, Finn KJELLBERG, Rodrigo A S PEREIRA

Specialization and generalization appear to contribute to the diversification of life on earth. In symbiotic communities, evolutionary diversification may be driven by these patterns, and could be shared by multiple trophic levels in a phylogenetic cascade. In the context of insect-plant networks, patterns of specialization may provide insights on the evolution and diversification of organisms and interactions such as plants, pollinators, gall-makers and parasitoids. The communities of micro-hymenopterans associated with figs may provide insights into processes that shape community structure. These wasps include a diversified set of gall inducing wasps, cleptoparasites and parasitoids that show different degrees of abundance and levels of host specificity. In this study, we described the community structure of non-pollinating fig wasps (NPFW) associated with *Ficus* section *Americana* in a set of semi-deciduous forest of São Paulo state, Brazil. Forty-two NPFW species belonging to eight genera were reared from six host species (*F. citrifolia, F. crocata, F. eximia, F. luschanathiana, F. obtusifolia* and *F. trigona*). Cleptoparasites and parasitoids represented at least 60% of the collected species. Each *Ficus* species hosted mainly non-strictly specific NPFW, and just 12–35% of the species occurring in each host were strictly specific; some NPFW species (3 spp.) occurred in all studied hosts. Quantitative food web analyses showed that the system NPFW–*Ficus* sect. *Americana* showed low

connectance (c = 0.33; $P < 10^{-4}$) and relatively high specialization (H'_2 = 0.65; $P < 10^{-4}$), and a modular structure. Species-level indices analyzed showed that despite some species have a rather broad resource range, they are mostly specific (PDI > 0.73; PDI > 0.9 for 85% of the spp.). The weighted network showed that despite most species (55%) utilized more than one host, the community structure is specialized, and NPFW species tend to show host preferences. The different levels of specialism and generalism observed indicate intricate patterns of diversification and cladogenesis of NPFW in their host species (Financial support: FAPESP – 2015/06430-2; 2007/06054-4).

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KEYNOTE TALK: Conflicts in the fig mutualism: host sanctions and fighting female fig wasps

Despite the ecological importance of mutualisms, factors that prevent one partner from reaping the benefits of the interaction without paying the cost are still poorly understood. Fig trees and their pollinating fig wasps present a powerful model system for studying mutualism stability: both partners depend on each other for reproduction, cooperation can be manipulated, and the resulting field-based fitness quantified. Previous work has shown that fig trees reduce the fitness of wasps that do not pollinate by fig abortion and reduced offspring numbers. I will show a third component of host sanctions. Through manipulative field experiments and surveys we show that offspring of pollen-free foundresses are only 50 – 90% the size of offspring of pollinating foundresses, making them less likely to reach a flowering fig to become foundresses themselves. I will further discuss what mechanisms could explain the fitness reduction caused by what we call host sanctions. For the Panamanian fig species, available evidence suggests that the reduction in wasp fitness is explained by selective resource allocation by the tree to more profitable (better pollinated) figs. Therefore a purely selfish and efficient resource allocation by the host can have the side effect of promoting cooperation in its symbionts.

I will also present a study of fighting among female fig wasps. Resources such as food, territories and access to mates are often contested among animals. As the value of a contested resource increases, animal aggression should increase and with it the equilibrium cost of fighting. However, these basic predictions have rarely been tested empirically. Here we studied aggression towards conspecifics, comparing closely related species of fig wasps where females experience dramatically different levels

of competition over the same type of resource, egg-laying sites. Through direct observations of egg-laying wasps and scoring of injuries of recently dead individuals we determined the probability and severity of aggression among the females, and quantified its effect on fitness. In nine of ten species females killed or maimed each other, and such injuries reduced wasps' lifetime reproductive success. Both the probability and severity of aggression increased with the level of competition for resources, supporting the theoretical predictions.

With these two very different examples I show how the fig tree – fig wasp mutualism can be a useful model system for widely different questions.

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TALK: The phenological resilience of *Ficus pedunculosa* var. *mearnsii* at two habitats of southern Taiwan

The ecosystem of the uplifted coral reef remnants on southern Taiwan coast is extremely sensitive to the consequences of current climate change. *Ficus pedunculosa* var. *mearnsii*, a rare fig taxon, is a shrub distributed along the seashores in Hengchun Peninsula. The phenological survey of *F. pedunculosa* var. *mearnsii* was conducted in Frog Rock Trail and Jialeshuei. The two locations are distant from about 10 km but the environmental conditions differ slightly as the Frog Rock trail is sheltered by the cliffs of the southernmost part of the peninsula while the individuals living in Jialeshuei undergo the strong East winds from the Pacific Ocean. We selected 50 trees in each area, and from May 2010 to November 2013 the leaf and fig productions were recorded every 2 weeks. Despite the short distance between two study sites, the leaf and fig phenologies showed different patterns. In Jialeshuei, *F. pedunculosa* var. *mearnsii* individuals, which are totally exposed to the winds, have in average more tender leaves and produced new leaves more often than the Frog Rock individuals. Moreover the Jialeshuei trees recovered more quickly after strong disturbances. Nevertheless the fig phenology showed typical seasonal changes with the production peak occurring in spring and the minimum in winter. In Jialeshuei, the onset of fig crops were more sensitive to temperature: the correlation of the new figs with temperature was greater in Jialeshuei than at the Frog Rock Trail. Some characteristics showed strong similarities, for example, the C-phase male figs represented the most common phase in both Jialeshuei (62.4% of the figs) and the Frog Rock Trail (62.3%), also in average 42.9% the male trees bore figs in Jialeshuei and 41.2% in Frog Rock Trail. Even when most of the branches were withered by typhoon or foehn winds (Four events during the survey period), the figs stayed on the branches while the leaves disappeared. Considering the small size of the *F. pedunculosa* var. *mearnsii* population in the South of Taiwan (the closest population is South-East offshore on

Orchid Island), the phenological characteristics revealed by this study permit to understand how *F. pedunculosa* var. *mearnsii* can maintain its pollinator population in such hazardous environment.

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POSTER: Phylogenetic analysis and taxonomic delimitation of the "Hairy-Fig" complex of *Ficus* sect. *Eriosycea* (Moraceae) in China

The hairy-fig complex of *Ficus* sect. *Eriosycea* (Moraceae) includes *F. hirta*, *F. esquiroliana*, *F. simplicissima* and a Chinese entity misidentified as F. fulva. These species are difficult to delimit because of the continuously varying morphological characteristics. In order to re-evaluate the status of these taxa, herbarium specimens were extensively examined and 118 samples of the complex were selected for anatomical and molecular analysis. ITS, ETS and trnH-psbA were applied for constructing phylogenetic trees and fluorescently labeled microsatellite primers were screened for cluster analysis. The results showed that all the four species show continuously variable morphological characters and make up one well supported clade on the phylogenetic trees, and that this similar genetic background was confirmed by the cluster analysis. In conclusion, all the four taxa of the hairy-fig complex recorded in China should be combined as one species *F. simplicissima*, with two varieties: var. *simplicissima* and var. *roxburghii*.

