Ovipositor Length of Three *Apocrypta* Species: Effect on Oviposition Behavior and Correlation with Syconial Thickness

Wen-Quan Zhen,[1,2,3] Da-Wei Huang,*[1,4] Jin-Hua Xiao,[1,3] Da-Rong Yang,[2]
Chao-Dong Zhu[1] and Hui Xiao[1]

We investigated oviposition behavior in three non-pollinating fig wasps: the sympatric species *Apocrypta bakeri* Joseph on *Ficus hispida* L., *A. westwoodi* Grandi on *F. racemosa* L., and *Apocrypta* sp. on *F. semicordata* Buch.-Ham. The oviposition behavior differs significantly between one pair of species (*A. bakeri* and *A. westwoodi*) and the other species (*Apocrypta* sp. on *F. semicordata*). *A. bakeri* and *A. westwoodi* were similar in the following aspects: the posture of the abdomen and the action of the hind legs before penetration, and the bending ovipositor sheath during penetration. In contrast, the oviposition behavior of *Apocrypta* sp. is quite different. This difference can be explained by the significant correlation between ovipositor length and syconial thickness. *Apocrypta* sp. has a shorter ovipositor than the two other species, which correlates with the thinner syconial wall of its host fig *F. semicordata*. It is deduced that the ovipositor length adapts to the syconial thickness and induces the oviposition behavior in the different species to diverge. For all three *Apocrypta* species, the midleg length and hindleg length are significantly correlated with their ovipositor lengths. This may be explained as due to the fact that body movement adjusting the hindlegs and midlegs up and down, or forward and backward, is also influenced by the ovipositor length.

KEY WORDS: Oviposition behavior; adaptation; *Apocrypta*; non-pollinating fig wasp.

INTRODUCTION

The obligate mutualism between figs and their species-specific pollinating fig wasps has long been considered as a classical case of co-evolution and co-speciation, in which the reproduction of the partners must be closely dependent on each other. Most fig species have their own unique pollinating wasp species, whose larvae feed on the fig ovules (7), although some figs have more than one pollinator (10). Many morphological characters of the partners are thought to be co-adapted (11,14,15). The biology of figs and their pollinators has led to a general idea that they have co-evolved extensively. Besides the pollinating fig wasps, several species of non-pollinating fig wasps (NPFWs) usually co-exist on one fig. Those NPFWs should not be artificially isolated from the mutualistic

---

Received March 9, 2004; accepted Nov. 22, 2004; http://www.phytoparasitica.org posting March 4, 2005.

[1] Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China. *Corresponding author [Fax: +86-10-62559639; e-mail: huangdw@ioz.ac.cn].
[2] Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan 666303, China.
[3] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China.
[4] Plant Protection College, Shandong Agricultural University, Tai'an, Shandong 271018, China.

relations occurring in a field, acting as gall makers, or parasites, or inquilines. They often play very important roles in the fig/fig wasp system (8,17).

Fig wasps (pollinators and non-pollinators) oviposit in figs by one of two approaches. One approach is that the female wasps enter the syconium through the ostiole; this includes pollinators and the Sycoecinae (Agaonidae) wasps (14). The other approach is that the female NPFWs penetrate the syconial wall from outside with their ovipositors and lay eggs. These NPFWs belong to subfamilies other than Sycoecinae and Agaoninae within the Agaonidae, and include such species as *Philotrypesis pilosa* (1), *Apocryptophagus* (*Idarnes*) *testacea* (3) and *Apocrypta westwoodi* Grandi (3), etc.

Wasps in the genus *Apocrypta* Coquerel (Sycoryctinae), characterized by bizarre telescopic proximal gastral segments to stabilize the long ovipositor, are parasitoids of *Ceratosolen* spp. that pollinate *Sycomorus, Sycocarpus* and *Neomorphe* sections of *Ficus* (13). *Apocrypta bakeri* Joseph is a parasitoid of *Ceratosolen solmsi* Mayr, the pollinator of *Ficus hispida* L. (2). *Apocrypta* spp. wasps may be parasitoids of *Ceratosolen* or gall-makers in the tribes of Sycophagini (5). After the fig pollinators enter the receptive syconia, the female *Apocrypta* lays eggs into syconia by penetrating the fig wall with long ovipositors. The oviposition behavior of *A. westwoodi* (in *F. racemosa* L.) (3) and of *A. bakeri* (2) has been described. The oviposition behavior of *Apocrypta* sp. was observed and the mechanism of the sterna shifting during oviposition has been discussed (13).

