BIOTROPICA 41(6): 717–720 2009 10.1111/j.1744-7429.2009.00536.x

Different Stimuli Reduce Attraction to Pollinators in Male and Female Figs in the Dioecious Fig *Ficus hispida*

Hao-Yuan Hu[1,2,3], **Zi-Feng Jiang**[4], **Li-Ming Niu**[1,2,5], **Yue-Guan Fu**[5], **Zheng-Qiang Peng**[5], and **Da-Wei Huang**[1,6,7]

[1] Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

[2] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

[3] Key Laboratory of Biotic Environment and Ecological Safety in Anhui Province, College of Life Sciences, Anhui Normal University, Wuhu 241000, Anhui, China

[4] Department of Biology, University of Maryland, College Park, MD 20742, U.S.A.

[5] Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, Hainan, China

[6] College of Plant Protection, Shandong Agricultural University, Tai'an 271018, Shandong, China

ABSTRACT

Fig trees (*Ficus*) and their obligate pollinating wasps (Hymenoptera, Chalcidoidea, Agaonidae) are a classic example of a coevolved mutualism. Pollinating wasps are attracted to figs only when figs are receptive. It has been shown that figs will lose their attraction to pollinators sooner in monoecious and male dioecious figs when multiple pollinators have entered the enclosed inflorescence. However, little is known about the nature of the stimulus inducing the loss of attraction. By conducting experiments on the functionally dioecious fig, *Ficus hispida*, we show that (1) different stimuli induce the loss of attraction in each sex, pollination in female figs, and oviposition in male figs; and (2) foundress number affects the loss of attraction in both sexes only when the prerequisites (*i.e.*, pollination in female figs and oviposition in male figs) have been satisfied. In general, the more foundresses that enter, the earlier the fig will lose its receptivity. We argue that the stimuli in male and female figs are adaptations to the fulfillment of its respective reproduction.

Abstract in Chinese is available at http://www.blackwell-synergy.com/loi/btp

Key words: behavior; co-evolution; mutualism; oviposition; pollen; pollinating fig wasp; receptivity; syconium.

MUTUALISMS ARE UBIQUITOUS IN NATURE. Partners invest individually but receive reciprocal benefits from each other (Sachs & Simms 2006). Fig trees (*Ficus*) and their minute pollinating wasps (Hymenoptera, Chalcidoidea, Agaonidae; Rasplus *et al.* 1998) are a classic example of a coevolved mutualism (Ramirez 1970, Janzen 1979, Wiebes 1979, Herre 1996, Weiblen 2002, Cook & Rasplus 2003, Cook & West 2005, Herre *et al.* 2008). Highly specific wasps pollinate fig trees while fig trees provide food and nurseries for the fig pollinator offspring. This mutualism originated 60–80 million years ago (Machado *et al.* 2001; Ronsted *et al.* 2005, 2008). The worldwide distribution of approximately 750 fig species and diverse characters within both partners (*e.g.*, wide range of fig life forms; monoecy and dioecy in figs; passive and active pollination modes in pollinators) demonstrate the success of this mutualism (Berg 1989, Herre 1996, Kjellberg *et al.* 2001, Machado *et al.* 2001, Jousselin *et al.* 2003b, Weiblen 2004, Harrison 2005).

Ficus is characterized by its enclosed urn-shaped floral receptacle, lined internally by uniovulate female flowers or male flowers. The ostiole is the only opening to the outside. Usually, the ostiole is closed by a cluster of bracts (Galil & Neeman 1977). Only at the receptive phase, figs attract short-lived pollinators (usually 1–3 d) by releasing specific volatiles, accompanied by a loosing of ostiolar bracts to let specialized pollinators force their ways through (Hossaert-Mckey *et al.* 1994, Grison-Pige *et al.* 2002, Proffit *et al.* 2008). Figs lose their attraction to pollinators after fig pollinators have entered (van Noort *et al.* 1989, Nefdt & Compton 1996, Borges *et al.* 2008). This process is irreversible. If figs lose their attraction without pollen or wasps inside, the figs would not produce seeds or pollinators. When pollinators are absent, figs' receptivity lasts several days or even several weeks (Ware & Compton 1994, Khadari *et al.* 1995).