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TALK: Signal utilization of three internally ovipositing female parasites associated with the same host *Ficus curtipes*

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ZONGBO Li and Yuan Zhang contributed equally to this work.

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Making sense of floral signals is essential to many mutualistic symbioses. Previously, we have shown that one main compound of floral scents, 6-methyl-5-hepten-2-ol, guides three internally ovipositing females, including an undescribed pollinator *Waterstoniella* sp., and two inquilines *Diaziella yangi* and *Lipothymus sp*., to locate receptive figs of their host *Ficus curtipes*. Yet, the selection by which three

parasites evaluate the additional signals of floral scents remained unknown. Here, headspace odors from the receptive figs of *F. curtipes* were analyzed by coupled gas chromatography electro-antennogram detection (GC-EAD). We found that four compounds produced consistent electrophysiological reactions in the antennae of the three wasp species and forty-six compounds could be identified with GC-MS. Behavioral tests in a Y-olfactometer indicated that the three wasp species responded to 6-methyl-5-hepten-2-ol and linalool by positive taxis, but did not respond to α-farnesene and 6-methyl-5-hepten-2-one. Furthermore, *Waterstoniella* sp. was more attracted by a mix of 6-methyl-5-hepten-2-ol and 6-methyl-5-hepten-2-one (in a 1:10 ratio) than by the single compound stimulus. Overall, our experimental results show the three internally ovipositing female wasps can recognize the additional chemical signal emitted by receptive figs, and that a minor non-active compounds are synthetic to the associated pollinators Waterstoniella sp., and can be used to increase host location on figs. Therefore, it appears that mutualistic and parasitic symbioses have an ability essential for maintaining the interspecific interactions in complex community.

Key words: Chemical signal. Internal inquiline. Waterstoniella sp.. GC-EAD. Minor compound

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POSTER: Diversity of fig glands is associated with nursery mutualism in fig trees

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Fig trees (Moraceae) have remarkable enclosed inflorescences called figs or syconia. The flowers are pollinated by host-specific fig wasps that enter the fig to lay their eggs. This nursery pollination system is one of the most studied of tropical mutualism interactions, but the source of the volatiles that attract fig wasps to their specific host figs has not been confirmed. The fragrance is the basis of host selection and, therefore, of reproductive isolation among sympatric *Ficus* species. This study locates and characterizes the glands likely to be responsible for pollinator attraction and also protection from herbivory in the figs of nine *Ficus* species representing all the major lineages within the genus. Figs with receptive pistillate flowers were examined using light and scanning electron microscopy. Tests for histolocalization of substances were employed to detect glandular activity throughout the figs. A great diversity of glands is found throughout the fig, and for the first time, the sites producing fragrances are identified. Scent glands are present on the ostiolar bracts and the outer layers of the fig receptacle. Laticifers and phenolic-producing idioblasts, epidermis, and trichomes associated with fig protection occur on the ostiolar bracts, the fig receptacle, and floral tissues. The volatiles produced by glands on the ostiolar bracts are candidate sources for the long-distance attraction of pollinator fig wasps. Scent glands on the outer layers of the receptacle may also play a role in chemical perception of the figs or may be related to their protection. The high cost to the plants if the figs are eaten and the temperature conditions required for nursery pollination are likely the factors that led to the selection of phenolic glands and laticifers during the group's evolution.

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TALK: Laticifers in syconia of *Ficus* L. species (Moraceae)

Cristina R. MARINHO, Simone P. TEIXEIRA

Laticifers are structures specialized in latex production, a type of exudate that acts on the sealing of injuries and on the defense of plants against herbivores. In Moraceae, the presence of laticifers is

considered to be a synapomorphy; however, few studies are available about the location of laticifers in reproductive organs. *Ficus* is the largest genus of the family and its representatives are characterized by an inflorescence known as syconium and by mutualistic interaction with pollinating wasps. The objective of this study was to evaluate the laticifer distribution in syconia of 36 *Ficus* species belonging to the sections *Americana*, *Conosycea*, *Ficus*, *Galoglychia*, *Pharmacosycea*, Sycidium, *Sycocarpus*, *Sycomorus* and *Urostigma*, in order to survey traits of taxonomic and adaptive value for the group. Syconia containing flowers in a receptive state were collected, fixed in FAA50 or buffered formalin and processed for anatomical analysis by light microscopy. All species studied have laticifers in the syconium receptacle, in the ostiolar bracts and in the pedicel of staminate flowers. Almost all show laticifers in the pedicel of pistillate flowers with a short style, which are the flowers that form the gall wasps (except for the species studied of the *Ficus* section). Laticifers also occur in the pedicel of long style flowers, except for *F. curtipes* (*Conosycea* section) and more than ¾ of species of the *Americana* section. Laticifers are observed in the sepals of 25 of the 36 species studied and in the pistil of *F. hispida*, *F. septica*, *F. religiosa* and *F. obtusiuscula*. The variation in the laticifer distribution in the syconium of *Ficus* species compared with the latest phylogeny of the group indicates that there was a loss of laticifers in the sepals, in the pedicel of pistillate flowers with a long or short style and in the pistil of some species. The ubiquity of laticifers in the pedicel of short style flowers and the absence of these structures in the pistil indicate that these secretory structures play a major role in the fig-fig wasp mutualism. The presence of laticifers in the pedicel must act in the protection of eggs and developing wasp larvae against parasitic wasps of the mutualism. However, the absence of these structures in the pistil may contribute to both the successful oviposition of fig pollinator wasps, which occurs through the style, and to the emergence of wasps from the ovary of the fig flower.

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TALK: Australian figs

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TALK: Systematics and biogeography of weevils (Curculionidae: Coleoptera) associated with figs (*Ficus*: Moraceae).

