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Contributed Paper

Interspecies Interactions of Fig Wasp Species and Their Association with the Fig Tree *Ficus schwarzii* Koord.

Jirapan Yimkaew*[a] and Chutamas Satasook [b]

[a] Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand.

[b] Princess Maha Chakri Sirindhorn Natural History Museum, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand.

*Author for correspondence; e-mail: jirapan.yk@gmail.com

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ABSTRACT

The interspecies interactions of fig wasps found in male figs, *Ficus schwarzii* Koord., including the influences of the fig diameter, the number of florets, and the wall thickness and focusing on the pollinator species were documented. The total number of individuals of the three non-pollinator species (*Apocryptophagus* sp., *Philotrypesis* sp., *Apocrypta* sp.) showed a significant negative correlation with the number of pollinators (*Ceratosolen vetustus*). The results from a path analysis indicated that *Apocryptophagus* sp. was an important non-pollinator species that competed for the same niche as the pollinator. The *Philotrypesis* sp. acted as a parasitoid of the pollinator, whereas *Apocrypta* sp. was considered to be a generalist for host selection. From the path model, an increase of pollinator numbers generally related to the bigger size of fig and the more plentiful florets that also favoured the pollinators. The wall thickness had a negative effect on the population number of the pollinators.

Key words: *Ficus schwarzii* Koord, fig wasp species, host utilisation, interspecies interaction, path diagram

1. INTRODUCTION

Hymenopteran insects in the superfamily Chalcidoidea play an important role in terrestrial ecosystems especially as pollinators and parasitoids. The Chalcidoid wasps that are only associated with fig trees (Moraceae, *Ficus* spp.) are classified as fig wasps. They can be generally separated into pollinator and non-pollinator wasps. The fig-fig wasp interaction covers the range from mutualism to parasitism [1]. An obligate mutualism between a pollinator and a fig tree allows for reproductive success for both species, and represents a classic example of co-evolution [2–4]. From molecular and fossil data, the symbiosis of figs and pollinator wasps diverged approximately 75 million years ago in order to maintain this specialized association [5]. The utilisation of the host by pollinator wasps is dependent on the fig breeding system [2]. About half of fig species are monoecious, that seeds and wasps can be found in the same tree. The other half are dioecious fig species of which seeds and wasps found separately within female and male trees, respectively.

Non-pollinator wasps are known to be the cause of fig and pollinator mutualistic instability [3]. Several studies have shown negative correlations between the number of pollinators and non-pollinators that result from competition for resources and can have either slight [6] or major impacts on pollinator populations [7–8]. Nevertheless, there are some cases when there is no correlation between pollinator and non-pollinator populations [9–10]. In addition, there is a positive relationship between them [11].

In Thailand, although knowledge of diversity and distribution of figs is well documented, the study on their association with fig wasp species, either pollinator or non-pollinator species, is still limited. Moreover, the relationship between fig trees, pollinators and non-pollinators is still poorly understood. The major goals of this study were to investigate the relationships between populations of pollinator and non-pollinator wasps that were associated in the same fig tree, and to elucidate the influences of some measured traits of the dioecious fig, *Ficus schwarzii* Koord. (Subgenus *Sycomorus*) on pollinator population. This study sought to investigate; (1.) how non-pollinator wasps affected the pollinator population in *F. schwarzii*, (2.) how fig diameters, wall thickness, and the numbers of florets of *F. schwarzii* related to the number of fig wasps, especially the pollinator populations.

2. MATERIALS AND METHODS

2.1 Study Site

As *F. schwarzii* is known as a pioneer species [12] and usually found in disturbed forests [13]. It is a common species and widely distributed from peninsular Thailand to peninsular Malaysia, Sumatra and Borneo [14]. Therefore, Ton Nga Chang Wildlife Sanctuary (TWS), which is part of the Ban-tad Mountain Range in southern Thailand, was chosen as a study site. The vegetation in TWS has been identified as a tropical semi-evergreen rain forest [15]. From a preliminary study, *F. schwarzii* was found at two sites around TWS at Ton Nga Chang Waterfall (TNC; N 6° 57'; E 100° 14') and Pha Dum Guard Station (PD; N 6° 47'; E 100° 13') that were about 15.5 km apart.

2.2 Collecting Data

Collection of data was divided into two parts. The first part was to collect one to three figs in an early emergence stage from 18 and 12 male (gall) fig trees at TNC and PD, respectively. Then, fig wasps from a total of 30 ripe figs collected from each site were identified. The diameter of a mature fig that represents the fig size was measured using a dial vernier caliper at the widest dimension of the fruit. Then, the fig was cut vertically into two pieces. The wall thickness was the mean value of the measurements taken from both sides of the fig. Finally, the number of female florets, including both galled and undeveloped female florets inside the fig, was counted to compare the host using capability of the fig wasp.