Although the loss of attraction plays a fundamental role in the fig–pollinator mutualism, it has been largely overlooked. A previous study suggests that figs lose their attraction sooner after multiple pollinators have entered into monoecious figs and male dioecious figs (Khadari *et al.* 1995). However, it is not known whether the pollinator's entry itself is enough to induce the loss of attraction for both monoecious and dioecious figs. In this study, for the first time we investigated the influence of several factors on the loss of attraction in a functionally dioecious *Ficus hispida* (both male figs and female figs), including pollinator entry, pollination, and oviposition. We found that different stimuli reduce attraction to pollinators in male and female figs: mainly by pollination in female figs and oviposition in male figs.

Received 16 July 2008; revision accepted 25 March 2009.

[7]Corresponding author; e-mail: huangdw@ioz.ac.cn

METHODS

STUDY SPECIES AND SITE.—Experiments on dioecious *F. hispida* Linn. were conducted at the Chinese Academy of Tropical Agricultural Sciences (CATAS), Danzhou, Hainan province, China (19°30.410′ S, 109°29.340′ E). Fig trees of *F. hispida* are free-standing, usually 5–8 m in height, with spherical figs (Corlett 2006) and they are actively pollinated by *Ceratosolen solmsi* Mayr (Shi *et al.* 2006). The fig fruit is *ca* 30–40 mm diam. when mature and *ca* 20 mm when receptive. The total number of female flowers in male fig fruits is about 1800. Hainan Island is located south of China's mainland. Its climate includes well-defined dry (November–April) and rainy (May–October) seasons. The annual mean temperature is 24.3°C, with the lowest and the highest mean temperature in February (18.2°C) and July (29.6°C), respectively.

FACTORS INDUCING LOSS OF ATTRACTION.—We investigated the factors affecting the loss of attraction to pollinators by controlling introductions of pollinating wasps. In female figs, we had five treatments: (1) one pollinator with pollen; (2) three pollinators with pollen; (3) one pollen-free pollinator; (4) three pollen-free pollinators; and (5) bagged inflorescences without pollinators as a negative control. In male trees, two additional no-oviposition treatments were added: one pollinator with and without pollen. About 20 figs were used for each treatment.

Pollen-bearing pollinators were collected from natural male figs and pollen-free pollinators were collected from male figs with stamens-removed following Jousselin *et al.* (2003a). Figs approaching the male phase were opened and their stamens (all around the ostiolar bracts) were removed with fine forceps. Those figs were reclosed in a fine-mesh bag. Pollen-free pollinators were collected when they emerged. Figs without oviposition were obtained by putting figs into warm water for 10 min *in situ* (8°C above ambient temperature), 30 min after the pollinator's entry (Patino *et al.* 1994). In this case, foundresses would die but the development of figs and wasps continues. The selection of 30 min was based on empirical observations that most pollinators (> 90%) pushed through the ostiolar bracts into the figs in this time period.

We chose figs reaching receptivity based on the change of fig size. We measured fig circumference to the nearest 1 mm with a soft plastic ruler every 24 h. On the first day of receptivity, figs of *F. hispida* always grow rapidly. Figs reaching receptivity were marked for the remaining experiments. The state of attraction of a fig was based on observations of the behavior of three wasps (Khadari *et al.* 1995). When a fig is receptive, pollinators will walk around on the fig, tapping the fig surface continuously with their distal antennal segments, searching for the ostiolar bracts. Then they push their antennae under the ostiolar bracts and attempt to enter. If any one of the three pollinators tried to enter, the fig was considered receptive. If the three wasps spent > 10 min walking on the fig or lifted the antennae and stopped tapping the surface or flew rapidly away, the fig was considered not receptive. The selection of 10 min was also based on empirical observations that almost all pollinators attempted to enter figs in < 10 min (> 90%).

Pollen-bearing and pollen-free pollinators were immediately moved to the marked receptive figs with a soft brush. Three-foundress figs were obtained by giving each pollinator 20 min to crawl through the ostiolar bracts. All figs were protected with nylon bags throughout the experiments to prevent other pollinators from entering. We checked the status of figs (*i.e.*, receptivity) every 24 h. Experiments were conducted until all figs lost their attraction. Duration of receptivity of each fig was recorded. All figs were taken to the laboratory and dissected under a microscope. Treatments with pollen-bearing or pollen-free wasps were verified by examining if there were yellowing stigmas in the syconial cavity (pollinated stigma turn yellow). Treatments with oviposition-free wasps were also verified by examining if there were dead wasps or swollen ovaries in the syconial cavity (oviposited ovaries swell several days later).

