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Coevolution of figs and fig wasps

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I declare that I worked out this thesis by myself under the guidance of my tutor Mgr. Petr Janšta. I have cited all the literature I used for writing this work.

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Abstract

This thesis reviews known morphological adaptations of pollinating fig wasps to figs (*Ficus* spp.). It was found out that they are obligatory mutualists of figs and they are selected to adapt their morphology to the morphology of fig's flowers and inflorescences. The selection influences figs as well because they adapt according to wasp's morphology. Further, adaptations in physiology and reproductive strategy in pollinating fig wasps are mentioned. All these coadaptations give them the opportunity to cospeciate with *Ficus*. Based on cospeciation and coadaptations in figs and wasps, their common coevolution is generally proclaimed.

Moreover, facts about known rates of cospeciation between figs and nonpollinating fig wasps are recapitulated. Based on current knowledge, it has been concluded that nonpollinating fig wasps show lesser or the same rate of cospeciation. The nonpollinating wasps do not coevolve with the figs as strictly as the pollinators.

Key words: Chalcidoidea, Agaonidae, fig wasp, nonpollinating fig wasp, *Ficus*, coevolution, cospeciation, morphological coadaptation

Abstrakt

Tato bakalářská práce zhodnocuje morfologické adaptace opylujících fíkových vosiček. U fíkových vosiček byl zjištěn obligatorní mutualismus s fíky (plody fíkusů – *Ficus* spp.). Opylující fíkové vosičky přizpůsobují morfologii svého těla morfologii květů a květenství fíků. Tato selekce působí i na fíky, které se adaptují dle různých morfologických struktur vosiček. Dále u opylujících fíkových vosiček existují adaptace fyziologické i reprodukční. Všechny tyto koadaptace jim umožňují kospeciovat s fíky. Kospeciace a koadaptace u fíků a fíkových vosiček předpokládají jejich společnou koevoluci.

Rovněž je v práci shrnuto, co je známo o míře kospeciace mezi fíky a neopylujícími fíkovými vosičkami. Na základě současných vědomostí se předpokládá, že neopylující fíkové vosičky kospeciují s fíky ve stejné nebo menší míře než opylující. Z toho vyplývá, že společná koevoluce není tak striktní jako u opylujících fíkových vosiček.

Klíčová slova: Chalcidoidea, Agaonidae, fíková vosička, neopylující fíková vosička, *Ficus*, koevoluce, kospeciace, morfologické koadaptace

1. Introduction

The fig wasps, an abundant group of insects from the superfamily Chalcidoidea (Hymenoptera), are all associated with fig trees and they reproduce in fig inflorescences. Fig wasps are not monophyletic since they belong to various families, e.g. Agaonidae, Pteromalidae, Torymidae, Orymyridae and Eurytomidae and to unplaced subfamilies Epichrysomallinae and Sycophaginae (Rasplus et al., 1998). Fig wasps depend on figs because they reproduce inside their organs. Figs depend on some fig wasps because they pollinate them. The pollinating fig wasps are monophyletic (Machado et al., 1996) and constitute the family Agaonidae (Rasplus et al., 1998).

Agaonidae represent the only group of pollinators for figs therefore they play an active role in sexual reproduction of figs. Thus there is a strong mutualism between figs and those wasps (Janzen, 1979a). The nonpollinating fig wasps come from all of the families mentioned above except of Agaonidae. Figs are not dependent upon these taxa because the wasps infest them by means of producing galls or they live in galls of other fig wasps. Here they either feed on plant tissues or on the larva (Kerdelhué and Rasplus, 1996). Thus they decrease the fitness of figs and pollinating wasps.

This bachelor thesis is focused mainly on the mutualism between pollinating fig wasps and figs, how it has evolved, how it is maintained and what types of morphological and other coadaptations were acquired to be fig's pollinators. Other part of this thesis deals with the descriptions how the mutualism is influenced by nonpollinating fig wasps as well as with a summary of phylogenetic relationships between pollinating and nonpollinating species and their host specificity.

This thesis is a literary basis for the future master thesis. It will deal with coevolution of parasitoids from the family Torymidae (Hymenoptera: Chalcidoidea) and their hosts, which are mostly belonging to the gall-forming insects. Fig wasps are the model for studying coevolution therefore many articles about them have been published. Other symbiotic relationships in the superfamily Chalcidoidea have not been described so well and that is why this work is mainly dealing with this mutualism of figs and fig wasps

2. Figs as an environment for development of fig wasps

Figs are trees from the genus *Ficus* Linnaeus, 1753 from the family Moraceae (Magnoliopsida: Urticales). This is the most numerous genus from the family and it contains more than 850 species (Ramírez, 1970). They grow in many woody forms for example as free standing, stranglers or epiphytes. They are distributed on all continents with tropical and subtropical climate but they are originally only from tropics (Janzen, 1979a). They have an inflorescence (syconium) which serves as an environment for developing fig wasps. Syconia are usually roundish and green when unripe. The flowers are inside (Fig. 1). Fig wasps pollinate the female flowers and oviposit into the ovaries of the flowers where their larvae develop.

There are two types of syconia which are differentiated in terms of types and sex of the flowers. The monoecious type of fig life strategy is characterized by inflorescences containing both sexes of flowers, male and female ones (Galil and Eisikowitch, 1968a). The gynodioecious (i. e. dioecious in means of many authors - Janzen, 1979a, Weiblen, 2002) figs contain only female flowers in one type of inflorescence which is called a female syconium. The other type, called a male syconium, contains female and male flowers (Galil, 1973).

Monoecious figs have female flowers with style lengths ranging from short to long and with different position of ovaries. On the opening of the syconium, called ostiole, there are scales that are closely overlapped forming narrow tunnel through which pollinators have to crawl. All the figs are strictly protogynous which means the female flowers mature 3 or 4 weeks before the male ones (Galil and Eisikowitch, 1968a).

In dioecious figs, male and female syconia grow either on the same tree or on different trees (Ramírez, 1980). The structure of the opening is the same as for monoecious figs (see above). The male syconia have short-styled female flowers and male flowers that grow near the opening. Female syconia have only long-styled female flowers and no male flowers. They do not need to produce any pollen because no wasps develop in them (Galil, 1973).

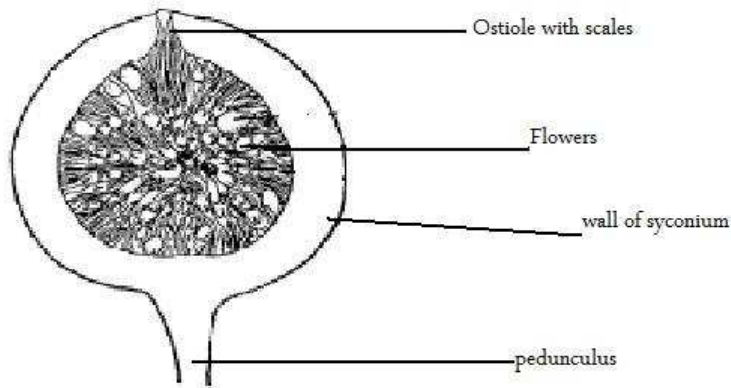


Fig. 1: Cross section of a ripe syconium (according to Ramírez, 1969, modified by author)

3. Evolution of the mutualism

The mutualism of figs and fig wasps has been developing for probably 90 million years old which corresponds with the Late Cretaceous period. It is estimated it started evolving during the break-up of Gondwana (Machado et al., 2001). It allowed the figs to distribute pantropically. The pre-*Ficus* was a monoecious terrestrial plant. Its inflorescence was not closed but convex (Ramírez, 1980). The insect pollination is advantageous in windless environments, therefore the pre-*Ficus* was probably a shrub growing in forest floors (Ramírez, 1976). However, the ancestral state for Moraceae was wind pollination.