Among the large number of examples of broad radiations of insects on classes of plants, the fig fig-wasp system is one of the most remarkable. Although this interaction has frequently been used as a model for the study of mutualism and coevolution, other groups of insects associated with fig trees have received less attention and have been neglected by fig-tree researchers. The weevils (Coleoptera: Curculionidae) associated with figs are one notable example, since they appear to be highly specialized on their host plants. We analyzed 325 fruit sets from more than 12% of the total *Ficus* species, from the Neotropical, Oriental and Afrotropical regions. The weevil larvae present in the figs were reared and the adults kept for identification. We also examined eight entomological collections (i.e. AMNH,

BMNH, GRA, INBIO, MNHN, MZUSP, NMNH, SAMC) searching for weevil species collected on figs. Our study material (collected + museum) totaled 95 fig tree species from 10 *Ficus* sections. At least 80 weevil species from four genera (*Cetatopus* Schonherr; *Omophorus* Schonherr; *Carponinus* Heller and *Curculio* Linnaeus) were found to be associated with figs. *Ceratopus* is exclusively Neotropical and appears to have two lineages occurring separately in section *Americana* and section *Pharmacosycea*. *Omophorus* occurs in many subgenera all over Africa, extending its distribution as far as the southern part of the Malayan archipelago. The distinction between *Carponinus* and *Curculio* is still not clear, both being associated with several sections of *Ficus*. *Curculio* distribution on *Ficus* is widespread over Africa and Asia and *Carponinus* is more concentrated within insular Asia. Morphological and molecular data of these weevil species are still being analyzed to estimate the phylogenetic relationships and divergence times among lineages in order to reconstruct the global biogeography of Curculionidae beetles associated with *Ficus*. The results of our study will encourage future research on the biology and ecology of these species and will help us understand the role that weevils may have played in the evolution of the fig-wasp-fig mutualism. (FAPESP grant nº 2012/23543-7 and nº 2015/04534-5)

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TALK: Pollinator-mediated selective reproduction in dioecious fig-fig wasp mutualism

The fig–fig pollinator association is a classic case of an obligate mutualism, and the figs are generally the 'controlling partner' in the mutualism. There is no study to show whether the pollinator also mediates the mutualistic reproduction. In this study, dioecious *Ficus hispida* and its pollinator (*Ceratosolen solmsi marchali*) were selected. The pollen grains produced by male flowers were firstly counted using a particle counter, and the pollen and egg loads of pollinators were also recorded in controlled experiments. The results showed that one fig produced about 4,532,882 pollen grains and 300.70±18.01 female pollinators. Each female pollinator averagely carried 592.50±34.79 pollen grains in its pollen pockets. Finally, only 3.93% pollen grains were transferred so that a lot of pollen grains were wasted. The egg loads of female pollinator were 225.69±5.49, and there was a significant positive relationship between pollinator size and egg load. However, large pollinators did not carry more pollen grains so that there was no correlation between egg and pollen loads. One and two foundresses were separately introduced to enter the fig during receptive phase. After 2h, 6h, 12h and 24h, the figs were opened and each foundress inside cavity was dissected to count eggs in the ovary and its pollen loads. The results exhibited that pollen loads significantly decreased with pollination time in female figs, but the eggs remained in the ovary did not reduced. In contrast, the pollen loads did not obviously change, while the eggs remained in the ovary significantly decreased with time in the male figs. This implies that pollinator can control the behaviour of pollination and oviposition when it enters female and male figs. It is first direct evidence that the pollinator mediates the mutualistic reproduction and opens an interesting perspective on maintaining fig–fig wasp mutualism.

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TALK: Phylogeography of Neotropical *Pharmacosycea* species supports the historical connections between Amazon and Atlantic Forests

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We investigated the phylogeographic patterns of two closely species of the genus *Ficus* (*F. insipida* and *F. adhatodifolia*) which occur in the Amazon and Atlantic Forests to address whether the paleoclimate changes influenced the geographic patterns of genetic distributions and the relationship between these two fig trees. We obtained molecular and bioclimatic data of *F. insipida* and *F. adhatodifolia* throughout of taxa's distributions. Genetic diversity, population structure, demographic parameters, and divergence time estimates were inferred using two plastid regions (trnH-psbA, trnS-trnG) and the internal transcribed spacers (ITS) of the nuclear ribosomal DNA region. Species distribution models were used to explore the congruence between temporal forest dynamics and the genetic structure. Most of *F. insipida* populations presented low genetic diversity despite the high number of haplotypes. Combined with paleoclimatic models, these results revealed that these species underwent retraction followed by expansion during Pleistocene climatic fluctuations. In turn, a very low genetic diversity was observed in *F. adhatodifolia* populations with four exclusive haplotypes and one shared with *F. insipida*, in the central region of South America. According to the paleoclimatic modeling, *F. adhatodifolia* underwent drastic change of distribution during LGM (Last Glacial Maximum), period in which this species probably had a disjunct distribution. Our results showed that geological events and paleoclimatic fluctuations influenced the spatial distributions of genetic diversity in the *F. insipida* and *F. adhatodifolia* populations, which occur in warm and dense vegetation areas. The probable contact zone between Amazon and Atlantic via gallery forest in southern of South America was reinforced by the detection of hybridization followed by isolation between these two *Ficus* species (Financial support: FAPESP 2011/01205-0; 2015/417-7).

POSTER: Past and future species distribution predictive modeling of three Neotropical fig trees.

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Species distribution predictive modeling uses associations between environmental variables and species occurrence records to estimate models that represent the environmental conditions favorable to the species. In the present study we chose three species of Neotropical *Ficus* with different ecological characteristics, representing two independent phylogenetic lineages. i.e., sections *Americanae* (*Ficus citrifolia*) and *Pharmacosycea* (*F. adhatodifolia* and *F. insipida*). We generated models for the past (interglacial 140,000 years ago and Glacial 21,000 years ago), present and future scenarios (2050 and 2070 in optimistic and pessimistic scenarios) for the three study species using Maxent 3.3.3k program. Our results showed that for *F. adhatodifolia* the most important variables in the models were minimum temperature in the coldest month and precipitation in the driest month. For *F. insipida* the most important variables in the models were minimum temperature in the coldest month and annual precipitation. For *F. cifrifolia* the most important variables in the models were minimum temperature in the coldest month and precipitation in the wettest month. The models designed for the interglacial stage showed areas of environmental suitability similar to the current scenario of the three species. During the glacial period *F. adhatodifolia* showed a considerable change in its range, occurring in regions considered species refuges. *Ficus insipida* had its environmental suitability decreased, but remained in the Amazon region, while *F. citrifolia* increased its area of suitability. In the future models (2050 and 2070) *F. adhatodifolia* showed a decrease in its range on both optimistic and pessimistic scenarios, *F. insipida* showed an increase in its area of environmental suitability and *F. citrifolia* has been decreasing and fragmentation in the Amazon region in the optimistic and pessimistic scenarios 2050 and optimistic 2070. The environmental requirements and

the potential phylogeographic patterns of the study species are discussed in the context of the generated predictive models (Financial support: CAPES; FAPESP 2015/417-7).