We conducted the experiments on a male fig tree and a female fig tree, respectively, in the rainy season from August to September 2007. We used nonparametric Kruskal–Wallis test to compare the receptive length among treatments on female and male fig trees. A Wilcoxon rank-sum test was used to compare the length within treatments or between the two sexual figs in SPSS version 15.0 (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

On the female fig tree, significant differences were observed among the duration of receptivity of the five fig treatments (two treatments with one pollinator pollen-bearing or pollen-free, two treatments with three pollinators pollen-bearing or pollen-free, and a control treatment without pollinators; $P < 0.0001$, df = 4, Kruskal–Wallis test; Fig. 1). Figs entered by pollen-bearing pollinators had significantly shorter duration of receptivity than those entered by pollen-free pollinators (both $P < 0.0001$ for treatment with one and three pollinators, Wilcoxon's rank-sum test). The duration of fig receptivity with three pollen-bearing pollinators was shorter than that of figs with one pollen-bearing pollinator ($P < 0.0001$). Figs entered

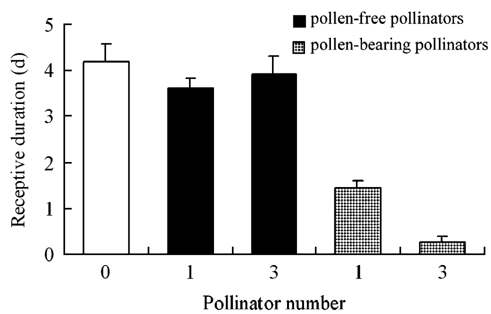

FIGURE 1. Duration of receptivity of female figs (means ± SE). Figs with pollen-free pollinators have similar duration of receptivity to figs without pollinator ($P = 0.305$ and 0.857 for treatment with one and three pollinators, respectively). Figs with pollen-carrying pollinators show shorter duration of receptivity than figs with pollen-free pollinators or negative control (both $P < 0.0001$ for treatment with one and three pollinators). Figs with three pollen-carrying pollinators show shorter duration of receptivity than figs with single pollen-carrying pollinator ($P < 0.0001$).

by pollinators with no pollen had similar duration of receptivity to the figs without pollinators introduced ($P = 0.305$ and 0.857 for treatment with one and three pollinators, respectively).

On the male fig tree, there was a significant difference among the seven fig treatments (two treatments with one pollinator pollen-bearing or pollen-free, two treatments with three pollinators pollen-bearing or pollen-free, two treatments without oviposition, and a control treatment without pollinators; $P < 0.0001$, df = 6; Fig. 2). Figs without oviposition had significantly longer duration of receptivity than those figs in which pollinators had oviposited (both $P < 0.0001$ for treatment with one pollen-free and one pollen-bearing pollinators, respectively), and were similar to the control treatment figs without any pollinators introduced ($P = 0.318$ and 0.155 for treatment with pollen-free and pollen-bearing pollinators, respectively). Figs with three pollinators had shorter receptivity than figs with one pollinator ($P < 0.005$ and < 0.05 for treatment with pollen-free and pollen-bearing pollinators, respectively).

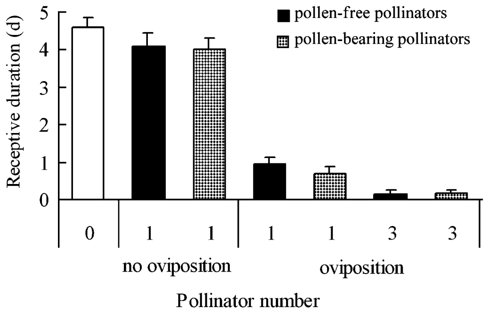

FIGURE 2. Length of receptivity of male figs (means ± SE). Figs without oviposition have significantly longer duration of receptivity than those figs oviposited by pollinators (both $P < 0.0001$ for treatment with pollen-free and pollen-bearing pollinators, respectively), and are similar to the control treatment figs without any pollinators introduced ($P = 0.318$ and 0.185 for treatment with pollen-free and pollen-bearing pollinators, respectively). Figs with three pollinators have a shorter duration of receptivity than figs with one pollinator ($P < 0.005$ and < 0.05 for treatment with pollen-free and pollen-bearing pollinators, respectively).