Present Agaonidae with primitive traits eat pollen. It was hypothesized that they evolved from pre-agaonid that visited open inflorescences to feed on pollen and maybe to lay eggs in fig's flowers, leaves or young shoots. Wasps that came on syconia brought probably some pollen grains that stuck on their bodies during a previous visit of another inflorescence. Agaonidae then took advantage of visiting the inflorescence and evolved oviposition into ovules of the fig flowers. They all oviposit through the whole female pistil, piercing the centre of the stigma with the ovipositor before the oviposition starts. It is because the ovules were not probably accessible from outside. Because the mutualism became advantageous also for figs, they evolved chemicals to attract the insects. It probably happened before the closure of syconia. Since the insect attractants drew many insects that were possibly harmful for the plant, the shape of syconium changed from convex to closed (Ramírez, 1976).

4. Flowering cycles and life strategies of figs

Flowering cycles of *Ficus* (Fig. 2) were studied on monoecious *Ficus sycomorus* Linnaeus, 1753 in Israel by Galil and Eisikowitch (1968a) and in East Africa by the same authors (1968b). It was found that in Israel *F. sycomorus* does not produce any seeds whereas in East Africa it reproduces sexually. It is because this tree is not native to Israel and its natural pollinator *Ceratosolen arabicus* Mayr, 1906 (Agaonidae) is absent there. Authors of the study observed flowering phases of figs and their responses to presence or absence of wasps. In Israel, *Sycophaga sycomori* Linnaeus, 1758 (Sycophaginae) was found inside the syconium. This is not a true pollinator of *F. sycomorus* but the fig responds to its presence.

The first phase of flowering cycles, phase A, called prefemale, is characterized by growing of the syconium while the ostiolar opening is still closed by scales. In the beginning of the phase B, called female, the scales relent and an opening is formed. It depends on the wasps whether they enter the syconium or not. If they do not enter, the syconium does not develop any further and after several days it drops from the tree. This is caused by the hormone ethephon which makes the syconium ripen fast (Zeroni et al., 1972) when there are no larvae developing in the syconia. If a female wasp comes inside the inflorescence, the fig goes through the whole developmental cycle. Figs are dependent on wasps and identify whether they were entered even though they were not pollinated. Afterwards, they let the wasps to finish their development even though the wasps are not their real pollinators but only gallers. Upon entrance wasps usually lose their wings and even distal parts of their antennae because the aperture is very narrow. Some of them even die during the passage. After burying into syconium, the wasp starts to push its ovipositor through the centre of the stigma, down the style as far as it reaches (Galil and Eisikowitch, 1969). One egg is generally oviposited into one flower but not all flowers are infested. Normally, those uninfested flowers develop into seeds. This is important for the maintenance of the mutualism. The reasons why wasps do not oviposit into all the flowers will be discussed in the chapter five. The phase C, called interfloral, encompasses the time of development of a gall harbouring the wasp larvae. The phase D, called male, occurs when male flowers mature. Male wasps hatch from galls, mate with females and gnaw holes for them to leave the inflorescence. Females then search for other syconia in phase B. Then phase E, called postfloral, follows. Now the releasing of the hormone ethephon is allowed again which means the figs ripen. Syconia color pink, red or

yellowish which makes them attractive to the frugivores. The whole flowering cycle takes six to seven weeks.

This has been short description of how receptacles of monoecious figs function. They did not produce any seeds, though, even after the researchers tested out artificial pollination by means *S. sycomori* covered with pollen grains. There was something missing in the wasp's behavior that would allow proper pollination (Galil and Eisikowitch, 1968a). This means that this is an example of utilization of coevolved features between mutualists of different species of the same family (Compton, 1990).

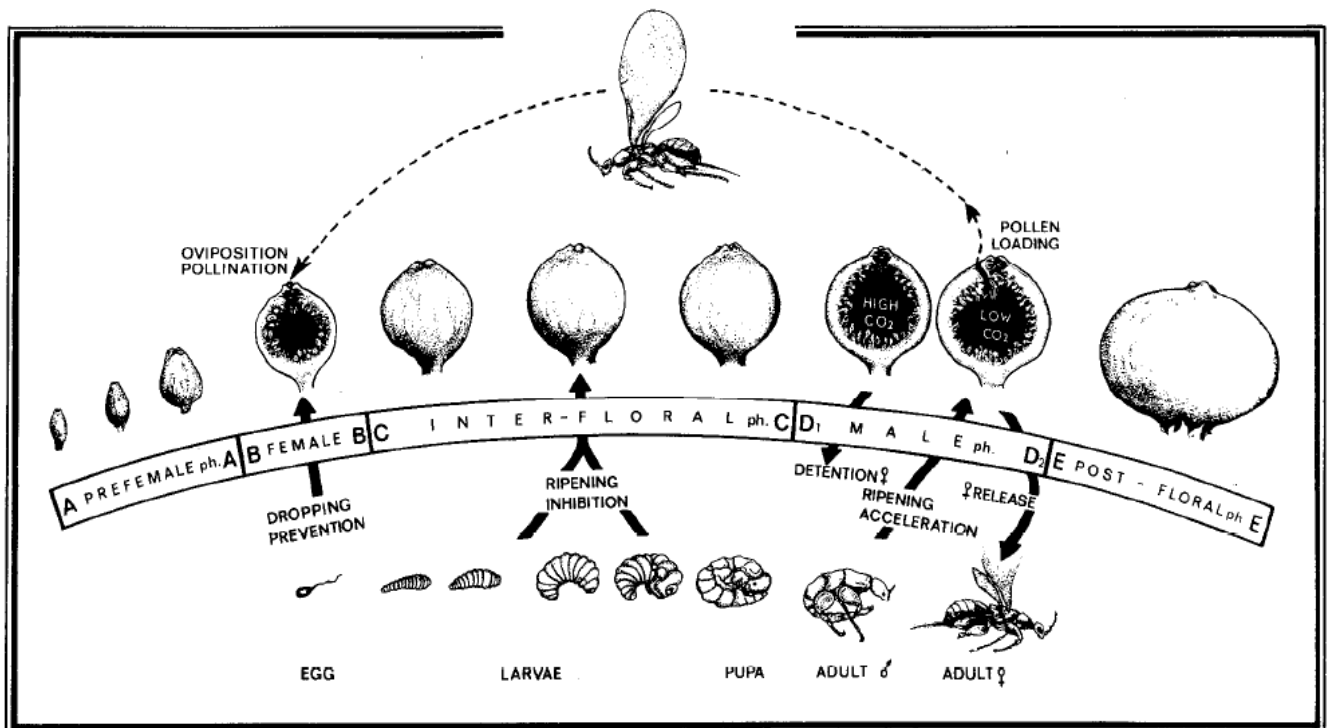


Fig. 2: Flowering cycles of monoecious fig and life cycle of its pollinator (according to Galil, 1977)