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TALK: Flowering variation and its effects on antagonists in a desert mutualism

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The phenology of flowering within individual fig trees is an important characteristic because it is relationship to the maintenance of their short-living obligate pollinating wasps. Typically, large crops of syconia are produced very synchronously within-trees, whereas syconia production is highly asynchronous and aseasonal at the population level. In large populations this flowering pattern provides receptive trees for dispersing pollinators and promotes fig tree outcrossing. However, some *Ficus* species, often in extreme environments where populations are small, produce relatively small crops of syconia asynchronously within trees. This flowering pattern increases reproductive overlap between trees and supports pollinators more reliably through the year, thus avoiding thus local extinction. Little is known, however, of how variation in the synchrony flowering impacts antagonists to the mutualism. We hypothesized that where asynchronous syconia production within trees benefits the pollinator, it would also benefit antagonists and thus incur an unappreciated cost to the system. We measured asynchrony, size of the crop, and fig tree density in nine populations of *Ficus petiolaris* and used generalized linear models to determine the effect of these variables on the proportion of pollinator and non-pollinator wasps per syconium, and rates of syconia attack by a larval lepidopteran pre-dispersal seed predator. The results show that (1) within-tree asynchrony did not significantly effect wasp counts but did reduce the loss of syconia to lepidopteran predators, (2) crop size did not influence wasp proportions but larger crops were associated with higher rates of lepidopteran damage, and (3) the aggregation of host trees was beneficial for the pollinators while also increasing the lepidopteran damage. These results suggest that where fig populations are small and isolated, asynchronous flowering within trees benefits pollinator populations and the dispersal and receipt of fig pollen, while also allows diminishing rates of fruit loss due to lepidopteran predators.

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KEYNOTE TALK: The ecological chemistry of the interactions between figs and fig-wasps: state of the art and perspectives

Magali PROFFIT, Bertrand SCHATZ, Martine HOSSAERT-MCKEY

RANGANATHAN Yuvaraj, tux.firefox@gmail.com

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TALK: Fig volatiles as taxonomic tools

Carl Linnaeus used floral traits to aid his classification of plants. Here, I intend to further this approach by using floral volatiles as traits to classify *Ficus* species. I used published data on the relative abundance of floral volatiles during the receptive stage of figs. Combining statistical methods of Random Forests and Hierarchical Clustering, I try to deduce relatedness among the *Ficus* species thereby suggesting the use of volatile profiles as tools for *Ficus* taxonomy. In addition, I make an attempt to determine the possible volatile profile of the ancestral species.

RASPLUS Jean-Yves, rasplus@supagro.inra.fr

KEYNOTE TALK: Fig and fig wasp phylogenies and taxonomy: where are we now?

Jean-Yves Rasplus and Astrid Cruaud

Since the last fig symposium new sequencing technologies (NGS) have been widely used to recover historical relationships among various insect groups. Here, for the first time, we used NGS to resolve the phylogeny of pollinating fig wasps. Using Ultra Conserved Elements (UCEs), we reconstructed a first phylogenomic hypothesis for the agaonids, encompassing 42 ingroup species and representing 19 out of the 21 genera known worldwide. Our results showed a fully resolved tree that was coherent with morphology. *Tetrapus* was recovered sister to all other agaonids, however observed substitutional saturation again suggests that Long Branch Attraction may lead to the artefactual early emergence of this fast-evolving lineage. A strongly supported clade grouping *Ceratosolen* + *Kradibia* + *Blastophaga* associated to *Ficus Frutescentiae* + some *Wiebesia* is recovered and is also supported by morphology. Another strong clade grouped *Blastophaga* and all *Dolichoris* + *Valisia* + some *Blastophaga* + some *Wiebesia*. The position of *Pleistodontes* is surprising and needs to be confirmed. Finally *Platyscapa* s.s. is recovered sister to a clade where *Pegoscapus* is sister to all Oriental genera associated to *Conosycea* + all Afrotopical genera. We will discuss our results in the light of fig phylogeny, morphology and taxonomy as well as Agaonid morphology.

In the meantime, several papers have addressed multiple pollinators observed in *Ficus* species, focusing mostly on local diversity and highlighting interesting examples of multiple pollinators. Twenty years after the publication of “The one-to-one species specificity of the Ficus-Agaoninae mutualism: how casual?”, we will discuss our recent results on the prevalence of multiple species of pollinators at a world level. We analysed an unprecedented dataset encompassing 448 *Ficus* species (188 dioecious + 260 monoecious species). We found multiple species of pollinators in nearly 30% of our sampled species. The number of pollinators per fig species ranged from 1 to 12 Agaonid species. We will discuss our results in the light of the fig taxonomy, the breeding systems, the sampling efforts and the known distribution ranges of the sampled figs. We also found that 23 pairs of fig species shared the same pollinators. We showed that the number of pollinators is not linked to the breeding systems but most probably to the distribution range of *Ficus* and to the sampling effort.

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TALK: Chemical communication between figs and wasps: Variation in wasp perception in *Ficus septica* and associated wasps

Lillian RODRIGUEZ, Benoit LAPEYRE, Lucie CONCHOU, Anthony BAIN, Astrid CRUAUD, Regielene GONZALES, Jean-Yves RASPLUS, Martine HOSSAERT-MCKEY, Magali PROFFIT, Finn KJELLBERG

Ficus and fig pollinating wasps constitute the most extreme case of plant-insect co-diversification documented to date. Each *Ficus* species is associated with one or some host-specific wasps that breed within their closed inflorescence (called fig). Receptive figs ready to be entered and pollinated emit species-specific floral odors that attract the short-lived insects. However, little is known about how receptive fig odors may diversify and whether this diversification is concomitant with variation in wasp response. In this study, we show the similarity of odor compound perception between wasps that share the same host (i.e. two pollinator species and two non-pollinator species of *Ficus septica* in Luzon, Philippines). We also show that the pollinator of *F. septica* in North Taiwan detects different compounds when compared to the Philippine pollinators. This probably implies that there is evolutionary convergence in odor perception between wasps that are constrained to recognize the same host plant. Ultimately, what makes the difference in host recognition is the ability of the wasps to respond to these odor compounds and behavioral tests should be performed to establish this aspect.

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TALK: Introducing Involucraoideae and its importance to understanding the evolutionary history of figs and their close relatives Castilleae (Moraceae)

Wendy L. CLEMENT, Sam BRUUN-LUND, Alanna COHEN, Finn KJELLBERG, George D. WEIBLEN, **Nina RØNSTED**.

