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A preliminary study on the life history of fig wasp, *Eupristina verticillata* and its key role in the pollination of fig tree, *Ficus microcarpa*

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Abstract
Fig wasps are apparently irrelevant insects which have an inseparable and delicate relationship with fig trees as they both try to maintain a delicate balance for survival. The fig trees are dependent on fig wasps for growth, reproduction and the perpetuation of their species. These wasps have considerably adapted to the morphological changes shown by the fig fruits. Hence a preliminary study had been made to study the life history of the fig wasp, *Eupristina verticillata* and its key role in the pollination of fig tree, *Ficus microcarpa.* There exists a mutualism between fig wasps and fig trees and the behavioural aspects shown by the wasps are highly suited for their living inside the fig fruit bodies. These wasps are the ones which pollinate the fig trees and in turn the fig trees provide nutrition and shelter to these wasps. The ratio of number of females to males is in such a way that many fig tree species can be pollinated at a time. The males occupy about only 25% of the total population. They show remarkable reproductive patterns which offer an environment for the interplay between the fig and the fig wasps.

Keywords: *Eupristina verticillata, Ficus microcarpa,* behavioural aspects, reproductive patterns

1. Introduction
Hymenoptera, which comprise wasps, ants, and bees, are a significant group to study because of their importance as parasites and pollinators [1]. They can be characterized by their four membranous wings, complete metamorphosis, and leg, antennal, thoracic, and abdominal characteristics [2]. Hymenopterans are also important for balancing and functioning of most of the ecosystems in the planet and are also useful for the human economy. They are not only diverse in terms of structure, size and numbers of species, but also in their habits and life histories. Of all the insect orders, the order Hymenoptera includes the commonest, diverse, best known insects and perhaps the most important insects for mankind. They are the most evolved and probably most diverse of all the terrestrial organisms.

Hymenopterans have a complete life cycle, which varies slightly depending on the species. Some females can produce young without mating, while others can store sperm and spread out their egg laying to coincide with available food [3]. Most species will lay their eggs on the appropriate host plant or on paralysed food sources they have gathered into specially constructed nests. A variation of this occurs with social insects such as ants and bees, where special castes provide food for the developing larvae. The larvae will moult several times before they pupate. Development may range from a few weeks for some parasitoids, too much longer in social species.

A permanent association between flowering plants and insects is pollination. Pollination is a mutualism in which two interactions reciprocally benefit: a host plant receives the service of insect pollination in return for a reward provided for its insect pollinator [4]. Typically, the reward is nectar or pollen, but occasionally the provision can be a mating site, resin for nest construction, floral aroma, or even the attraction of plant-generated heat. The plants are visited by lots of pollinators and they differ from species to species. Each plant has a specific species of pollinator [5].

Figs are deciduous tree growing to a height of about 10-30 cm and spreading wider than their height. They often grow as multi-branched shrubs. Fig wood is weak and decays rapidly and the twigs are pithy rather than woody. The succulent trunk and branches are unusually sensitive to heat and sun damage, and should be whitewashed if particularly exposed. Roots are invasive and greedy, travelling far beyond the tree canopy [6]. The sap contains copious milky latex that is irritating to human skin.

The tree under study was *Ficus microcarpa*. This tree is native to India and also called as Indian laurel. They are also found in Southern China, Australia and New Caledonia. This tree is native to India and also called as Indian laurel. They are also found in Southern China, Australia and New Caledonia. *Ficus microcarpa* is grown in all habitats and on all major islands of the world. The outer covering of the fig is the syconium. The pore which will lead into the syconium is the ostiole. A fig fruit is derived from a specially adapted type of inflorescence. The fleshy, fruit like body commonly called the fruit technically is a specialized structure, or accessory fruit called a syconium: an involuted receptacle with many small flowers arranged on the inner surface. Thus, the flowers of the fruit are not are not seen unless the fruit is cut open. Hence the fig is also called "fruit without flower".