In the dioecious figs, wasps develop in the male syconia. In the female inflorescences only seeds can develop due to the length of styles which does not allow wasps to reach the ovary (Galil, 1973). Anyway, a female pollinator has to enter to ensure pollination of the long-styled flowers. The cycles in this life strategy are similar to the cycles in monoecious figs, even though they fully conform only to the male syconia. Since the female syconia do not have any male flowers and do not have larvae inside, they do not flower exactly in the phases described above. The behaviour of the wasps is also very similar to behaviour of pollinators in the monoecious figs (Galil, 1973).

5. Conflict of interests

In the evolution of the whole mutualistic system, there has been one important feature of figs that made wasps to coevolve with. It is the length of the styles of female flowers (Weiblen, 2002). The monoecious life strategy of the trees is the ancestral state (Ramírez, 1980). Change from monoecy to dioecy was accompanied by shortening of the ovipositor. The life strategy of *Ficus* changed more than once and the length of ovipositor always changed as well (Ramírez, 1980).

It was supposed that monoecious figs evolved two types of female flowers with different lengths of styles (Galil and Eisikowitch, 1968a). It was thought that females of pollinators oviposit only into the short styled flowers because their ovipositors could not reach the ovary of the long styled ones (Galil and Eisikowitch, 1968b). This would mean that the fig itself controls the number of seeds infested by pollinators. Bimodal distribution of lengths of styles would be expected. However, the whole theory broke up when it was found that lengths of styles of female flowers are distributed normally. Moreover, wasps have ovipositors long enough to reach most of the flowers (Bronstein, 1988). Those two facts have refuted that figs can control proportion of sites for oviposition.

Anyway, it is true that wasps oviposit only into the layer of flowers which is closer to the lumen of the fig (Galil and Eisikowitch, 1969). What is then the selection pressure on the wasps not to oviposit into all available ovules and to suppress their own individual interests? How is it possible that the mutualism is still stable? Several hypotheses have been stated to answer the questions. Abortion of young syconia where too many wasps developed was proposed by Murray (1985). If too many wasps lay eggs, there are not enough flowers to produce seeds therefore the fig could drop these syconia. This would create a selection pressure on the wasps not to over-reproduce because the foundresses that would lay eggs in all flowers would have zero fitness. However, no evidence was found for this hypothesis (Murray, 1985, Nefdt and Compton, 1996). Nevertheless, it was found that if there are more foundresses in one syconium, the clutch size tends to be decreased (Bronstein, 1988).

Hypothesis called unbeatable seeds was proposed by West and Herre (1994). They observed a situation when pollinators and galling parasites of the tree competed for some flowers meanwhile others stayed untouched. This happened even though the gallers oviposited from the outside. It could mean that some flowers are sequestered for seed production while others for wasp production.

Another theory is called optimization of the ovipositor length. The variance in lengths of flower styles could be increased as a response to the length of ovipositors and the wasps would be able to reach lower regions of ovaries. Furthermore, the costs on evolving longer ovipositor for wasps could be too high in terms of difficulties in flight and energy allocation. In this situation there would be a selection pressure on optimizing ovipositor length (Ganeshaiah et al, 1995).

The ostiolar opening into the syconium could play a role in selecting only wasps up a specific size (Nefdt and Compton, 1996). The body size correlates with number of eggs a female is able to develop (Herre, 1989). The selection pressure on a wasp's size could result in lowering the number of eggs one female is able to develop and the proportion of infested flowers would decrease. Another role of the ostiole is its closing short after entering of a pollinator (Bronstein, 1987). It blocks the opening and prevents other pollinators from entering (Nefdt and Compton, 1996). The fig also regulates the number of foundresses by termination of the attractive substance production. Mainly if more than one wasp passes through the ostiole, the fig has to arrest its receptive state (Khadari et al, 1995).

Ansett (2001) linked the hypotheses and she published a theory that flowers are of different quality in terms of size. Resolving unbeatable seeds hypothesis supposed that flowers closer to the wall of fig are smaller due to space limitations. Therefore it is more advantageous for the wasps to lay eggs into ovaries closer to the lumen. They compete for this site with gallers that also want their larvae to develop in galls of higher quality. The optimization of the ovipositor length was also included. The ovipositor lengthening would be costly not only in energy allocation but also in the availability of the long styled ovaries. If a wasp oviposited into the long styled flowers, it would secure fewer sources for the larvae. Moreover, there would be lower chance of being fertilized because males do not easily move in the layer of flowers which is by the syconium wall. They prefer to mate with females in the other layer of flowers.

Pollination is so important for figs that they sacrifice 55 % of their seeds which are killed by larval activity (counting in parasites which could mean half of the seeds). This surprisingly high percentage can be explained by the need of figs to be pollinated and by the fact that the more fig wasps develop inside of one syconium, the more pollen is carried away and fitness of the tree is increased (Janzen, 1979b). Janzen (1979b) and later Murray (1985) found out that this applies only to a certain extent. They said that the number of pollinator female wasps is advantageous only to a certain number. In case of too many wasps the benefits of pollen distribution would not exceed the benefits of having more seeds. The value of 55 % could be

the value when both partners optimize their fitness. Wasps have enough offspring and figs still have enough seeds to reproduce successfully.

6. Conflict of dioecious figs and their pollinators

The other conflict of interests is between the pollinator wasps and dioecious figs. The wasps enter both types of syconia despite the fact they cannot leave any offspring in the female ones. They die there without possibility to escape (Kjelberg et al., 1987). They even keep on performing pollinating movements inside of their 'grave'. It is a paradox why the female wasps enter the syconia and why they take an active part in pollinating it (Grafen and Godfray, 1991). It contravenes the individual selection.

Firstly, it was hypothesized that females of pollinators do not have possibility to enter any other fig than the female one because of flowering phenology (Kjelberg et al., 1987). This study was conducted on *Ficus carica* Linnaeus, 1753. Only female syconia were found to be receptive when male syconia released the pollinator females. No male syconia in B phase were found at that time. It would mean that there is no selection on wasps to enter female syconia since they do not have any other option. Grafen and Godfray (1991) opposed that there has to be some selection pressure on the wasps otherwise the pollination movements in female figs would have vanished due to neutral evolution. Furthermore, this specific phenology does not apply to all fig species (Moore et al., 2003).

The interests of male and female figs are the same so they have to mimic each other and must not compete. If they competed, the male figs could be recognizable for wasps and the mutualism would collapse. For explanation a conception has to be made. Hypothetically, female fig undergoes a mutation that generates selection pressure on the wasps to change their morphology. Wasps, which emerge from male syconia with similar mutation and therefore similar characteristics to the female syconia, react better to the change. They would be able to pollinate the female fig and the mutualism would be maintained (Grafen and Godfray, 1991).