Figs and fig wasps have long been a text-book example of coevolution, and to this end much research has been dedicated to understanding this interaction from ecological and evolutionary perspectives. Figs, comprising 800 species are among some of the larger genera of angiosperms. Recent studies of the mulberry family, Moraceae, to which figs belong have identified the sister lineage of figs, the Castilleae. This is a small group of approximately 60 species, which help us see figs not only as a large lineage, but also one that likely experienced increased rates of diversification throughout its history. Little work has been done on Castilleae, and we argue that a better understanding of the clade, hereafter Involucraoideae, to which both figs and Castilleae belong, provides a better vantage point for studying the evolutionary history of the figs. In this study, we define Involucraoideae and provide a phylogenetic framework for its members making use of the most comprehensive fig and Castilleae phylogenies currently available. Finally we name clades within Involucraoideae to provide a common framework for understanding the origin and evolution of pollination and diversification patterns within the group.

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TALK: Divergent chemical syndromes in species rich plant genera: the case in *Ficus* local community in Papua New Guinea

Large, speciose tropical plant genera harbour a large diversity of insect herbivores and such systems are therefore important in understanding the generation of tropical diversity. Here we extend previous food web studies across *Ficus* to include not only insect herbivore data from several guilds but also detailed trait and plant chemistry data in a phylogenetic context. We generated a comprehensive multi-gene phylogeny of 21 species representing a sympatric lowland rainforest community in Papua New Guinea. We then investigated the trade-offs between several key defensive traits (triterpene diversity and content, cysteine protease activity, trichome density) and palatability (represented by specific leaf area, Nitrogen content and Carbon content) as well as studying the influence of all of these traits on caterpillar abundance and diversity. We tested for phylogenetic signal in each trait, and found that all traits apart from total triterpene content and specific leaf area, showed significant levels of phylogenetic signal. Using phylogenetic least squares regression we found several significant negative correlations between traits, notably between triterpene diversity and total triterpene content. The most effective trait in reducing caterpillar abundance was the activity of cysteine proteases found in *Ficus* latex; we suggest that other traits may have a more important role in influencing caterpillar community structure. Furthermore, we show that traits are generally labile at the tips of the phylogenetic tree, with the major influence of phylogeny occurring at mid-level nodes and that sister species tend to be more divergent in trait space than expected by chance. In combination with the fact that we found limited evidence for trait escalation across our phylogeny these results suggest that the evolutionary dynamics of herbivore pressure acting in local communities may force divergence in defensive traits between closely related species. This situation, with labile suites of defensive traits being adopted at a community level suggests a system in flux, which may be a more realistic hypothesis for species rich plant communities growing in sympatry than a constant escalation of defensive traits across time.

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TALK: Elevation as a barrier to geneflow through fig-wasp dispersal in Mt. Wilhelm altitudinal gradient in Papua New Guinea.

Discrete geographic barriers are considered an initial step towards reproductive isolation, leading to the formation of new species. Mountains have often been regarded as speciation pumps due to the rapidly changing environmental conditions along relatively short vertical distances and species adaptation to particular conditions along the gradient. Such altitudinal gradients offer a unique opportunity for exploring evolutionary adaptation, gene flow and speciation in action. Inter-specific interactions, such as pollination services, are an ideal system for studying the effect of altitude as a barrier to gene flow and the diversification of species. This project is focused on using molecular tools to identify population and species differences of figs and corresponding pollinating fig-wasps in the continuously forested Mt. Wilhelm altitudinal gradient in Papua New Guinea (200 – 2,700 m.a.s.l.). Recent microsatellite analysis for two *Sycocarpus Ficus* species have revealed, contrary to previous studies across large lowland rainforest areas, distinct clustering between highland and lowland populations. Since pollinating fig-wasps are the sole means of pollen dispersal in *Ficus*, we expect these tiny wasps to play a major role in regulating gene flow between plant populations. Fig-wasp sensitivity to temperature variation could delimit their range and thus may play a crucial role in gene flow among

Ficus populations. Their limited dispersal could potentially unlock reproductive isolation mechanisms that would culminate in formation of new species. Likewise, since generally speaking, each *Ficus* species is pollinated by an exclusive fig-wasp species, we expect fig-wasp's genetic patterns to mirror those in the *Ficus* populations and perhaps be able to identify some directionality in dispersal patterns. Using high throughput sequencing techniques for a number of *Ficus* species along the species continuum, we intend to identify population genomic patterns between both figs and their pollinating fig-wasps along the altitudinal gradient.

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POSTER: Compared with the leaf traits between *Ficus pedunculosa* var. *mearnsii* and *F. tinctoria* ssp. *swinhoei* at Hengchun Peninsula uplifted coral reefs southern Taiwan

Leaf traits which reflect one of the key indicators of the survival strategy of plant adaptation are the most correlated closely to the acquisition, utilization and efficiency of resources for plant biomass. Uplifted coral reefs are one of the most sensitive ecosystems impact by global climate change. Plants which live in such harsh uplifted coral reefs how to adapt and survive are worth concentrated issue. In this study, we compared with leaf traits between deciduous *Ficus pedunculosa* var. *mearnsii* (mearnsii fig) and evergreen *F. tinctoria* ssp. *swinhoei* (swinhoei fig) in Hengchun Peninsula uplifted coral reefs southern Taiwan. Most of leaf structure traits showed difference significant between figs but leaf dry weight not. According to the results of leaf structure traits, the leaf thickness, upper and lower epidermis thickness, layers of upper epidermis, spongy tissue thickness and stomata density were greater in *swinhoei* fig than *mearnsii* fig. Those leaf characteristics were correlative and useful to efficiency of water use in such harsh, as dry, salty, high radiation and temperature environment. Specific leaf area (SLA) was also significantly greater in *swinhoei* fig than *mearnsii* fig, which might indicate the former could have higher net photosynthetic rate. The leaf structure characteristics reflect on its leaf production pattern for each fig species, which showed that *swinhoei* fig has higher leaf resistivity on impact of climate events as typhoons and foehn. On the contrary, the *mearnsii* figs adapt and survive in such harsh unstable environment with rapid leaf production. In summary, our results showed that the adaption strategies were significant between two fig species in Hengchun Peninsula uplifted coral reefs to maintain their mutualism-pollination fig wasps, respectively.