The syconium has a bulbous shape with a small opening called the ostiole at the apex that allows access to the pollinators. The flowers are pollinated by the tiny wasps that crawl through the opening in search of a suitable place to reproduce (lay eggs). Without this pollinator service, fig trees cannot reproduce by seed. In turn, the flowers provide a safe haven and nourishment for the next generation of wasps.

Ficus microcarpa is monoecious; both male and female flowers are present in the same individual. Inside each of the "fruits" of many of the monoecious species are three kinds of flowers: male, short female and a long female. Female fig wasps can reach the ovaries of the short female flowers with their ovipositors, it cannot reach the ovaries of the long female flowers [7]. Hence the short flowers grow wasps whereas the long flowers if pollinated grow into fruits which can be eaten. There are several commercial and ornamental varieties of fig that are self-fertile and don't require pollination; these varieties are not visited by fig wasps.

Fig trees exhibit remarkably varied reproductive patterns which provide backdrop for the complex, symbiotic interplay between figs and fig wasps [8]. The dependence of female flowers on pollinating services and the dependence of wasps on figs for nutrition and habitat tells us about a symbiotic relationship called obligate mutualism. Each species depends on the other for the survival in this biological world.

The female wasps are the ones which have an important role to fulfil as they are responsible for creating future generations of fig wasps and pollinating a new fig. They collect some of the pollen from the fig in their pouches or on their bodies after they mate. Once they smell the aroma from a fig ready to be pollinated, they leave and begin a new life cycle in the new fig.

The various adaptations that the wasps have undergone to live inside the fig fruit bodies is due to the evolution. This mutualism between fig and fig wasps is about 70 million year old. In due course of time, the wasps have started to adapt to live in the fig fruit. This mutualism is thought to as old as the earliest fossils of *Ficus* that date back to 50-90 million years ago [9].Over this vast span of evolutionary time it is thought that members from each of these groups have developed distinct fig and wasp traits via coadaptation [10].There is increasing support for the traditional view that figs and agaonids have highly correlated speciation histories. However, the famous one-to-one rule is often broken, suggesting that fig and pollinator speciation is not always tightly linked. Many fig-parasitic wasps might be as host-specific as the pollinators, but host shifts probably playing a superior role in their speciation. Comparative studies are beginning to discover how key traits have co-evolved as figs, pollinators and parasites have radiated, but the stability of the mutualism remains divisive and mechanisms might differ between lineages.

An introduction on the behavioural aspects of fig wasps would reveal that even though a lot of work has been done on other species of fig wasps, not much work has been done on *Eupristina verticillata*. Hence an attempt has been made to study the behavioural aspects of the wasp *Eupristina* which is associated with the fig species *Ficus microcarpa.*

2. Material and Methods

2.1 Collection of fig fruits

The fig trees were located in places in and around Coimbatore, Tamilnadu. About 200 syconia were collected at random from four individuals (50 syconia from each fig tree) between June-September 2015. The fruits were cut open to check for dead wasps inside the fruit. The syconia were separated in plastic flasks covered with a voile type cloth, and kept for 48 hours to collect all the wasps.

2.2 Dissection of fig fruit

Due to the high abundance of flowers per fig, the figs were dissected into 8 equal parts and one of them was randomly selected for examination each time. To minimize the error due to position effects, the figs were dissected longitudinally along the opening of the figs. For convenient identification, all flowers were scratched off. Number of flowers of different stages was counted under 20X magnification using a dissecting microscope. It was recorded and then multiplied by 8 to represent the total number of the whole figs. For the male figs, each gall flower is dissected to identify whether the gall flowers were parasitized or not and the parasitic species identified.

2.3 Observation of fig wasps

Two to three fruits were cut open and placed inside a jar and it was closed with a cloth so that wasps wouldn't escape. The insects were killed by freezing, and subsequently a solution of 70% alcohol was added to the flasks, where the specimens were kept with their respective syconia before sorting. The wasps that emerged from the syconia were sorted with the help of a dissection microscope. The dead female wasps were identified with the presence of ovipositor and wings.