However, some differences between the types of syconia can be found. The chemical substances released by male and female figs are not identical in *Ficus carica* (Hossaert-McKey et al., 1994). The diameters of figs at a peak of receptivity are neither identical. Wasps could therefore find a way how to differentiate between sexes if they learned how to link receptivity with diameter (Patel et al., 1995).

It was then hypothesized that wasps searching for a syconium do not have time to search for a male one due to their life-span which is about 2 days (Kjellberg et al., 1988) and enter

any receptive syconium they find. This hypothesis is called selection to rush (Patel et al., 1995).

Finally, Moore et al. (2003) found another synthesis of some theories of conflict of dioecious figs and their pollinators. They hypothesized that pollinators of figs whose sexes are receptive at different time (as is *F. carica*) can recognize male figs from female. However, there is strong pressure on them to enter the first encountered inflorescence because they would not be able to find the one they prefer. This means that there is not such a strong pressure on the figs to disguise their sex. Figs whose sexes flower synchronously are selected to mimic each other so that wasps cannot choose only male figs.

7. Evolution of dioecy

The evolution of dioecious life strategy in figs could be linked with adaptation to seasonality as was thought for *F. carica* in south France (Kjellberg, 1987). Trees with male syconia were found all year round but trees with female syconia were found only when the weather was feasible for production of seeds. However, *Ficus* is generally a genus from tropics where no seasonality occurs and dispersion of *Ficus* into seasonal environment is rather consequence of dioecy than its cause (Kerdelhué and Rasplus, 1996).

Kerdelhué and Rasplus (1996) proposed that evolution of dioecy was selected by presence of nonpollinating wasps. Monoecious figs have 3 or 4 layers of female flowers with different style lengths. The two middle layers are often occupied by galls that are parasites of figs. When the number of galls exceeds a threshold, there will be a selection pressure on the monoecious figs to get rid of these flowers and to develop two types of figs with two types of flowers, each dedicated to other component of reproduction (pollen transfer and seed development). This hypothesis was supported by the fact that dioecious figs do not have any galls larvae that would oviposit from outside after pollination of the fig. Conversely, in monoecious figs half of nonpollinating wasps were found to be galls (Kerdelhué and Rasplus, 1996).

8. Morphological coadaptations of pollinating fig wasps and figs

There are many morphological and physiological adaptations in Agaonidae which have evolved due to their life strategy. The most remarkable adaptation is the sexual dimorphism, mainly in relation to male body form. The males have evolved to their present appearance due to conditions of closed and isolated syconium that they never leave (Galil, 1977). They hatch

as first and search for the galls with female individuals that are still in. When a male finds a gall of a conspecific female, it perforates it and mate. Abdominal segments 7 to 9 are modified to a tubular structure in males. It is inserted into the female's gall telescopically (Ramírez, 1976). The female stays in its gall during copulation (Askew, 1968). After mating, the male of most species gnaws a hole through the wall of the syconium allowing females to emerge from their native syconium to oviposit in others. However, in *F. carica*, the ostiole widens and females leave syconia without the help of males (Galil and Neeman, 1977). Males of pollinator wasps are always wingless, their eyes are not developed, the mid legs and antennae are shortened (Compton, 1989). Some species did not develop the mid legs at all, for example some species of the genus *Tetrapus* Mayr, 1885 (Ramírez, 1991).

In figs of the subgenus *Sycomorus* and subgenus *Ficus*, the males of some fig wasps have enlarged cuticular plates, called peritremata, which are located around spiracles. The pollinator (*Ceratosolen capensis* Grandi, 1955) and some nonpollinating wasps from the subfamily Sycophaginae (for example *Sycophaga cyclostigma* Waterson, 1916) and from the family Pteromalidae (for example *Apocrypta guineensis* Grandi, 1916) were amongst the studied wasp species. The reason for having peritremata is that the figs are filled with fluid during ripening (phase C and beginning of the phase D) and the time when males hatch and have to search for a mate conforms this period. The male wasps have to survive these conditions (Compton, 1989). *Sycophaga cyclostigma* was the species with the most modified peritremata even though they do not pollinate the figs. Shape of the peritrema is elongated and the inner surface is covered with tiny setae which play role in repelling water and capturing air bubbles. The part of the insect's body with modified peritremata always stays above the fluid and serves as breathing siphon. In case the male covers itself with the fluid completely, there will still be bubbles of air on setae so that the wasp does not die (Compton, 1989).

The sexual dimorphism is not only morphological but in some species also a physiological state. Males of *Platyscapa quadraticeps* (Mayr, 1885) (Agaonidae) inhabit syconia of *Ficus religiosa* Linnaeus, 1753. The internal atmosphere of the syconium does not interchange with the external in this species. Due to respiration, concentrations of gases change inside of the syconium compared to the outside. In the phase D, there is 10 % CO₂ and 10 % O₂. The male wasps have evolved adaptations to a higher carbon dioxide content and they perform all the needed actions to reproduce even in this atmosphere while the females stay intact. When the males gnaw exit holes in the syconium wall and the gas contents inside and outside balance, the female wasps start to fulfill their function (Fig. 2). This implies that lowering of

CO₂ concentration serves as a signal for females to leave the gall, collect pollen and exit the fig. This process has also consequences for the figs because they ripen very fast after wasps leave them. It was proven that ripening is linked with releasing of the hormone as well as with changing of the CO₂ content (Galil et al., 1973).

Another morphological and behavioral adaptation was found in the way how the flowers in syconia were pollinated. There are wasps that are passive pollinators as the genus *Tetrapus* in the New World (Ramírez, 1970) and some *Blastophaga* Gravenhorst, 1829 species in the Old World (Ramírez, 1980). Females of these genera do not have to perform any activity to get pollen grains onto themselves and therefore it is called a passive pollination. Females of *Blastophaga psenes* Linnaeus, 1758, the passive pollinator of the common fig (*Ficus carica*), emerge from the galls and crawl towards the ostiole. The ostiole is opened and the scales are loosen in phase D (the males in this species of fig do not gnaw the escape hole). They have to pass through flush of shed pollen if they want to leave the syconium. When they leave it, they clean their bodies from pollen and they fly away to search for receptive syconia. Galil and Neeman (1977) explored how enough pollen can be loaded on a female wasp to pollinate enough flowers even though they clean themselves. After hatching, a female's abdomen is swollen due to large water content in its body. Later as the water evaporates and intersegmental membranes infold, the abdomen shrinks by 20-30 %. This happens in the cavity of syconium when the wasp moves through the released pollen. As the intersegmental membranes infold, they create concave space for pollen load which automatically gets onto the wasps' body.

Unloading pollen and pollination in B phase syconia occurs also passively for the wasp. As the female enters the syconium, the ostiole exerts pressure on it which might push some pollen away from the infolds between segments of the abdomen. For the rest of the pollen, it is the moisture in the syconium that secures it's unloading. Swelling of the pollinator's abdomen restores (Galil and Neeman, 1977).