DISCUSSION

Precise control of fig receptivity is fundamental for the fig–pollinator mutualism. However, the nature of the stimulus causing loss of attraction to pollinators has largely been overlooked for both monoecious and dioecious fig species. Although it has been observed that monoecious figs and male figs of a dioecious species lose their attraction sooner when more pollinators have entered the syconium (Khadari *et al.* 1995), it is not known whether the loss of attraction is initiated by the pollinator's entry itself or by other factors. In this study, for the first time we investigated the influence of several factors on the duration of receptivity of a dioecious fig species, *F. hispida*, including pollinator entry, pollination, and oviposition.

FACTORS INDUCING LOSS OF ATTRACTION IN MALE AND FEMALE FIGS.—Our data suggest that female figs remain receptive even after pollen-free pollinators entered (Fig. 1). Further, male figs remain receptive until oviposition is conducted, regardless of the presence or absence of pollinators (Fig. 2). Clearly, the entry of wasps is in itself not the cue to initiate the loss of attraction to pollinators in both male and female figs. Instead, different stimuli cause the loss of attraction to pollinators, mainly by pollination in female figs and oviposition in male figs.

For dioecious figs, the ultimate reproductive functions of male and female figs are to produce wasps and seeds, respectively (Kjellberg *et al.* 1987, Grafen & Godfray 1991, Kerdelhué & Rasplus 1996, Patel 1996, Weiblen 2002, Harrison & Yamamura 2003); hence, the critical checkpoint for male figs is to make sure that they have wasp eggs inside (oviposition) and the critical checkpoint for female figs is to make sure that the figs are fertilized (pollination). Thus, the main stimuli for loss of receptivity in male and female figs seem to be adaptations to maximize their respective reproductive functions, suggesting again that the fig's interests generally dominate this mutualism (Herre 1989, Herre *et al.* 2008).

FOUNDRESS NUMBER AND THE LOSS OF ATTRACTION TO POLLINATORS.—In monoecious fig species, control of the number of pollinators entering figs is an important way to resolve resource conflicts between figs and fig pollinators because seeds and wasps are produced in the same syconia and they compete with each other for ovules (Nefdt & Compton 1996, Anstett *et al.* 1997, Weiblen 2002, Cook & Rasplus 2003). In dioecious fig species, there are no obvious resource conflicts between figs and fig pollinators due to the separate production of pollinators and seeds in different figs. Some researchers even suggest that figs would wait for enough pollinators to fully fertilize all female flowers and lay eggs in all the available ovules in male figs (Patel & Hossaert-McKey 2000). Thus, the duration of receptivity of a dioecious fig is expected to be less sensitive to the number of foundresses. Contrary to this, our data show that foundress number and the duration of fig receptivity are negatively correlated in both sexes when the process of loss of attraction to pollinators is initiated. In general, the more foundresses that enter, the earlier figs lose their attraction in both male and female figs. A similar pattern was also observed in monoecious and in male dioecious figs (Khadari *et al.* 1995). This unexpected pattern could be the result of an interaction between the pollinator and the fig. The level of attraction is mostly likely to decline in proportion to the number of wasps that enter and depends on the condition of the remaining receptive stigmas, which may produce chemical signals and are getting older. Alternatively, it could also be that the rapid attraction loss after enough pollinators have entered may save pollinators for unpollinated figs because pollinators are generally a rare resource (Nefdt & Compton 1996), or that the quick loss of attraction allows stopping releasing costly chemical signals as soon as they are not required.

This is the first study to investigate the stimuli inducing the loss of attraction in figs. We observed that male and female figs respond to different stimuli to reduce attraction and figs will lose their attraction faster if there are more foundresses in *F. hispida*. Whether patterns observed here can be generalized to other dioecious fig species requires further study. It would be also interesting to know what kind of stimulus (*e.g.*, pollen, oviposition, or entry of pollinators) causes the loss of attraction in monoecious fig species. The comparison between monoecious and dioecious fig species would help us to further understand the evolutionary significance of the patterns observed here and might shed light on the evolution of the dioecious mating system in figs.