TALK: Recovery of *Ficus pedunculosa* Miq. var. *mearnsii* (Merr.) Corner in in Southern Taiwan after Typhoon Morakot

Typhoons are one of the most important meteorological disturbances for forest regeneration, plant reproduction and vegetation succession in Taiwan. Affected by global climate change, extreme heavy rainfalls occur increasingly and the probability of powerful typhoons also increases every year. As they have considerable impact on terrestrial ecosystems, the recovering abilities of the coastal plants after major meteorological disturbances need to be investigated. Hengchun Peninsula is the southernmost part of Taiwan Island where the coastline is constituted by uplifted coral reef remnants. After the passage of Typhoon Morakot in August 8, 2009, the Hengchun Peninsula was impacted by severely dry and warm foehn-like winds. Phenological surveys of the dioecious species *Ficus pedunculosa* var. *mearnsii* were conducted every two weeks from September 2009 to August 2011. Most of fig trees appeared to have withered after the typhoon, and only a few individuals located in sheltering depressions were less damaged. Recover of leaf and fig production for both genders showed seasonal and appeared highly timing overlap between genders. Correlation showed that mature leaves and fig

production for both genders were positively related with temperature, whereas only female fig abundance was significantly associated with the cumulative rainfalls. Male tree fig crops (4-7 fig crops with an average of 5.2) were more frequent than females (2-7 fig crops with an average of 4.7) during the survey period but not different significantly. However, fig-leaf ratio showed significant differences between genders. More specifically, the number of fig per branch on male plants was greater than on female plants during the early weeks after Typhoon Morakot. Our results show that male trees could produce more figs than female trees for the same leaf number. This sexual differentiation in the fig production strategy permits to produce male figs quickly in order to provide oviposition sites for its mutualistic pollinating wasps. The rapid recovery of *Ficus pedunculosa* var. *mearnsii* in the harsh environment of the Hengchun Peninsula coast may be the key to understand how the fig tree and its pollinator may have colonized the area. However, under the growing number of powerful typhoons and the sea level rise, the recovery capacity of *Ficus pedunculosa* var. *mearnsii* may not be sufficient, the *Ficus* and its associated symbiotic insects may risk local extinction.

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TALK: Figs, fig wasps, and *Parasitodiplogaster* nematodes: rates of nematode attack and consequences for wasp fitness

Justin VAN GOOR, John D. NASON

Fig wasp nematodes are omnipresent members of fig-fig wasp communities and pose ecologically relevant fitness limitations for both wasp hosts and their plant mutualists. While much is understood about the morphology of these nematodes, our understanding of nematode biology and ecology has been limited primarily to host-locating mechanisms within in a species and the influence of host wasp population structure on the evolution of virulence. The research presented here evaluates the interaction between a *Parasitodiplogaster* (Diplogastridae) nematode species and its *Pegoscapus* wasp host, which is the pollinator of *Ficus petiolaris* (subgenus *Urostigma*, section *Americana*) in Northern Mexico. Observational data and experiments identify multiple mechanisms through which nematodes limit the fitness of their wasp hosts. In addition to negatively influencing wasp offspring production, nematode infection is shown to reduce wasp longevity. In particular, high levels of infection by dauer-stage nematodes significantly reduce wasp lifespan, and the finding of fewer nematodes in successfully pollinating than in newly emerging wasps indicates high infection rates diminish host dispersal ability as well. Surprisingly, nematodes were also observed infecting non-pollinating wasps. Microdissection of four non-pollinating taxa (two species each of *Heterandrium* and *Idarnes*) reared from nematode infected syconia revealed 10-30% of individuals to contain dauer-stage nematodes. This frequent attack of non-pollinators is surprising given that such host choice behavior should be strongly selected against: these hosts do not enter a new synconium after exiting the natal one and, consequently, the offspring of nematodes that infect them are unlikely to have access to new host individuals emerging within syconia.. These findings highlight gaps in our knowledge of *Parasitodiplogaster* ecology and suggest previously unappreciated ways in which this nematode may influence non-pollinator community dynamics, fig-pollinator fitness, and mutualism persistence.

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TALK: Converting FigWeb to a multiple input research database on Scratchpads

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FigWeb is a bioinformatics resource for figs (*Ficus*, Moraceae) and fig wasps (Chalcidoidea, Hymenoptera) of the world (www.figweb.org). First developed in 2004 using FrontPage, FigWeb is a static html (Hyper-text Mark-up language) driven site, currently comprising 1650 pages. Static html creates a user-friendly online web interface that loads much faster than a database driven, or CSM (Content Management System) website. With html the developer has much stricter control of structure and design of individual pages, all browsers support html and it is very search engine friendly. Static websites, however, have limitations in terms of tedious site development and logistical restrictions on remote content population by multiple personnel, hindering efficient growth and development of complex, large sites such as FigWeb. With this in mind a database driven version of FigWeb (http://figweb.myspecies.info) has been initiated, using the Scratchpads interface, allowing for the development of a virtual online resource for the fig research community (character matrices, media, taxon descriptions, specimen records, localities, maps, literature, classifications etc). This resource includes facilities to interact directly with peer reviewed journals for publication of data; provides links to external genetic databases such as NCBI; allows for mobilisation and linking of data to multiple biodiversity resources such as EOL, using Nexus formatting and Darwin Core Archive standards; as well as providing a portal for internal peer collaboration on communal projects that remain private until ready for publication. The vision is to develop a dynamic, centrally situated resource that will facilitate collaboration among the fig and fig wasp research community in our ongoing endeavours to unravel the intricate interactions of this fascinating obligate mutualism.

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TALK: Seasonal variation of trophic cascade strength in an ant-fig wasps-fig system

Bo WANG, Yan-Qiong PENG, Da-Rong YANG

Studies on trophic cascade strength have showed that variation in trophic cascade strength among different ecosystem is due to the traits of the system, such as: food chain length and spatial heterogeneity. However, for the same ecosystem, seasonal dynamic and disturbances (such as extreme weather, epidemics, etc.) can greatly influence the way species interacted, interaction strength, and ultimately the strength of trophic cascade. However, dynamic characteristics and mechanism of the cascading effect strength in the same system remains unclear. A wide variety of insects lived in fig trees that form a relatively stable arthropod community, which is an ideal model to study indirect interspecies interaction. To date, only few studies have reported the variation of trophic cascades, and the underlying mechanisms are largely unknown. In the fig tree, *Ficus racemosa*, the