The other way of carrying pollen is called active pollination and it can be observed for example in genera *Pegoscapus* Cameron, 1906 (Ramírez, 1970), *Ceratosolen* Mayr, 1885 (Galil and Eisikowitch, 1968b) and many *Blastophaga* species (Ramírez, 1980). In *Pegoscapus*, the wasps use mandibles and front legs to gain enough pollen from the anthers. It means that they take active part in the pollination because those movements are goal-oriented to pick up pollen (Ramírez, 1970). They move the pollen to special concavities in the front coxae where there is one on each of them and to the paired mesosternal (Ramírez 1969). Ramírez (1969) called these concavities corbiculae as in honey bees (*Apis mellifera* Linnaeus,

1758). Some actively pollinating species possess either mesosternal or coxal corbiculae (Ramírez 1969). Corbiculae have evolved at least three or four times convergently because their structures in some agaonid lineages are slightly different. They evolved in order to increase the load of pollen and to facilitate pollination (Ramírez, 1978).

The movements for collecting of pollen were observed by Galil and Eiskowitch (1969) in *Ceratosolen arabicus*. The wasps alternate movements of forelegs and push pollen grains between the thorax and the forelegs. There are setae on coxae of those legs and they are used to put the lifted pollen from inbetween the thorax and legs to the thoracic corbiculae. The setae are called coxal combs (Galil and Eisikowitch, 1969). It seems that there was simultaneous evolution for the coxal combs and sternal corbiculae because they are connected functionally. The coxal corbiculae are absent in this species.

The wasps after entering the syconium pollinate the flowers of figs also by goal oriented movements because the corbiculae were found empty in dead female wasps inside of the inflorescences. It has been proven that pollen does not come out of the pockets spontaneously because it resisted 20 minutes of shaking in ethanol (Ramírez, 1969). When the wasp comes to a receptive inflorescence, it uses tarsi, mainly arolia (Galil 1973) of folded forelegs to attach pollen grains on stigmata and inserts ovipositor into the flower parts (Galil and Eisikowitch, 1969). This behaviour is different niether in short and long styled flowers in monoecious figs (Galil and Eisikowitch, 1969) nor in male or female figs in dioecious figs (Galil, 1973). That is why Galil (1973) proposed for the female Agaonidae that there is a need for a stimulus such as a contact of the ovipositor with the flower to complete pollination behavior successfully. Other ways of pollination are eating the pollen and regurgitating it in B phase syconium which can also be called active pollination. However, this indication is not entirely confirmed, it is possible that the wasp just feeds itself and the pollen covers its body as in the process of passive pollination (Ramírez, 1969).

The evolution of such structures and such behaviour has been induced by the selection pressure for bringing enough pollen into the syconium. Pollen grains are shed away from the body surface by the scales of the ostiole while wasps are squeezinng inside. Therefore only buccal cavity, digestive track, inner sides of coxae, sternum and petiolar area are hidden from the pressure of scales. Therefore it was positively selected for these parts to carry more pollen (Ramírez, 1978).

Figs have certainly coevolved with their pollinators and there are some adaptations that have evolved primarily or secondarily as a response to the wasps' behaviour. The mode of pollination mentioned above is linked to the number of anthers and the amount of pollen. In

the passively pollinated figs, there is much greater number of anthers than in the actively pollinated ones. In the actively pollinated figs, the wasps load enough pollen to pollinate the other generation and the fig does not have to invest so much energy into pollen production. In the passively pollinated figs, there is so much pollen that the wasp has to clean its body after leaving the syconium (Galil and Neeman, 1977, Galil and Meiri, 1981). Other coadaptation to facilitate pollination might be softening of the wall of syconium in phase D. It helps the male pollinating wasps to hole it afterwards (Ramírez, 1970).

The ostiole is a very important part of the fig because its tightness selects for many features of wasps (Galil, 1977). It serves as a barrier for other insects and prevents them from entering the syconium (Ramírez, 1970). It also selects very specifically the species of wasps which can pass through (Galil, 1977). As a response to the ostiolar shape, the wasps have evolved special head shape, modified antennae and mandibles to get inside of the fig (Galil, 1977). Bigger figs have thicker walls and longer passage for the fig wasps to go through so that their pollinators and other wasps that pass through the ostiole possess elongate and flattened head shapes. Smaller figs did not exert such a selection pressure on their associated wasps, which is why they have square heads (van Noort and Compton, 1996). Other reasons for different shapes of head might be the tightness of the scales lining the inner part of the ostiole. The evidence that head shape has coevolved due to the opening at top of fig follow. Square shaped and elongated heads seem to be convergent in non related species of wasps, in the family Agaonidae and in the subfamily Sycoecinae (Pteromalidae). The feature had been lost and then reappeared in history as size of the figs fluctuated. There is a counter pressure on wasps to have smaller heads because flying with long heads is not efficient and that is why wasps tend to lose this feature when the figs grow smaller (van Noort and Compton, 1996). Other coadaptation induced by the ostiole characteristics are mandibular appendages and tibial teeth or lamellar structures on fore legs. They have evolved because they help the wasps to crawl through the ostiole (Ramírez, 1991).

As mentioned above, a very important interaction between figs and wasps is the length of ovipositors in correlation with the life strategy of figs and therefore with the style length and position of the ovary (Ramírez, 1980).

9. Species specificity

The mutualism of figs and pollinator fig wasps appeared to be very species specific. It has been supposed that one section or subsection of figs is pollinated by just one genus of wasps.

The other assumption was that each fig species has only one species of a pollinator that is specific for it. In other words, the mutualism obeys one to one rule to some extent (Ramírez, 1970). For introducing figs to new areas, it is necessary the specific pollinator follows the figs otherwise no pollination occurs, the syconia do not mature and fall down from the tree (Ramírez, 1970). There is one example with *Ficus sycomorus* which has been introduced from East Africa to east and south Mediterranean area and its pollinator *Ceratosolen arabicus* did not follow it. *Sycophaga sycomori* uses this species in Egypt and Israel to develop in the seeds. However, it does not pollinate the flowers and the trees reproduce only asexually (Galil and Eisikowitch, 1968a, Ramírez 1970). On the other hand, Ramírez (1970) published an example of a pollinator that was able to travel the distance from Costa Rica to Venezuela where just one isolated fig tree specific for this pollinator was planted and therefore this lonely tree could bear viable seeds and reproduce sexually. It means that in some cases pollinators are able to travel long distances but it probably depends on chance whether they reach their host as well as on abiotic factors.