ACKNOWLEDGMENTS

This project was supported by the National Natural Science Foundation of China (NSFC grant no. 30770302, 30570970), partially by Program of Ministry of Science and Technology of the Republic of China (2006FY110500), and by National Science Fund for Fostering Talents in Basic Research (Special subjects in animal taxonomy, NSFC-J0630964/J0109). We thank C. Machado, E. Suurmeyer, A. Himler, and especially A. Herre and R. Harrison for their insightful comments and suggestions. All experiments reported here comply with the current laws of China. H.-Y. Hu, Y.-G. Fu, Z.-Q. Peng, and D.-W. Huang designed the experiment. H.-Y. Hu and L.-M. Niu conducted the experiment. H.-Y. Hu analyzed data. H.-Y. Hu, Z.-F. Jiang, and D.-W. Huang wrote the paper.

LITERATURE CITED

ANSTETT, M. C., M. HOSSAERT-MCKEY, AND F. KJELLBERG. 1997. Figs and fig pollinators: Evolutionary conflicts in a coevolved mutualism. Trends Ecol. Evol. 12: 94–99.

BERG, C. C. 1989. Classification and distribution of *Ficus*. Experientia 45: 605–611.

BORGES, R., J. BESSIÈRE, AND M. HOSSAERT-MCKEY. 2008. The chemical ecology of seed dispersal in monoecious and dioecious figs. Fun. Ecol. 22: 484–493.

COOK, J. M., AND J.-Y. RASPLUS. 2003. Mutualist with attitude: Coevolving fig wasps and figs. Trends Ecol. Evol. 18: 241–248.

COOK, J. M., AND S. A. WEST. 2005. Figs and fig wasps. Curr. Biol. 15: R978–R980.

CORLETT, R. T. 2006. Figs (*Ficus*, Moraceae) in Urban Hong Kong, South China. Biotropica 38: 116–121.

GALIL, J., AND G. NEEMAN. 1977. Pollen transfer and pollination in the common fig (*Ficus carica* L.). New Phytol. 79: 163–171.

GRAFEN, A., AND H. C. J. GODFRAY. 1991. Vicarious selection explains some paradoxes in dioecious fig pollinator systems. Proc. R. Soc. Lond. B 245: 73–76.

GRISON-PIGE, L., J. M. BESSIERE, AND M. HOSSAERT-MCKEY. 2002. Specific attraction of fig-pollinating wasps: Role of volatile compounds released by tropical figs. J. Chem. Ecol. 28: 283–295.

HARRISON, R. D. 2005. Figs and the diversity of tropical rainforests. Bioscience 55: 1053–1064.

HARRISON, R. D., AND N. YAMAMURA. 2003. A few more hypotheses for the evolution of dioecy in figs (*Ficus*, Moraceae). Oikos 100: 628–635.

HERRE, E., K. JANDER, AND C. MACHADO. 2008. Evolutionary ecology of figs and their associates: Recent progress and outstanding puzzles. Annu. Rev. Ecol. Evol. Syst. 39: 439–458.

HERRE, E. A. 1989. Coevolution of reproductive characteristics in 12 species of New World figs and their pollinator wasps. Experientia 45: 637–647.

HERRE, E. A. 1996. An overview of studies on a community of Panamanian figs. J. Biogeogr. 23: 593–607.

HOSSAERT-MCKEY, M., M. GIBERNAU, AND J. E. FREY. 1994. Chemosensory attraction of fig wasps to substances produced by receptive figs. Entomol. Exp. Appl. 70: 185–191.

JANZEN, D. H. 1979. How to be a fig. Annu. Rev. Ecol. Syst. 10: 13–51.

JOUSSELIN, E., M. HOSSAERT-MCKEY, E. A. HERRE, AND F. KJELLBERG. 2003a. Why do fig wasps actively pollinate monoecious figs? Oecologia 134: 381–387.

JOUSSELIN, E., J.-Y. RASPLUS, AND F. KJELLBERG. 2003b. Convergence and coevolution in a mutualism: Evidence from a molecular phylogeny of *Ficus*. Evolution 57: 1255–1269.

KERDELHUÉ, C., AND J. Y. RASPLUS. 1996. The evolution of dioecy among *Ficus* (Moraceae): An alternative hypothesis involving non-pollinating fig wasp pressure in the fig–pollinator mutualism. Oikos 77: 163–166.