Correlations between behavioral and morphological characteristics in the wasps and morphological characteristics in the figs are often striking (9,16,17). There is much variability in fig inflorescence morphology, with concomitant variability in fig wasp morphology (11) and behavior (6). The morphology of female pollinators reflects the adaptation of entering the fig through the ostiole (15); females have smooth bodies and often some modifications that prevent them from slipping back out when squeezing into the fig cavity through the ostiole. Male morphological characters reflect respiration adaptation in figs for both pollinators and NPFWs (4). The NPFW females laying eggs from outside the fig wall often have conspicuously long ovipositors and are apt to attack the pollinated syconia.

Focusing on the three species of *Apocrypta*, we attempt to analyze the correlation between the oviposition behavior and morphological characters relating to oviposition, and to speculate on the divergence in behavior and morphology.

MATERIALS AND METHODS

Study site The study was conducted in the Xishuangbanna Tropical Botanical Garden (21°41'N, 101°25'E), Chinese Academy of Sciences, Yunnan province, China.

Fig wasps and figs Although cryptic fig pollinator species are common (11), we assert that the three studied *Apocrypta* species are morphologically and behaviorally different.

Ficus racemosa L. (section *Sycomorus*, subgenus *Sycomorus*) is a monoecious fig, actively pollinated by *Ceratosolen fusciceps*. The trees are common in villages and along streamlets. Three or four crops are harvested a year in south China. The fruits hang down on boughs. *Apocrypta westwoodi* is a parasitoid of *C. fusciceps*.

Ficus hispida L. (section *Sycocarpa*, subgenus *Ficus*) is a dioecious fig, actively pollinated by *Ceratosolen solmsi*. It occurs in forest gaps and waste places. Many twigs with fig fruit hang down from boughs. *Apocrypta bakeri* is a parasitoid of *C. solmsi*.

Ficus semicordata Buch.-Ham. ex J. E. Sm. (section *Sycidium*, subgenus *Ficus*) is also a dioecious fig, actively pollinated by *Ceratosolen gravelyi*. The trees grow in fields and along roadsides, and long and slender twigs with fruits hang down near to the ground. *Apocrypta* sp. is a parasitoid of *C. gravelyi*.

The figs hosting *Apocrypta* spp. wasps were obtained in fields and taken to the laboratory for dissection and measurements. When *Apocrypta* spp. oviposited, the thickness of the syconial wall was measured from fig surface to the interface of the ovary layer with a digital caliper, to the nearest 0.01 mm.

Fig wasp morphological measurement Adults were obtained by placing figs in transparent plastic cups covered with a fine mesh screen. Dead adults were removed from the cups, mounted onto cards, or put into 75% alcohol solution. Dissection and measurements were done under a Leica (MZ APO) stereomicroscope with an ocular micrometer, to the nearest 0.02 mm. Voucher specimens were deposited in the Insect Collection of the Institute of Zoology, Chinese Academy of Sciences, Beijing.

Thorax length was considered to be the distance from the front margin of the pronotum to the hind margin of the propodeum; thorax height was considered as the maximum in lateral view; abdomen length was from the front margin of the first tergite to the basal part of the last tergite, not counting the unusually narrow and tubular part; abdomen height was regarded as the maximum in lateral view; leg length was the sum of the length of the parts of the leg. Sample size was ten.

Fig wasp behavior All photos were taken *in situ* using a digital camera Nikon Coolpix 995. All together 355 individuals of *A. westwoodi*, 291 of *A. bakeri*, and 211 of *Apocrypta* sp. were observed during oviposition.

Statistical analysis SAS software was used for statistics (12). For each of the three *Apocrypta* spp., we analyzed means and standard error for all measured variables. The t-test was performed for both comparison of ovipositor length and comparison of fig wall thicknesses between *A. westwoodi* and *A. bakeri*. CORR was performed for ovipositor length and several other morphological data, with Pearson's test. GLM was performed for multiple comparisons of morphological characters among each of the measured items of a fig wasp, and the syconial wall thickness, with Tukey's Studentized Range (HSD) Test. The syconial thicknesses among three fig fruit species, and the ovipositor lengths of three *Apocrypta* spp. wasps, were analyzed and plotted by the cluster procedure of STATISTICA, with an option of unweighted pair-group average. Then two cluster trees were put together artificially.