This specialization brings up a question of how the species specificity of pollinators and figs is maintained. There is selection pressure on wasps to enter the right fig (Bronstein, 1987). If the insect enters a wrong species of fig, the pollen would not be compatible, eggs could not develop in nonpollinated flowers because their larvae feed mostly on endosperm which originates only after pollination (Galil and Eisikowitch, 1968a, 1971). Moreover, newly appeared pollinator would have to compete with the established one (Silvieus et al., 2007). Firstly, it had been predicted that there might be some species-specific odor (Ramírez, 1970, Bronstein, 1987) and afterwards it was found out that the odor really existed. It is released through the ostiolar opening and it is attractive for the species of wasp which pollinate the given fig (van Noort et al., 1989) as well as for other nonpollinating wasps (Bronstein, 1987). Further research showed that the chemicals are released mainly from the inside of the inflorescence and that figs are most attractive at the beginning of their period of receptivity (beginning of B phase) when they release a special chemical compound of the chemical which is not released at any other stage (Ware et al., 1993). Fig wasps respond to presence of the specific chemical by change in their behaviour and display movements typical for pollination (Hossaert-McKey et al., 1994). It is not only maintaining the species specificity of the interaction but also a way how to ensure that newly emerged wasp would find syconia in B phase. Tree with such inflorescences could be far away because all the syconia in one tree are always found just in one phase of flowering and it could be difficult to find one (Janzen, 1979a). Exceptions to this rule can be found only on islands,

edges of tropics and other places where long dry seasons occur (Janzen, 1979b). In stable conditions, this adaptation serves as a tool how to prevent self-pollination.

There are many exceptions, though, to the one to one rule which firstly did not cross the minds of researchers that the specificity does not have to be so strict (Ramírez, 1970). Wasps sometimes mistake the species of fig they are supposed to enter. Those errors occur when a tree is too far from the other conspecific figs and therefore there is lack of natural pollinators. These errors are possibly frequent but they are not visible since many syconia are pollinated and do not fall from the tree. In case of absence of pollinators, only inflorescences with other wasp inside stay on the tree. Those can be examined afterwards (Compton, 1990). This means that it is similar to the case of *Ficus sycomorus* and *Sycophaga sycomori*. The tree is able to detect the presence of any wasp, mainly its gall (Galil and Eisikowitch, 1971). Compton (1990) described a case with *Ficus lutea* Vahl, 1805 and wasps specific to other fig trees that were found inside. Pollinators of related species of figs were able to produce progeny in the nonspecific flowers but other wasps from unrelated species did not have this capability. Even the seeds were hybrid but viable. Since errors by pollinators lead to progeny, it also gives opportunity to adaptation to a newly colonized fig species, mainly if there is free niche (Weiblen and Bush, 2002).

After the modern molecular methods were introduced, we can observe congruence of phylogenetic trees of wasps and figs. Examining how phylogenies reflect each other leads to inferences about host specificity and cospeciation. Since the phylogenetic trees are not based only on morphology they are more exact. The morphological traits in wasps might have arisen as an adaptation to figs and that is why the phylogeny based only on morphology could show polyphyly due to convergence (Herre et al., 1996). It was refuted by Weiblen (2001) because the phylogenies based on both, DNA sequence and morphology, showed greater accuracy. Anyway, it was proven that molecular markers give more information than morphological characters (Weiblen, 2001).

There is a strong objection in finding a relationship between phylogenies of figs and wasps. We have to be sure that the cladograms have been made accurately. If not, the inferences we would make about species specificity would be incorrect either. It was already stated by Wiebes (1979) that if one expects species specificity in the relationship, it is much easier to find such features in morphology of both sites that would help to reconstruct the history in the way one expects.

The first study on pollinating wasps and figs based on molecular markers showed that there is one to one rule concerning the subsections of figs and genera of Agaonidae. However,

colonization events (see chapter 10. Speciation) could play role in a lower-scale relationships (Herre et al., 1996). Other studies confirmed that Agaonidae are mostly species specific to their hosts and they obey the one to one rule (Silvieus et al., 2007). It was proposed that it is because of chemicals released by figs that maintain the species specificity of the mutualism (Silvieus et al., 2007).

On the other hand, one to one rule was not kept in Africa by pollinators of *Galoglychia* section of *Ficus*. More genera pollinate one section in this case. The pollinators of this section showed that they are monophyletic but many host switches or extinctions happened on lower taxonomic scale. The host specificity is not held further than on section level, subsections already show incongruence between phylogenies (Erasmus et al., 2007, Jousselin et al, 2008).

Other recent studies have revealed much greater number of exceptions from one to one rule, reaching 50 % of species of pollinators that do not obey it. It implies that the extent of common cospeciations between figs and pollinators is not as high as it was thought (Molbo et al., 2003). It is because modern techniques of sequencing microsatellites and mitochondrial DNA can easily show cryptic species of pollinators associated with one species of fig (Molbo et al., 2003). Species undistinguishable from morphological characters are found in many of the pollinator genera. They are in the primitive *Tetrapus* and also in the derived *Pegoscapus* (Molbo et al., 2003). The speciation was proposed to occur in two possible ways in this case. One of them is host shifts. It occurred mainly if the cryptic species are found in allopatry or they have deep divergences in DNA when found on the same species of *Ficus* (Molbo et al., 2003). The other proposed way is that the wasps speciate with help of *Wolbachia* bacteria (Haine et al., 2006). Moreover, it was shown that the pollinating as well as nonpollinating fig wasps have much higher prevalence of *Wolbachia* infection than other insects and arthropods (Shoemaker et al., 2002) which would favor the theory.

10. Speciation

Generally, the whole superfamily Chalcidoidea is supposed to be in the period of speciation because many sibling species can be found in each family (Askew, 1968). Speciation is ensured by many mechanisms (two of them were already mentioned in the previous chapter. Other way is the sib-mating which gives a chance to speciate to the fig wasps by itself. It gives an opportunity to colonize isolated areas because females will always have mating partner inside the syconium. Populations under conditions of disruptive selection pressure might be able to speciate fast because of inbreeding. Wasps will not get to

an inbreeding depression because of small a rate of outbreeding in allopatric populations (Askew, 1968).

Cospeciation due to colonization of another habitat by figs is discussed by Michaloud et al. (1996). It was concluded that there could be two ways of cospeciation due to a change in a fig's ecology and possibly geography. Firstly, figs colonize new environment (drier or wetter) in parapatric or allopatric way. Only wasps which can comply the change due to their alleles follow it. Then due to the genetic drift or selection pressures exerted by the environment, the volatile attractants can change and cospeciation can occur (Fig. 3-left). The other way of cospeciation can involve two sister species or subspecies of wasps that can partly overlap in the process of pollination on two species of figs. The wasps should be both adapted to 'their' specific tree but since they are related to each other they can be found on the same tree erroneously. One species of fig then changes its habitat and it is followed by the other wasp species than is adapted to it. This results in third species of wasps and figs (Fig. 3-right).