weaver ants (*Oecophylla smaragdina*) can induce trophic cascade effects that indirectly benefit the *Ficus racemosa*–pollinating fig wasp mutualism by predation on non-pollinating fig wasps. However, the population and behavior activity of both *O. smaragdina* and fig wasps vary across season, which may result in dynamics of trophic cascade strength. In this study, we conducted across-season monitoring and field experiments to reveal the patterns, processes and mechanisms of trophic cascade strength dynamics in Xishuangbanna tropical botanical garden. Here, we observed the behavior dynamics of weaver ants and fig wasps in different seasons (dry-hot season, rainy season, fog-cold season) and analyzed the correlation between behavior and temperature and light. The results were as follows: (1) During the rainy season and dry-hot season, both the weaver ants and pollinating fig wasps showed stronger behavior activity than in fog-cold season. The behavior activity of non-pollinating fig wasps showed little change among different seasons. In both dry-hot season and rainy season, pollinating fig wasps population is higher than in fog-cold season. Ant nest number is not significantly difference among different seasons. (2) Behavior activity of weaver ants showed significant positive correlation with temperature, but not significantly correlation with light. Behavior activity of pollinating fig wasps showed significant positive correlation with temperature and light. Behavior activity of non-pollinating fig wasps showed no significant correlation between temperature and light. These results indicate that predators's and prey's behavior will changed greatly in different seasons. Such changes would result in variation of interspecific interaction and finally affecting the cascading effect strength. In dry-hot season and the rainy season, ant showed high behavior activity, the effects of ants have on non-pollinating fig wasps is strong, which will lead to a strong cascading effects. While in fog-cold season, the ant behavior activity is relative low, which will have weak effects on non-pollinating fig wasps. Therefore, in fog-cold season, the system will show weak cascading effects. These results support our predictions on trophic cascade strength in fig-fig wasps system among seasons. However, count number of fig wasps in different seasons and isolation experimental results is still being calculated, and therefore the final conclusions still need to wait for the results of further analysis of counting results.

Chemical camouflage: a key process in maintaining a mutualism and shaping a mutualistic network.

Bo WANG, Min LU, James M. COOK, Da-Rong YANG, Derek W. DUNN, Rui-Wu WANG

Different types of mutualisms may interact, co-evolve and form complex networks of interdependences, but how species interact in networks of a mutualistic community and maintain its stability remains unclear. In a mutualistic network between treehoppers-weaver ants and fig-pollinating wasps, we found that the cuticular hydrocarbons of the treehoppers are more similar to the surface chemical profiles of fig inflorescence branches (FIB) than the cuticular hydrocarbons of the fig wasps. Behavioral assays showed that the cuticular hydrocarbons from both treehoppers and FIBs reduce the propensity of weaver ants to attack treehoppers even in the absence of honeydew rewards, suggesting that chemical camouflage helps enforce the mutualism between weaver ants and treehoppers. High levels of weaver ant and treehopper abundances help maintain the dominance of pollinating fig wasps in the fig wasp community and also increase fig seed production, as a result of discriminative predation and disturbance by weaver ants of ovipositing non-pollinating fig wasps (NPFWs). Ants therefore help preserve this fig-pollinating wasp mutualism from over exploitation by NPFWs. Our results imply that in this mutualistic network chemical camouflage plays a decisive role in regulating the behavior of a key species and indirectly shaping the architecture of complex arthropod-plant interactions.

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TALK: *Ficus* diversification via hybrid and introgression under the diffuse coevolution, preliminary results.

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Whether and how coevolution between plant and insect pollinator has facilitated plant diversification is one of the central questions for both ecology and evolution researching. The extreme diversity of figs (*Ficus*) and their obligate pollinators, the fig wasps (Agaonidae), provides a nice opportunity to target that question. Here, following the diffuse coevolution mode, in which, a group of figs species coevolution with a group of pollinating fig wasps and the hybridization and introgression among fig species mediated by pollinator sharing or pollinator shifts are allowed, we try to test whether diffuse coevolution is common in this obligate pollination system and how it contribute to the species diversification of the system.

Using five sympatric dioecious fig taxa and their pollinators, we examine the degree of pollinator sharing and inter-taxa gene flow. We experimentally test pollinator preference for floral volatiles, the main host recognition signal, from different figs. All five fig taxa shared pollinators with other taxa and gene flow occurred between fig taxa within and between sections. Floral volatiles of each taxon attracted more than one pollinator species. Floral volatiles were more similar between closely related figs, which experienced higher levels of pollinator sharing and inter-taxa gene flow. This study demonstrates that pollinator sharing and inter-taxa gene flow occurs among closely related sympatric dioecious fig taxa and that pollinators choose the floral volatiles of multiple fig taxa. The diffuse coevolution should be common in this system.

We further tested how many “good” fig species may speciation with hybridization and introgression via pollinator sharing or shifts as above diffuse coevolution. A chloroplast *Ficus* phylogeny with de novo assembled full cp genome sequences of 27 species of 16 sections of 6 subgenera was established and compared with recent nuclear *Ficus* phylogeny. Primary result shows chloroplast genome *Ficus* phylogeny get very high support in almost all deep and tip nodes. However, the cyto-nuclear discordance was repeatedly detected in both phylogeny at species and section level, which may suggest the role of hybridization speciation via above diffuse coevolution should be one of important way for *Ficus* diversification.

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TALK: Fighting, hiding and mating in male fig wasps.

Da-Mien WONG, Anthony BAIN, Shiuh-Feng SHIAO & Lien-Siang CHOU

Fighting over limited resources leads to serious injuries is well documented in fig wasp community. Population and injury level in fig wasp species associated with *Ficus benguetensis* were investigated from Fuyang Eco Park, Taipei. About 88% of *Philotrypesis* sp.1 males have been injured during their mating period. Interestingly, male fig wasps display a wide range of variations in their morphologies and mating strategies. The males of *Philotrypesis* sp.1 ex. *Ficus benguetensis* showed two types of morphology according to their mandible shape: falcate (atypical) and sawtoothed (typical). There is 7% of males bearing the atypical mandible.

Since signals are thought to be linked with costs and are considered to be honest indicators, the atypical morph in male fig wasps has been proved to be a dishonesty in previous studies. However, there is no superiority of being atypical over other males during mate competition in our study. Firstly, individuals with longer mandible tend to start the fight, regardless of their morph. Secondly, atypical males tend to hide within empty gall when they had injured, these injuries may impaired the mating success of an individual male. Thirdly, males with different body sizes seem to have equal chance to mate with receptive females. Hence, we conclude that the atypical males of *Philotrypesis* sp.1 ex. *Ficus benguetensis* do not have any impunity or advantage when competing with typical males.

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TALK: Comparison of sensillar morphology in female pollinators and non-pollinators in the hots *Ficus auriculata*

Pei YANG[1], Zongbo LI [2], Yanqiong PENG [1]✉, Darong YANG[1], Finn KJELLBERG[3]

Pei YANG and Zongbo LI contributed equally to this work.