RESULTS

Fig wasps and figs In 2002 we measured the syconial wall thickness of three fig species oviposited by *Apocrypta* spp. The thickness of *F. racemosa* fruit is on average 4.97 mm (4.01–5.23 mm, n=235). The thickness of *F. hispida* fruit is ~4.54 mm (4.00–5.18 mm, n=176). However, the thickness of *F. semicordata* fruit averages 1.88 mm (1.76–2.23 mm, n=143). Five fig samples for each fig were selected in 2003, to ensure 10% error around mean thickness of fig wall measured in 2002.

Fig wasp morphological measurements Our preliminary investigation showed that the correlation between fig wasp ovipositor stylets and ovipositor sheaths was highly significant in the three *Apocrypta* species: *A. bakeri*, *A. westwoodi*, and *Apocrypta* sp., being 0.98

($P<0.0001$), 0.99 ($P<0.0001$) and 0.96 ($P<0.0001$), respectively. Therefore, we use the length of ovipositor sheaths instead of ovipositor length for ease of measurement.

TABLE 1. Comparison of three *Apocrypta* species' ovipositor length and fig wall thickness

		A. westwoodi		*A. bakeri*	*Apocrypta* sp.
	n	Mean±S.E.		Mean±S.E.	Mean±S.E.
Ovipositor length	10	5.45±0.19		5.20±0.05	2.63±0.06
Thickness of fig wall	5	5.20±0.25		4.90±0.23	1.88±0.02
$t^1{}_{(9,9)}$			-1.27		
$t^2{}_{(4,4)}$			-1.35		
$F^1{}_{(2,27)}$				167.50	
$F_{2(2,12)}$				189.53	

A t-test was performed for both ovipositor length comparison (between *A. westwoodi* and *A. bakeri*) and the comparison of their host fig wall thickness. $t^1{}_{(9,9)}$ for ovipositor length, $P>0.22$; and $t^2{}_{(4,4)}$ for fig wall thickness, $P>0.21$. GLM was performed for both ovipositor length comparison (among *A. westwoodi*, *A. bakeri* and *Apocrypta* sp.) and their host fig wall thickness comparison, with Tukey's Studentized Range test. $F^1{}_{(2,27)}$ for ovipositor length, $P<0.0001$, and $F^2{}_{(2,12)}$ for fig wall thickness, $P<0.0001$.

TABLE 2. Correlation of *Apocrypta* species' ovipositor length *vs* measured variables

Species		Mesosoma length	Mesosoma width	Metasoma length	Metasoma width	Foreleg length	Midleg length	Hindleg length
A. bakeri	mean	0.3503	0.4417	0.5094	0.1660	0.4186	0.9160	0.7929
	P	0.3211	0.2012	0.1326	0.6467	0.2286	0.0002	0.0062
A. westwoodi	mean	0.6657	0.4220	1.1586	0.8414	1.4682	2.1106	2.4377
	P	0.0055	0.0798	0.2655	0.4422	0.0125	0.0008	0.0020
Apocrypta sp.	mean	0.6507	0.5086	0.6323	0.3556	0.6628	0.8336	0.7432
	P	0.0416	0.1333	0.0498	0.3132	0.0367	0.0027	0.0138

P is probability of Pearson correlation coefficient of ovipositor length with respective mean. Ten fig wasps of each *Apocrypta* species were dissected and measured.

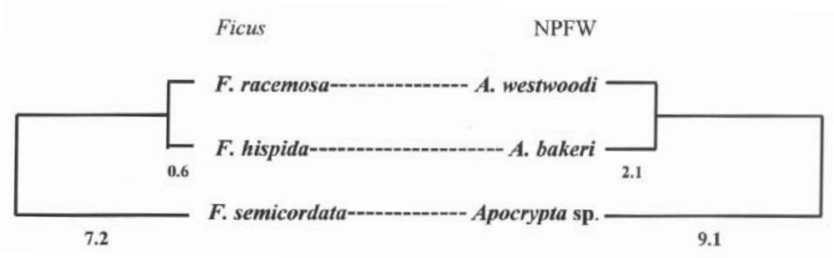

Fig. 1. The cluster analysis tree comparison between *Ficus* species and *Apocrypta* species (linkage distances are marked under branches).