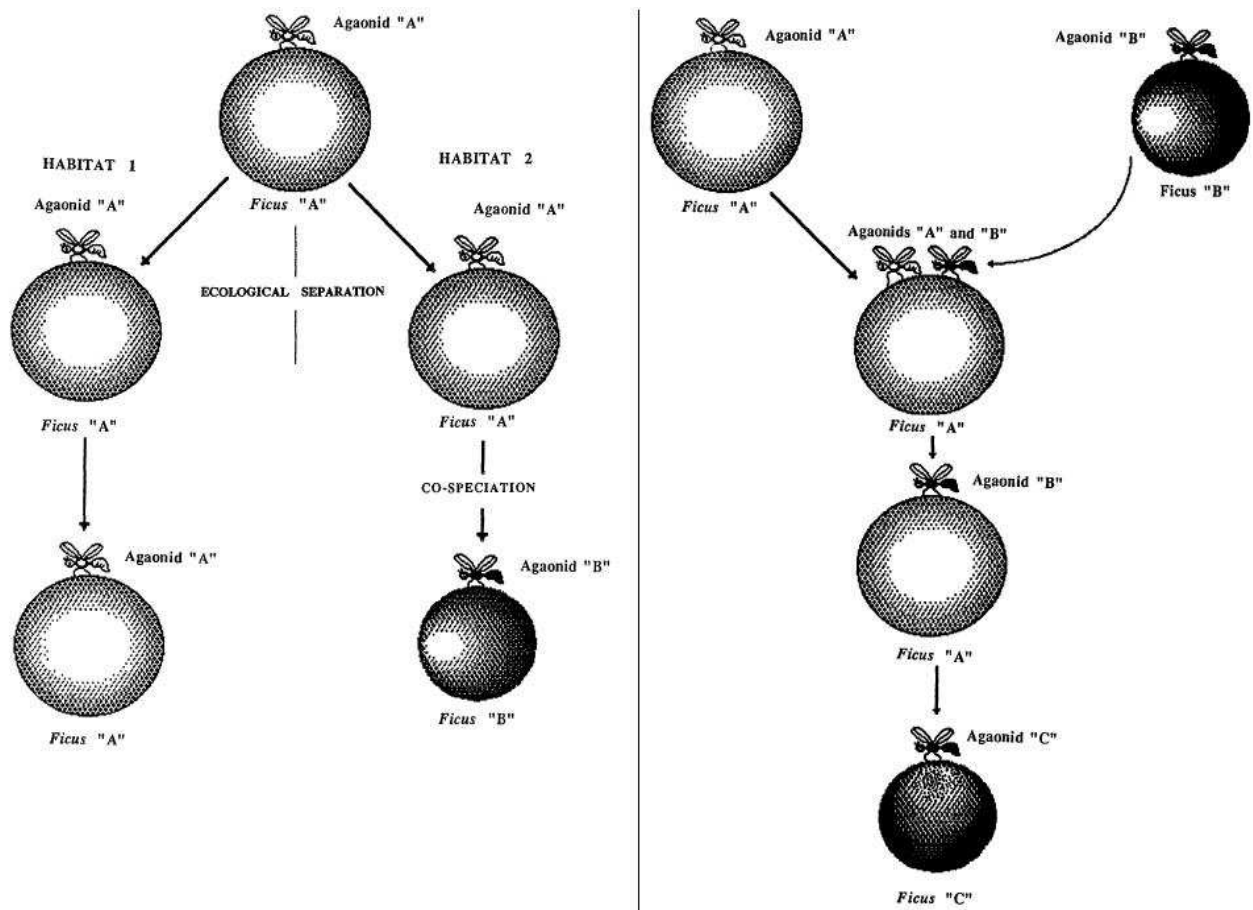


Fig. 3: Parapatric or allopatric speciation. Ficus A changes its habitat from A to B. It adapts and speciates to Ficus B. An Agaonid follows the change so that it speciates to Agaonid B (left). Speciation involving sister species of wasps. Agaonid A and B overlap in their geographic distribution and both pollinate Ficus A. Ficus A changes its habitat and only Agaonid B is able to adapt to such environment. They both cospeciate to Ficus C and Agaonid C (right) (according to Michaloud et al., 1996)

Speciation in figs had certainly a high rate since the genus has about 850 species. The point is that the closed pollination (only few female pollinating wasps entering) offers a chance for speciation without geographic fragmentation even though it is not sure whether it can work in complete sympatry (Ramírez, 1970). This example is similar to the one mentioned by Michaloud et al. (1996). Pollinators for which a fig is attractive enter it, mating occurs inside the syconium, often between brothers and sisters (Askew, 1968). Their descendants will possess the ability to find figs with specific volatile chemicals that were attractive for their parents. Just one mutation in the preferences of the wasps or in the chemical compound released by the figs is enough for the pollinator to fail to find its host (Ramírez, 1970, Silvieus et al., 2007).

11. Species specificity and speciation in nonpollinating fig wasps

Recently the nonpollinating wasps have become a subject to many studies. Their ecology (West and Herre, 1994) and phylogenies (Machado et al., 1996) have been examined. I will focus mainly on species specificity and ways of speciation between gallers and figs and between parasitoids of pollinators or these gallers. Those categories have to be separated because differences in ecology and morphology can cause different results in phylogenies. Moreover, some incongruence in phylogenies of hosts and associated animals can lead to new hypotheses about ecology of the relationship (Jousselin, 2008).

Gallers (*Apocryptophagus* Ashmead 1904, Sycophaginae), that were studied by Weiblen and Bush (2002) and by Silvieus et al. (2007), oviposit from the outside of the wall of syconium. They have very long ovipositors in order to pierce the wall of syconium. Their larvae feed on proliferating nucellar cells that are produced with no need for pollination. For gallers and figs, the species specificity was not found to obey the one to one rule since many non-sister species of wasps were found on one species of fig (Silvieus et al., 2007). It was reasoned by different rate of speciation in nonpollinating wasps from pollinating wasps. Larvae of gallers are not dependent on pollination of figs thus the host shifts are easier for them. In pollinating species, the host shifts are limited by compatibility of pollen and developing of endosperm as food for larvae. Moreover, gallers can speciate in sympatry by means of changing the ovipositor length and therefore their niche (Weiblen and Bush, 2002). It was found that some sister species of *Apocryptophagus* differ in ovipositor length by 2 mm. Species with shorter ovipositors lay eggs through younger syconia because their walls are thinner. This sympatric speciation therefore occurred due to change in timing of oviposition (Weiblen and Bush, 2002).

Other examination of fig's gallers was conducted in Otitesellinae (Pteromalidae). They also oviposit from the outside of the fig. They show greater host specificity to figs than *Apocryptophagus* in the previous study. The reason might be that they are attracted by the same chemicals as pollinators because they all visit the fig in B phase when it is receptive for pollinators (Jousselin et al., 2006).

Lopez-Vaamonde (2001) examined species specificity between pollinators and their parasitoids. It showed that parasitoids do not strictly cospeciate with their hosts. Anyway, some cospeciations occurred since their frequency was significantly higher than could be caused by chance. Silvieus et al. (2007) found that parasitoids of pollinators were even less species specific than the gallers in the same study. It is because too narrow specialization is

a risk to extinction (Silvieus et al., 2007). This can also explain the trend in very wide host specificity in parasitoids of gallers (*Apocrypta* Wiebes 1966, Sycorectinae, Pteromalidae). The gallers have orders of magnitude smaller population sizes than pollinators. Parasitoid thus cannot specialize; they have to adjust the host range to their ability to find a host and to the probability of extinction of the host (Silvieus et al., 2007).