The second part was a field observation in order to identify the oviposition sequences of the fig wasp species. A gall tree in an early receptive phase was selected to determine the oviposition time sequences of each fig wasp species. The oviposition sequence can be defined as the order and period of egg-laying of each fig wasp species that was related to the fig development based on the release of different volatile compounds at each fig state [16–18]. The timing of the oviposition sequence refers to the niche partition among the fig wasps and the stability of the species coexistence [17]. The figs in every branch from the height at the level of the observer's head down to the ground were observed. The diameter of figs which had been probed by fig wasps was measured. This observation was undertaken about two hours per day in the morning for 30 days until the fig became ripe.

2.3 Data Analysis

The number of pollinators and non-pollinators was transformed by using a square root transformation. The correlation between these two variables from both sites was determined using the Pearson's Correlation. The frequency curve of fig diameters of each fig wasp species was plotted to show the oviposition time sequences. The statistical analyses were computed in a Minitab 14 (Minitab Inc., State College, PA., USA).

A path analysis was conducted to determine the actual relationships between and within fig wasp species, and as well as with some measured fig traits by using AMOS 5 (student version) that was represented by an over identified model [19]. The model was modified from an original model [20] by adding the variables of the numbers of florets and wall thickness, and deleting seed variability because there was no seed in the selected gall figs. All pathways were tested to eliminate non-causal pathways with zero coefficients or non-significance and were built in an 'over identified model'. A standardised path coefficient (P) indicated either a positive or a negative effect. The positive effect values were defined into three levels [21]; a small effect ($P < 0.10$), a medium effect ($P = 0.30$), and a large effect ($P > 0.50$). The negative effect was indicated by negative P (between -1.0 and -0.1). The criterion for indicating a good model fit in the over identified model was described by using the chi-square (χ^2) and fit indices [19,22].

3. RESULTS AND DISCUSSION

3.1 The Inter-relationship of Fig Wasp Species

Four fig wasps species; *C. vetustus, Philotrypesis* sp., *Apocryptophagus* sp., and *Apocrypta* sp., were found in *F. schwarzii*. A recent study on fig wasp species composition in this fig species also defined *C. vetustus* as pollinator species with proportionally more than 80% of the total wasp population, while other species defined as non-pollinator species was less than 7% of each species population [23]. The correlation between the transformed number of pollinator and non-pollinator species was significantly negatively correlated ($r = -0.448$, $n = 60$, $p < 0.001$, Figure 1). An increase in the number of pollinator individuals led to a decrease in the non-pollinator numbers. This finding coincided with several studies that had concentrated on both monoecious and dioecious figs [6–8]. However, a positive correlation [11] and non-correlation [9–10] between the number of non-pollinators and pollinators were also reported. Peng et al. [11] observed that the non-pollinators found in the dioecious fig species (*F. hispida* L.) were all parasitoids or inquilines that oviposited after the pollinator's oviposition. Therefore the number of non-pollinators increased with the number of pollinators. In this study, however, it was not only the non-pollinators *(Philotrypesis* sp. and *Apocrypta* sp.) that oviposited after the presence of the pollinator species (*C. vetustus*), another important non-pollinator species (*Apocryptophagus* sp.) was recorded as an early ovipositing galler that oviposited before the pollinator (Figure 2). In this case, there was some competition between the non-pollinator, *Apocryptophagus* sp. and the pollinator, *C. vetustus* for the oviposition sites (flower) that occurred. Thus, this non-pollinator species may have a greater negative effect on the pollinator ($r = -0.413$, $n = 60$, $p < 0.01$), although it has lowest

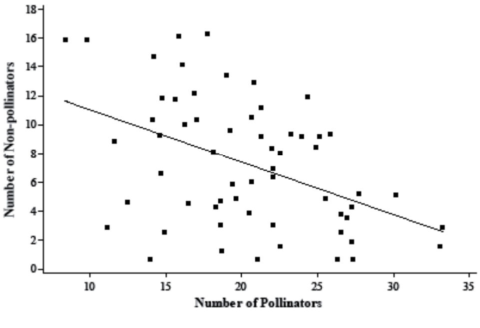

Figure 1. Negative correlation between pollinator and non-pollinator fig wasps.