Analysis of figs and fig wasp morphologies There was no significant difference between ovipositor lengths of *A. westwoodi* and *A. bakeri* ($P>0.22$), or between thicknesses of the fig walls of *F. racemosa* and *F. hispida* ($P>0.21$). However, the ovipositor length of

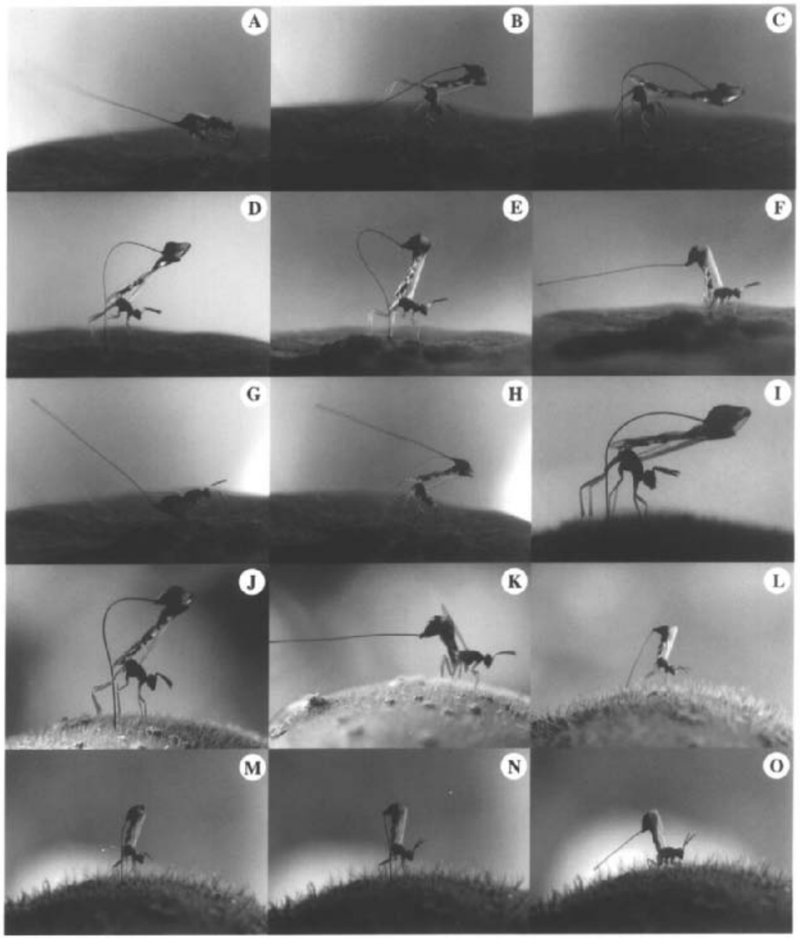

Fig. 2. Three *Apocrypta* wasps oviposit in their own figs. **A** to **H** show the process of oviposition behavior of *A. westwoodi* on *Ficus racemosa*. **I** to **K** show the key postures of *A. bakeri* on *F. hispida*. **L** to **O** show the different postures of *Apocrypta* wasp on *F. semicordata*.

Apocrypta sp. ($F_{(2,27)}$=167.50, $P<0.001$) and syconial wall thickness of *F. semicordata* ($F_{(2,12)}$=189.53, $P<0.001$) are significantly different from the former (Table 1).

The correlation between the ovipositor length and the wasps' other morphological characters is analyzed (Table 2). For all three *Apocrypta* species, the midleg length and hindleg length are significantly correlated with their ovipositor lengths, but the mesosoma height and the metasoma height are not. The foreleg length of *A. bakeri* is not correlated with the length of the ovipositor, nor is its mesosoma length. The metasoma length of *A. bakeri* and *A. westwoodi* are not correlated with their ovipositor lengths, but, in contrast, that of *Apocrypta* sp. is.

Behavior observations The process of oviposition of *Apocrypta* spp. can be divided into three different successive phases: searching for a receptive syconium, penetrating the syconium, and oviposition and withdrawal of the ovipositor (Fig. 2A-H). The female wasps often fly from one syconium to another in the search for a suitable host in which to lay eggs. When finding a suitable syconium, the female lifts her abdomen up and then forward and draws the ovipositor tip down to the syconial surface (Fig. 2C, I, M), and then starts to penetrate. After oviposition, she withdraws her ovipositor stylets from the syconium.