3. Results

The life history of the fig wasps were observed and recorded. The figs are totally dependent on these minute wasps for pollination. The wasps are found inside the fig fruit. The female wasp enters into the fruit through an ostiolar opening and enters the syconium and lays its eggs with the help of its ovipositor. The males remain inside the fruit and the only job of the male is to mate and dig a hole for the female to escape so that the females can deposit the pollen into the fruit of another host fig. These female wasps are provided with concavities to carry pollen.

The life cycle of the wasp begins with the female entering into the fruit through the ostiole of the syconium. The ostiole is a small opening which is sensed by the female wasps with the help of antennae. The ostiolar passage is very narrow that the female loses its wings and its antennae in this process and is determined to remain inside the fruit.

A female pollinator wasp (1.2-1.8 mm in length) arrives at and immediately begins antennating a syconium. The wasp assumes a characteristic pose during this "assessment behavior." The legs are extended and the head and sternum are well clear of the syconium surface. The antennae are

arched up and the distal portion of the antennae contact the syconium surface perpendicularly, with only the distal antennal segment actually touching the syconium.

The female wasp lowers her antennae, and the distal five or six antennal segments are pressed flat against the syconium surface. The wasp is searching for the ostiole, which she recognizes as a lip on the smooth syconium surface. When she finds the ostiole she loosens the topmost scale with one of the sharp, sclerotized horns on the third antennal segment. These are the series of events that take place when the female wasp attempts entry. Then sSe makes her way past the male flowers that are not yet mature and have no pollen. She moves further into the hollow down to the female flowers and dusts them with the pollen that she brought with her from the fig in which she grew up.

The figs have three types of flowers: short female flowers, long female flowers and male flowers. The female wasps can reach only the ovary of the short female flower with its ovipositor and so she deposits her eggs there. The baby wasps are nurtured by the short female flowers. The long female flowers develop into seeds. The eggs hatch and the larvae develop rapidly. In a day or two after the pollination and oviposition had taken place the female dies inside the cavity of the syconium.

The male wasps are born first, and emerge looking nothing like a wasp: no eyes and no wings. Yet they are soon able to detect the baby females and mate with them. About 9 to 18 male wasps mate with 100 females. Before perishing, they perform one other duty – to enlarge the original entrance to a wider tunnel so that the females can exit without losing their wings. As the female wasps squeeze past to exit the fig, they are dusted with this pollen enabling them to carry it to another green fig, where they in turn will lay their eggs.

Before the wasp becomes an adult it has to undergo three stages-larval stage, inactive pupa and imago (adult). The wasp, immediately after coming out of the egg is the larval stage. They don't have well developed compound eyes, antennae. They actively move about inside the fruit during this stage. The next stage is the inactive pupal stage. After the wasp goes through this stage the male mates with the adult female. They remain dormant throughout this period. When the wasps reach the adult stage, they develop wings, antennae and legs. They fly through the hole dug by the male in search of another fig tree to deposit and for the purpose of oviposition. After 2 or 3 days the male wasp present inside the fruit dies.

The fig fruit bodies were observed closely and it showed lot of adaptations and it also had the necessary conditions so that the wasps could survive inside the fruit bodies. When the eggs were laid by the wasps, the fig had special chemical secreted with which it can identify that the eggs had been laid and it had to nourish the eggs and baby wasps. Hence the food is provided by the endosperm for nourishment. The narrow pathway of the ostiole also illustrates a special adaptation. If the passage was big, other insects would also start entering and parasitise the tree. So, the nature has created in such a way that only wasps should enter.

The wasps also show lot of adaptations to live and survive inside the fig fruit bodies. The wasps also have to change with the changing world and adapt itself to the surroundings. In that case, the male wasps have very short limbs or no limbs at all, as they have to move inside the fruit. If the males have had wings and long limbs it would have been difficult for the wasps to move inside the fruit.