Compared to previously mentioned examples, Jusselin et al. (2008) found out that nonpollinating fig wasps reflect the host phylogeny at least to the same extent as pollinators. It was studied on figs from section *Galoglychia*, their pollinators and both, gallers (Otitesellinae) and parasitoids (species of the genus *Philotrypesis* Förster, 1878, Pteromalidae). They argued that nonpollinating fig wasps show also many adaptations that preclude frequent host shifts: the ovipositors are definitely adapted to fig wall thickness. The gallers had to develop specific galling process. As was already mentioned, many fig wasps tend to recognize figs thanks to the volatile attractants they release. The species specificity of nonpollinating fig wasps can then be comparable to the pollinator's one.

12. Sex ratio and male dimorphism in fig wasps

The sex ratio of fig wasps is biased towards females because of local mate competition in closed syconia (Hamilton, 1967) and because of inbreeding (Herre, 1985). The female would waste its life resources if it oviposited more male eggs than would be needed to fertilize all its daughters. Its sons would be exposed to greater competition for mates if the ratio was 0.5. Herre (1985) published that the number of females of various species of Agaonidae (two species of *Blastophaga* and *Tetrapus costaricanus* Grandi, 1925) entering the syconium is negatively correlated with level of local mate competition. He confirmed it by an experiment where species of wasps with higher mean number of females entering one fig demonstrated less female-biased sex ratio. There were about 10 % of males in case of one foundress. When five or six foundresses entered the syconium, proportion of males turned to 30-40 %. Those females were selected to lay more male eggs because their sons had to stand the competition for sexual partners. The sex ratio is maintained by females who choose whether to oviposit fertilized or unfertilized egg. The actual sex ratio a wasp should lay is estimated due to known size of clutch that can be laid. Clutch size gets smaller when another conspecific female enters the same fig (Bronstein, 1988, Moore et al., 2005). Females even had to evolve a strategy how to count in the number of females that leave syconium as virgins and they will have only sons. Thus all females have to perform oviposition in further female-

biased sex ratio (Godfray, 1988). When molecular analyses on cryptic species of pollinating fig wasps were made, it revealed that the hypothetical sex ratio (Hamilton, 1967) even better corresponds to the actual one (Haine et al., 2006). The cryptic species do not mate with each other and therefore the females have to adjust the sex ratio in the described way.

The sex ratio is adjusted by all wasps that develop inside the fig, not only pollinating wasps (Godfray, 1988). The reason is they all share the same environment for their development therefore the local mate competition and inbreeding apply for them as well.

As the sex ratio links with the competition for mates it should also have an effect on rate of wingless male wasp's fights of nonpollinators. This study was conducted by Frank (1987) on *Philotrypesis caricae* Linnaeus, 1762 (Pteromalidae) which is a parasitoid of *Blastophaga psenes*. The more females there are per one male, the less numerous and less injuring the fights are. Then, males would not meet so often and there are still lots of females left for them. If they meet, they fight using strong mandibles. Even a special shape of head for bearing the mandibles had to evolve. Why males of pollinator species do not fight is unknown. It could be because they are mostly brothers or because there is a selection pressure on females to possess elongate head shape that allows them to enter the syconium. This could constrain the development of different head shape in males (Frank, 1987).

Sex dimorphism of males can be observed in some species of nonpollinating fig wasps. It has been studied on many species from the genera *Sycoscapter* Saunders, 1883 (Pteromalidae), *Philotrypesis* Förster, 1878 (Pteromalidae) and *Idarnes* Walker, 1843 (Sycophaginae) and some other genera. It has been proven that species with large broods have only wingless males because there will always be a male to mate with females. Oppositely, species with small broods tend to have both winged and wingless males due to a female-biased sex ratio. Possibly, there would not be enough males in the syconium to mate with all the females. Wasps secure sexual partners for their daughters in form of winged males that search for females outside the syconia. This feature in fig wasp communities was shown to be convergent and has evolved at least five times. Winged males are the ancestors, sex dimorphism is more derived situation and winglessness is the most advanced. Winglessness occurs in all pollinator wasps, there are no dimorphic males (Cook et al., 1997).

13. Discussion and conclusion

According to published data, it is possible to conclude that figs and their associated wasps from superfamily Chalcidoidea undergo a common coevolution. It could be hypothesized that the symbiosis is interlacing all the species involved, e.g. figs, pollinators, galls and parasitoids. Generally, it was supposed (Silvieus et al., 2007) that pollinating wasps show closer cospeciation with the figs than nonpollinating fig wasps. However, some molecular analyses reject this inference. Therefore more experiments should be done to verify this trend in Agaonidae. The number of cryptic species has to be found out for more wasps. It was studied on three genera on two continents only (Molbo et al., 2003, Haine et al., 2006). It should be found out whether the community of cryptic species on one fig species underwent rather host shifts or duplications. I suppose that if rather host shifts occurred, the host specificity might be the same for pollinating and nonpollinating wasps.

Morphological coadaptations are the visible confirmation of the coevolution. Wasps evolved head shapes, mandibular appendages and corbiculae. Figs evolved different length of styles of female flowers. However, they possess the syconium shape because of nonpollinating fig wasps rather than pollinators. The adaptations have been well studied but there is still some equivocalness about the male body of Agaonidae. It is known that they have shortened midlegs. Compton (pers. comm.) assumes that it has evolved because four legs are better for walking in figs. I suppose that the other reason might be the energy allocation. Since it is enough to develop four legs for moving about, it would be wasting in terms of resources to invest in growth of all six. Other problem that it is not explored is how the males find their conspecific females in the galls. This would be interesting to study mainly in complexes of cryptic species.

It would also be challenging to review all the morphological, physiological and other characteristics of nonpollinating fig wasps. It is likely that many of them have evolved due to wasps interaction with figs. Jousselin et al. (2008) concludes that those characteristics might cause coevolution of nonpollinating wasps with their hosts at similar level as species of the family Agaonidae and the genus *Ficus*. The reproductive biology and sex ratio have been observed on pollinating fig wasps but a few studies have been carried out on those subjects in other families. It is known that sex ratio of nonpollinators is also biased towards females. It is known that there is male dimorphism in species with small clutch sizes. However, there are still some more features that need to be studied as the actual sex ratio in various species, the course of copulation, number of foundresses and rate of inbreeding. Generally, the behaviour

and ecology of fig wasps inside the syconium is much better described for pollinators than nonpollinators. It still is not known for some species of nonpollinating fig wasps, what they feed on and whether they are parasitoids or inquilines.

The hypotheses described in the chapter 5. Conflict of interests were studied mainly for monoecious figs. For a dioecious fig conflict only one hypothesis was proposed but it was not tested. That is why it is not included into the previous text. Dioecious figs have flowers in male syconia whose ovaries are all available to wasps due to the length of style. Since the male syconia flower more frequently than the female ones, the probability a wasp encounters fig with short styles is high (Ganeshiah et al., 1995). This seems to be the reason why there is not a strong selection pressure on the wasps to develop long ovipositors. It should be examined whether the theory works for dioecious figs.

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