Although the processes of oviposition were similar for each species studied, there were obvious differences among them.

(i) Posture of the abdomen prior to the penetration: *A. westwoodi* and *A. bakeri* lift the abdomen upward and forward beyond the head, leaving the abdomen parallel with the syconium surface (Fig. 2C, I). However, *Apocrypta* sp. on *F. semicordata* lifts the abdomen up at a right angle to the thorax, so that it never turns forward and beyond the head (Fig. 2L-M).

(ii) Action of the hind legs before penetration: *A. westwoodi* and *A. bakeri* free the hind legs to draw the ovipositor sheath down to the syconial surface (Fig. 2B); in contrast, *Apocrypta* sp. always stands on the fig surface on all its legs (Fig. 2L).

(iii) Angle of the ovipositor during penetration: *A. westwoodi* and *A. bakeri* strongly bend the ovipositor in a distinct arc (Fig. 2C-E, I-J), but *Apocrypta* sp. pierces straight into the fig wall (Fig. 2M).

(iv) Duration of ovipositor penetration: for *A. westwoodi* it is $\sim$40 min (range, 25–55 min, n=45) and for *A. bakeri* it is 38 min (25–51 min, n=55), whereas for *Apocrypta* sp. it is $\sim$12 min (6–23 min, n=36).

DISCUSSION

Two simple cluster analyses were performed on both ovipositor lengths of *Apocrypta* wasps and syconial wall thicknesses of their fig hosts. The two cluster trees were artificially combined into Figure 1, which shows that the ovipositor lengths of the three *Apocrypta* wasps are correlate with the syconial wall thicknesses of their respective host species. In reference to *Ficus*, the syconial wall thickness of *F. racemosa* is similar to that of *F. hispida*, forming a clade. However, the syconial wall thickness of *F. semicordata* is significantly thinner than, and separated from the two others. In reference to the fig wasps, the ovipositor lengths of *A. westwoodi* and *A. bakeri* are almost the same, forming a clade. However, the ovipositor length of *Apocrypta* sp. on *F. semicordata* is significantly shorter than that of the two others, so that it stands alone in a clade.

For these three *Apocrypta* spp., oviposition behavior is divided significantly into two groups, one for *A. bakeri* and *A. westwoodi*, and the other for *Apocrypta* sp. (Fig. 1). The two in the first group show similarity in the following aspects: the posture of the abdomen and the action of the hind legs before penetration and the ovipositor bending during penetration. In contrast, *Apocrypta* sp. behaves in quite a different way. These observations coincide with the cluster analysis in Figure 1, which can be interpreted by the correlation between the ovipositor length and syconial thickness. We can deduce that the ovipositor length adapts to the syconial thickness and induces a divergence in the oviposition behavior. A comparative study of the oviposition timing in fig wasps associated with section *Sycomorus* (*F. sur* and *F. vallis-choudae*) (8) suggested that there was a close and convincing relationship between the ovipositor length and the fig size those fig wasps had selected.

For all three *Apocrypta* species, the midleg length and hindleg length are significantly correlated with their ovipositor lengths. The movement of the abdomen in preparation for penetrating the fig is affected by the ovipositor length and the length of the hind- and midlegs. Legs may co-evolve with the ovipositor.

The genus *Ficus* has hundreds of species, with syconia differing greatly in shape, size and inner structure. The correlation between the ovipositor length and the syconial thickness implies adaptation among them. The thickened syconial wall is a physical barrier to prevent the NPFWs from laying eggs inside the fig. The NPFWs have to develop a long enough ovipositor to reach the fig ovaries. The thicker the syconial wall, the longer the wasp's ovipositor. Ovipositor lengths of *Sycoscapter* species were strongly correlated with the thickness of fig wall (16). The fig species with thin syconial walls have the ovaries close to the syconial surface, so that the ovaries can be reached by a short ovipositor through the fig wall, such as by *Apocrypta* sp. on *F. semicordata*. However, the fig wasps ovipositing through a thick fig wall have to develop a long ovipositor, as done by *A. westwoodi* and *A. bakeri*. As a result, the various ovipositor lengths lead to diverse oviposition behavior.