The females are provided with compound eyes, long antennae and long limbs. The antennae are multi-segmented and has sense organs on it, which is used to sense the ostiolar opening and to move through the inside the fruit. Whereas the males have reduced antennae as they have no other job other than to mate and dig a way for the females to escape.

The preserved wasps were taken and dissected. The separation of ovipositor from the rest of the body of the fig was a bit difficult to dissect and hence scissors was used for dissection. This shows that the ovipositor was hard as steel, so when the wasps attempt to lay eggs inside the ovary of the short flower, it may damage the style canal and further laying of the eggs becomes difficult.

When the wasps came out of the fruit, it couldn't live for a long time. This shows that the food was provided by the endosperm of the fig. hence the wasps are totally dependent on the fig for food and shelter. Both ripe and unripe fruits were taken for counting the number of wasp present inside it. The ripe fruits had more wasps than the unripe ones. This shows that the wasps grow along with the fig fruits. When the fig ripened the wasps also grew along with it, and became adults. In unripe fruits the number of wasps per fruit was observed to be between 5-6 in number. But the presence of wasps varied from fruit to fruit and in ripe fig fruits, the ratio of wasps were observed to be in the ratio 100:120 (Tab-1, Fig-1).

The ratio of female wasps was more when compared to the male wasps. There were very less number of males. This shows that one male mates with many females. Hence about 9 to 18 males mate with 100 females. The size of wasps were measured and a comparison was made. The male wasp was smaller than the female wasp. This adaptation is acquired by the males so that they can move easily inside the fruit. If it is going to be big in size, the movement becomes difficult.

4. Discussion

Mutualistic interactions between plants and insects are of particular interest because the exchange of resources involved attracts a great variety of other organisms that benefit from the relationship [11]. A classic example is the relationship between the 750 species of the genus *Ficus* (Moraceae) and its pollinating wasps. The figs (syconia) are pollinated exclusively by specific wasps from the family Agaonidae (Chalcidoidea), which in turn reproduce by laying eggs in the fig's flowers (by preference the longer-styled flowers), where the larvae feed and develop [12]. This mutualism is exploited by a number of other parasitic wasps - denominated non-pollinating wasps and by innumerable species of ants, Homoptera, Coleoptera, Lepidoptera and Diptera [13].

A similar study on fig pollinators done by K.J. Joseph [14] on the reproductive strategies of fig wasps showed that the males and females show sexual dimorphism externally. The male wasps were vermiform, apterous and pale-coloured. Their chitinized integument, strongly developed prothorax, well developed anterior and posterior legs and small middle legs are meant for easy locomotion inside the cavity of the fig in order to search for the fig ovaries containing their females for copulating with them. The peculiar telescopic arrangement of the seventh, eighth and ninth abdominal segments is a strategy for facilitating their function during copulation.

The females of the Agonidae are fully winged and the penetration through the ostiolar canal is an arduous task for them for which they are fully adapted. The peculiar wedge shaped head with a soft and deformable cranium is an adaptation in these wasps force themselves through the ostiolar canal. The interaction between *Ficus microcarpa* and

the fig wasp *Odontofro gattiagalili* has been studied by Galil and Copland [15]. Although there is sexual dimorphism in Odontofrogattia, it is not well marked as shown in other aganoid wasps. The males of these species are fully winged and free living unlike the larviform, apterous, almost blind males of other Agonidae and other torymid fig wasps.

A study was conducted in the Everglades National Park during June 1981 regarding the entry of the female wasps inside the fig fruit bodies. According to Steven A. Frank, the wasps' entry into the syconia is a fixed sequence of behavioral events. A female pollinator wasp (1.2-1.8 mm in length) arrives at and immediately begins antennating a syconium. The wasp assumes a characteristic pose during this "assessment behavior [16].

A research done by Stephen G. Compton on undescribed species of the genus *Watshamiella* with a characteristic black transverse bar on the dorsal surface of the thorax and black bars across the gaster.. The female *Watshamiella* were active on the surface of the figs, paying close attention to any *Apocrypta longitarsus* females that were in the process of ovipositing through the fig wall. *Watshamiella* were active on the surface of the figs, paying close attention to any *A. longitarsus* females that were in the process of ovipositing through the fig wall. As soon the wasp species finished ovipositing, the species *Watshamiella* came in search of the pore through which it had oviposited before [17].