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Antennal sensilla were compared in foundress association of the four agaonid species, *Ceratosolen emarginatus*, *Sycophaga* sp., *Sycoscapter roxburghi* and *Philotrypesis longicaudata* which are, generally, specific and obligatory parasitoids on *Ficus auriculata*. The four species are different in oviposition tactics in the same fig where they hatch offspring. The morphology and ultrastructure of the sensilla were presented using scanning and transmission electron microscopy. Foundress have 11 types of antennal sensilla, morphologically similar in the four species: the aporous trichoid sensilla, sensilla obscura, chaetica sensilla 1 and 2, which have mechanosensory functions, uniporous basiconic

sensilla, which are presumably contact chemosensilla, and basiconic capitate peg sensilla and coeloconic sensilla 1 and multiporous basiconic and placoid sensilla, which may be regarded as olfactory sensilla, while coeloconic sensilla 2 and 3, which are presumed to be thermohygroreceptor or pressure receptor. The significant difference among the four agaonids is the abundance and arrangement of trichoid sensilla, of which extensive number are found on the flagellum in *C. emarginatus* and *Sycophaga* sp. that lay eggs in early soft figs, while *S. roxburghi* and *P. longicaudata* in lately solid fig are rare, with the chaetica sensilla on the flagellum. The coeloconic sensilla and sensilla obscura only occur on the antennae of *C.emarginatus* entering into the fig cavity. The chemosensilla involved in host selection, i.e. basiconic sensilla, multiporous placoid sensilla and basiconic capitate peg sensilla, are similar in ultrastructure and in abundance and arrangement. These findings will serve as a prerequisite for next electrophysiological studies on the chemoreception of these fig wasps.

Key words: *Ficus auriculata*, Agaonid, Foundres, Antennal sensilla, Ultrastructure

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TALK: Species speciation of the pollinators of the dioecious fig, *Ficus hirta* Vahl. from Southeast Asia

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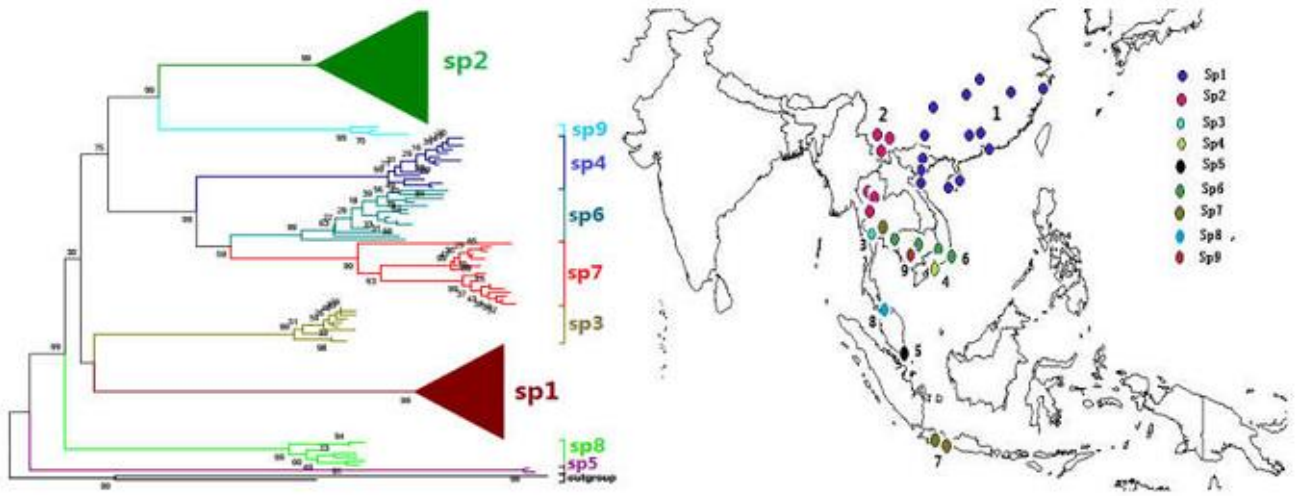
Figs (*Ficus*, Moraceae) and their pollinating fig wasps (Agaonidae) constitute a classic example of an obligate plant-pollinator mutualism, and have become an ideal system to address questions on coevolution, speciation, and maintenance of mutualisms. The fig-fig wasp mutualism was a classic example of the strict-sense cospeciation based on coevolution. However, more and more findings of cryptic fig wasp challenged species cospeciation and imply that fig wasp speciation maybe have the other ways, such as geographic isolation, or host-shift. In this study, we focused on the pollinator of a dioecious fig (*Valasia javana* Hill) from Southeast Asian countries (including China, Vietnam, Thailand, Singapore and Indonesia) to explore species diversification of the fig pollinating wasp using gene sequences and analyze phylogenetic relationship among them. We employed partial mitochondrial COI and nuclear ITS2 gene sequences to investigate the genetic structure and demographic histories of the wasps. Deep genetic divergence in both mitochondrial (5.4%-28.1%) and nuclear genes (0.8%-42.5%) indicates that nine pollinator species are present and they have largely allopatric distribution (Fig 1). Most of the species are sister species that may have speciated through geographic isolation except that sp5 in Singapore maybe from host-shift. The morphology of these species are different from each other. Our study is helpful to understand species speciation and evolutionary mechanism better.



A **B**

Fig 1. Phylogenetic tree (A) and distribution (B) of the co-occurring pollinators of *F. hirta*

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TALK: Estimating divergence times and ancestral breeding systems in *Ficus* and Moraceae

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There are two breeding systems in *Ficus* (monoecy and gynodioecy). Monoecy has been suggested to be ancestral in *Ficus*, with one origin of gynodioecy and at least one reversal to monoecy. Although several hypotheses have been proposed for the origin of gynodioecy in *Ficus*, the evolution of breeding systems in the genus is still incompletely understood. Here, we reconstruct ancestral states of breeding systems in *Ficus* and Moraceae as a whole using parsimony, maximum likelihood and Bayesian approaches. To do so, we first revised the timescale of Moraceae diversification using new family-wide phylogenetic analyses calibrated with a revised set of 10 internal and outgroup fossil calibrations. We find that ancestral states for breeding systems in Moraceae in general, and the ancestral breeding system for *Ficus* in particular are especially sensitive to: 1) models of morphological evolution; 2) phylogenetic and dating uncertainty; and 3) taxonomic sampling (e.g., whether outgroups of *Ficus* are included or not). For instance, when considering *Ficus* only (without outgroups), ancestral monoecy is inferred with parsimony and the equal-rates (ER/Mk1) model in maximum likelihood (P=0.96), and dioecy with the unequal-rates (ARD/Mk2) model (P=0.87). Several possible scenarios for breeding system evolution in *Ficus* emerge including: 1) ancestral monoecy with one transition to gynodioecy and four reversals to monoecy; 2) ancestral gynodioecy with seven transitions to monoecy. These results are a first step towards investigating the relationship among breeding system evolution, geographic events and historical climate change in *Ficus* and Moraceae.