According to our present knowledge, the *Apocrypta* wasps are fig species-specific, which may explain their co-existence. The wasp with a long ovipositor, such as *A. westwoodi*, might lay its eggs in figs with thinner syconial walls, as in *F. semicordata*. However, this does not happen because the wasp is host-fig specific and its larvae cannot survive in the fig of *F. semicordata*. It can survive only in its natural host fig, *F. racemosa*. On the other hand, *Apocrypta* sp. on *F. semicordata* can not oviposit through the thicker syconial wall as *F. racemosa* and *F. hispida* do, because its ovipositor length is shorter than the thickness of the wall. Therefore, there is no opportunity to lay its eggs into those syconia. We have provided one explanation for the co-existence of these three species of *Apocrypta*. More evidence of genetic and ecological importance is needed to strengthen or refute this explanation.

ACKNOWLEDGMENTS

This project was supported by the National Natural Science Foundation of China (NSFC grant no. 30330090), and in part also by the CAS Innovation Program (KSCX2-SW-105) and the National Science Fund for Fostering Talents in Basic Research (NSFC-J0030092). We would like to thank Dr. Jean-Yves Rasplus, Centre de Biologie et de Gestion des Populations, Montferrier-sur-Lez, France, for identification of fig wasps. Prof. Zachary Huang and Mr. Ostein Berg checked the manuscript. Our special appreciation is expressed to the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, for hosting the first and second authors and enabling them to carry out the field investigation.

REFERENCES

1. Abdurahiman, U.C. (1980) Observations on the oviposition behaviour in *Philotrypesis pilosa* Mayr (Torymidae: Hymenoptera). *Proc. Symp. Environmental Biology* (Trivandrum, India), pp. 146-150.
2. Abdurahiman, U.C. and Joseph, K.J. (1979) Observation on the oviposition behaviour in *Apocrypta bakeri* Joseph (Torymidae: Hymenoptera). *J. Bombay Nat. Hist. Soc.* 76:219-223.
3. Ansari, M.H. (1967) The process of egg laying in Idarninae (Chalcidoidea: Hymenoptera). *Indian J. Entomol.* 29:380-384.
4. Compton, S.G. and McLaren, F.A.C. (1989) Respiratory adaptations in some male fig wasps. *Proc. K. Ned. Akad. Wet* 92:57-71.
5. Galil, J. and Eisikowitch, D. (1968) On the pollination ecology of *Ficus sycomorus* in East Africa. *Ecology* 49:259-269.
6. Galil, J. and Meiri, L. (1981) Number and structure of anthers in fig syconia in relation to behaviour of the pollen vectors. *New Phytol*. 88:83-87.
7. Jousselin, E. and Kjellberg, F. (2001) The functional implications of active and passive pollination in dioecious figs. *Ecol. Lett*. 4:151-158.
8. Kerdelhue, C. and Rasplus, J.-Y. (1996) Non-pollinating Afrotropical fig wasps affect the fig-pollinator mutualism in *Ficus* within the subgenus *Sycomorus. Oikos* 75:3-14.

9. Machado, C.A., Jousselin, E., Kjellberg, F., Compton, S.G. and Herre, E.A. (2001) Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proc. R. Soc. Lond. B. Biol. Sci.* 268:685-694.
10. Molbo, D., Machado, C.A., Sevenster, J.G., Keller, L. and Herre, E.A. (2003) Cryptic species of fig-pollinating wasps: implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. *Proc. Nat. Acad. Sci. U.S.A.* 100:5867-5872.
11. Ramirez, B.W. (1974) Coevolution of *Ficus* and Agaonidae. *Ann. Mo. Bot. Gard.* 61:770-780.
12. SAS (1992) SAS User's Guide. SAS Institute Inc., Cary, NC, USA.
13. Ulenberg, S.A. (1985) The Systematics of the Fig Wasp Parasites of the Genus *Apocrypta* Coquerel. North-Holland Publishing Company, Amsterdam, the Netherlands.
14. van Noort, S. and Compton, S.G. (1996) Convergent evolution of agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. *J. Biogeogr.* 23: 415-424.
15. Weiblen, G.D. (2002) How to be a fig wasp. *Annu. Rev. Entomol.* 47:299-330.
16. Weiblen, G.D. (2004) Correlated evolution in fig pollination. *Syst. Biol.* 53:128-139.
17. West, S.A., Herre, E.A., Windsor, D.M. and Green, P.R.S. (1996) The ecology and evolution of the New World non-pollinating fig wasp communities. *J. Biogeogr.* 23:447-458.