Therefore the species Watshamiella, an African species oviposits through the hole drilled by the another species *Apocrypta longitarsus.* A research by James M. Cook on fig wasp mutualism "the famous one-to-one rule is often broken, suggesting that fig and pollinator speciation is not always tightly linked. Many fig-parasitic wasps might be as host-specific as the pollinators, but host (and niche) shifts probably play a greater role in their speciation [18].

According to JT Wiebes, "When the syconium are too ripe the female fig wasp have emrged already and the wingless males have been dragged away by ants. No wasp can propagate its kind outside of figs and no fig can thrive where there are no figs. The figs are dependent on these *hymenopterous* agonidae which are the sole agents for the pollination of the flowers hidden in their urn- shaped inflorescence. The ecology of fig wasp is for the greater part of flower ecology of figs and there is but one biogeography for both [19].

In the present study, it was hence proved that the fig wasps are the ones which pollinate the fig trees. There exists a mutualism between these two species. The behavioural aspects and the adaptations shown by the wasps are highly suited for their living inside the fig fruit bodies. The male wasps have very short limbs or no limbs at all, as they have to move inside the fruit. If the males have had wings and long limbs it would have been difficult for the wasps to move inside the fruit.

The females are provided with compound eyes, long antennae and long limbs. The antennae are multi-segmented and has sense organs on it, which is used to sense the ostiolar opening and to move through the inside the fruit. Whereas the males have reduced antennae as they have no other job other than to mate and dig a way for the females to escape.The ratio of number of females to males is in such a way that many fig tree species can be pollinated at a time. The ratio of female wasps were more when compared to the male wasps. There were very less number of males. This shows that one male mated with many females and about 9 to 18 males must have mated with 100 females.

Table 1: Total number of fig wasps collected from the fig tree, *Ficus microcarpa*

Dissected fig fruit	No: of wasps in unripe fruit	No: of wasps in ripe fruit	No: of females per Fig fruit	No: of males per fig fruit
Fruit I	8	120	100	20
Fruit II	5	114	102	12
Fruit III	7	110	100	10
Fruit IV	9	129	104	25
Fruit V	10	135	102	30

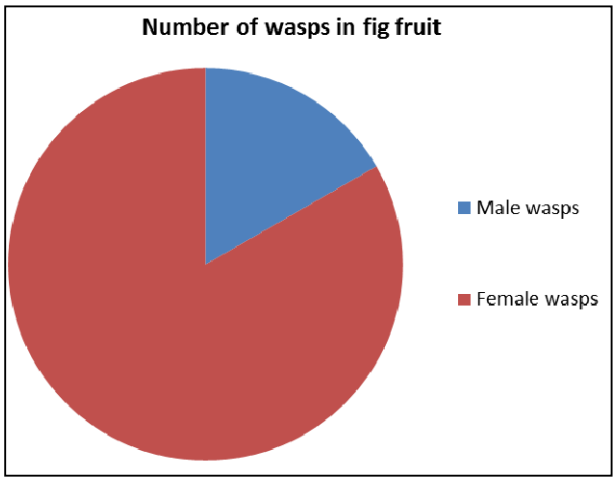

Fig 1: Number of wasps in fig fruit

Plate 1: Fig tree

Plate 2: Fig fruit cut open

Plate 3: Fig wasps

5. Conclusion

The findings during the present investigations helped to evaluate the life history of fig wasp, E*upristina verticillata* and its interaction with the fig tree. The present findings are illuminatory and emphasize the relationship between the fig wasp and the fig tree that had occurred for than a million years and it has led to co-speciation. Thus the research on fig wasps is incessant since there are more than 750 species of fig wasps are found all around the world [20].