KHADARI, B., M. GIBERNAU, M. C. ANSTETT, F. KJELLBERG, AND M. HOSSAERT-MCKEY. 1995. When figs wait for pollinators: The length of fig receptivity. Am. J. Bot. 82: 992–999.

KJELLBERG, F., P.-H. GOUYON, M. IBRAHIM, AND G. VALDEYRON. 1987. The stability of the symbiosis between dioecious figs and their pollinators: A study of *Ficus carica* L. and *Blastophaga psenes* L. Evolution 41: 693–704.

KJELLBERG, F., E. JOUSSELIN, J. L. BRONSTEIN, A. PATEL, J. YOKOYAMA, AND J. Y. RASPLUS. 2001. Pollination mode in fig wasps: The predictive power of correlated traits. Proc. R. Soc. Lond. B 268: 1113–1121.

MACHADO, C. A., E. JOUSSELIN, F. KJELLBERG, S. G. COMPTON, AND E. A. HERRE. 2001. Phylogenetic relationships, historical biogeography, and character evolution of fig-pollinating wasps. Proc. R. Soc. Lond. B 268: 685–694.

NEFDT, R. J. C., AND S. G. COMPTON. 1996. Regulation of seed and pollinator production in the fig–fig wasp mutualism. J. Anim. Ecol. 65: 170–182.

PATEL, A. 1996. Variation in a mutualism: Phenology and the maintenance gynodioecy in two Indian fig species. J. Ecol. 84: 667–680.

PATEL, A., AND M. HOSSAERT-MCKEY. 2000. Components of reproductive success in two dioecious fig species, *Ficus exasperata* and *Ficus hispida*. Ecology 81: 2850–2866.

PATINO, S., E. A. HERRE, AND M. T. TYREE. 1994. Physiological determinants of *Ficus* fruit temperature and implications for survival of pollinator wasp species: Comparative physiology through an energy budget approach. Oecologia 100: 13–20.

PROFFIT, M., B. SCHATZ, J.-M. BESSIÈRE, C. CHEN, C. SOLER, AND M. HOSSAERT-MCKEY. 2008. Signalling receptivity: Comparison of the emission of volatile compounds by figs of *Ficus hispida* before, during and after the phase of receptivity to pollinators. Symbiosis 45: 15–24.

RAMIREZ, W. 1970. Host specificity of fig wasps (Agaonidae). Evolution 24: 681–691.

RASPLUS, J.-Y., C. KERDELHUÉ, I. LE CLAINCHE, AND G. MONDOR. 1998. Molecular phylogeny of fig wasps: Agaonidae are not monophyletic. C. R. Acad. Sci., Ser. III 321: 517–527.

RONSTED, N., G. D. WEIBLEN, W. L. CLEMENT, N. J. ZEREGA, AND V. SAVOLAINEN. 2008. Reconstructing the phylogeny of figs (*Ficus*, Moraceae) to reveal the history of the fig pollination mutualism. Symbiosis 45: 45–56.

RONSTED, N., G. D. WEIBLEN, J. M. COOK, N. SALAMIN, C. A. MACHADO, AND V. SAVOLAINEN. 2005. 60 million years of co-divergence in the fig-wasp symbiosis. Proc. R. Soc. Lond. B 272: 2593–2599.

SACHS, J. L., AND E. L. SIMMS. 2006. Pathways to mutualism breakdown. Trends Ecol. Evol. 21: 585–592.

SHI, Z.-H., D.-R. YANG, AND Y.-Q. PENG. 2006. The style–length of the female florets and their fate in two dioecious species of Xishuangbanna, China. Trees—Struct. Funct. 20: 410–415.

VAN NOORT, S., A. B. WARE, AND S. G. COMPTON. 1989. Pollinator-specific volatile attractants released from the figs of *Ficus burtt-davyi*. S. Afr. J. Sci. 85: 323–324.

WARE, A. B., AND S. G. COMPTON. 1994. Responses of fig wasps to host-plant volatile cues. J. Chem. Ecol. 20: 785–802.

WEIBLEN, G. D. 2002. How to be a fig wasp. Annu. Rev. Entomol. 47: 299–330.

WEIBLEN, G. D. 2004. Correlated evolution in fig pollination. Syst. Biol. 53: 128–139.

WIEBES, J. T. 1979. Co-evolution of figs and their insect pollinators. Annu. Rev. Ecol. Syst. 10: 1–12.