6. References

1. Kjellberg F, Jousselin E, Bronstein JL, Patel A, Yokoyama J, Rasplus JY. Pollination Mode in Fig Wasps: The Predictive Power of Correlated Traits. Proceedings: Biological Sciences. 2001; 268(1472):1113-1121.
2. Price PW. Reproductive strategies in parasitoid wasps. American Naturalist. 1973b; 107:684-693.
3. Price PW. Reproductive strategies of parasitoids. Evolutionary Strategies of Parasitoids (ed. by P. W. Price). Plenum, New York, New York. 1975; 87-111.
4. Ma WJ, Yang DR, Peng YQ. Disturbance effects on community structure of *Ficus tinctoria* fig wasps in Xishuangbanna, China: implications for the fig/fig wasp mutualism. Insect Science. 2009; 16:417-424.
5. Proffit M, Schatz B, Borges RM, Hossaert-McKey, M. Chemical mediation and niche partitioning in non-pollinating figwasp communities. Journal of Animal Ecology. 2007; 76:296-303.
6. Compton SG, Rasplus J Y, Ware AB. African fig wasp parasitoid communities. In B. Hawkins and W. Sheehan (Eds.), Parasitoid community ecology. Oxford University Press, 1994, 343-368.
7. Hui Wen Yang, Hsy-Yu Tzeng, Lien Siong Chou, Yang. Botanical studies, Phenology and pollinating wasps dynamics of *Ficus microcarpa* L. f: Adaptation of seasonality. 2013; 54(11):1-11.
8. Martine Hossaert-McKey, Jorg Ernst Frey, Marc Gibernau. Chemosensory attraction of fig wasps to substance produced by Receptive figs, Entomologiae Experimentalis. 1994; 70:185-191.
9. Ripu M Kunwar, Rainer W Bussmann. Fig species in Nepal, A review of Diversity and Indigenous uses, Lyonia: A journal of ecology and application. 2006; 11(1):85-97.
10. William Ramirez B. Evolution of mandibular appendages in fig wasps (Hymenoptera: Agonidae), Revista De Biologia Tropical. 1991; 39(1):87-91.
11. Joseph KJ. The reproductive strategies in fig wasp (Chalcidoidea: Hymenoptera), Indian National Science Academy, University of Calicut, Kerala. 1984, 449-460,
12. William Ramirez B, Jollyanna Malavasi. Fig wasps: Mechanism of pollen transfer in malvanthera and Pharmacosycea fig (Moraceae), Revista De Biologia Tropical. 1997; 45(4):1635-1640.
13. Pereira RAS, Semir J, Menezes Jr, AOB. Pollination and other biotic interactions in figs of Ficus eximia Schott (Moraceae). Brazilian Journal of Botany. 2000; 23:217-24.
14. Joseph KJ. The reproductive strategies in fig wasp (Chalcidoidea: Hymenoptera), Indian National Science Academy, University of Calicut, Kerala. 1984, 449-460.
15. Galil J, Eisikowitch D. Further studies on the pollination ecology of *Ficus sycomorus* L. Tijdschr. Enlomol. 1969; 112:1-13.
16. Steven A Frank. The Behaviour Morphology of the Fig wasp: Descriptions and suggested behaviour characters for phylogenetic studies, Psyche, University of Florida. 1984; 91:289-308.
17. Stephen G Compton, Simon Van Noort, Michael Meleish, Mark Deeble, Victoria Stone. Sneaky African Fig wasp that oviposits through holes drilled by other species, African Natural History. 2009; 5:9-15.
18. Cook JM, Rasplus JY. Mutualists with attitude: coevolving fig wasps and figs. Trends in Ecology & Evolution, 2003; 18:241-248.
19. Wiebes JT. Co-evolution of figs and their insect pollinators, Annual reviews of Ecology and systematics, Leiden University, Netherlands. 1979; 10:1-12,
20. Segar TS, Derek WD, Clive TD, James MC. How to be a fig wasp down under: The diversity and structure of an Australian fig wasp community. Acta Oecologica. 2013; 57:17-27