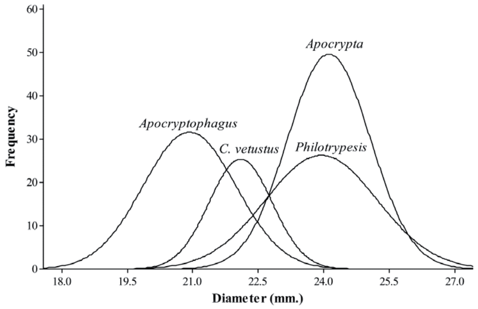

Figure 2. Ovipositing time sequences of the four fig wasp species related to fig development.

percentage of presence in comparison to the other two non-pollinator species [23].

3.2 Association of Fig Wasp Species with Fig Development

The pattern of an ovipositing time sequence for each fig wasp species is shown in Figure 2. *Apocryptophagus* sp. was an early galler that oviposited on small figs with an average diameter of 20.93±1.07 mm (range = 18.1–24.2 mm, n = 169). Any increase in the diameter of the fig reduced the number of *Apocryptophagus*'s ovipositions and allowed the second ovipositor to invade. An average diameter of 22.10±0.72 mm (range = 20.6–24.4 mm, n = 92) favoured the oviposition of *C. vetustus*. It indicated that *Apocryptophagus* sp. was the first species that oviposited before pollination. From field observations, an oviposition time of *Apocryptophagus* sp. was two days prior to the oviposition by the pollinator (*C. vetustus*). This agrees with the result reported by Cruaud et al. [24] who found that a pollinator was the second species that oviposited after the galler group. Many studies in other *Ficus* species have reported similar results for an early ovipositing time by gallers [17,24,25]. However, some gallers showed an overlapping oviposition time with pollinators [26]. Proffit et al. [17] proposed that the sequences of oviposition by different fig wasp species were dependent on volatile signals released at different stages of fig development. Moreover, they reported that three species of *Apocryptophagus* were the first group to oviposit in *Ficus racemosa*, and they were attracted by a chemical signal produced from the plant tissue. Hossaert-McKey et al. [27] suggested that pollinators responded to specific floral scents.

Philotrypesis sp. and *Apocrypta* sp. were the last two species that oviposited in the gall fig at an average diameter of 23.95±1.28 mm (range = 18.9–27.1 mm, n = 196) and 24.13± 0.95 mm (range = 21.4–27.1 mm, n = 236), respectively. These species were found to oviposit after the pollinator (Figure 2) at the interfloral phase. Elias et al. [18] stated that non-pollinators which oviposited later after the pollinators were assumed to be parasitoids or inquilines that probably responded to scent from another wasp larvae [17]. Although the function of *Philotrypesis* sp. is still unclear, either as an inquiline or a parasitoid [1,10], Harrison and Shanahan [28] suggested that *Philotrypesis* sp. may be an inquiline of a pollinator while *Apocrypta* sp. was probably a parasitoid of *Apocryptophagus* sp. on *F. schwarzii*. Nonetheless, Compton and Robertson [29] reported *Apocrypta* sp. attacked both *Apocryptophagus* sp. and *Ceratosolen* sp. in an African fig (*Ficus sur* Forsk.). However, there was no evidence to support their actual functions such as any experiment on their feeding behaviour, and histological studies [18] as these would be necessary to understand the biology of *Philotrypesis* sp. and *Apocrypta* sp. in this fig. In addition, Chen et al. [30] reported that a definition by the inquiline is not properly fit for non-pollinators, and also suggested to describe their biology as parasitoids. Therefore, in this study, these non-pollinator species were referred as parasitoids.

3.3 Association of Fig Wasp Species with Fig Diameter, Number of Florets, and Wall Thickness

Path analysis demonstrated a well fitted 'over identified model' ($\chi^2 = 2.854$, df = 6, n = 59, p = 0.827, CFI = 1.000, TLI = 1.116, NFI = 0.965, RMSEA = 0.000, Figure 3) with an elimination of *Apocrypta* sp. from all pathways because of its insignificant statistical results. It could be implied that this parasitoid species has much less relationship within this *Ficus* species. Cook and Segar [4] also suggested that *Apocrypta* species is a generalist in host selection. The effect of variables on the pollinator was represented by path coefficient values (P) (Table 1.). *Apocryptophagus* sp. (P = -0.32) and *Philotrypesis* sp. (P = -0.28) had a negative direct effect on the pollinator. The results indicated that *Apocryptophagus* sp., as a galler, may compete with a pollinator for the same niche (flowers), while only *Philotrypesis* sp. was expected to be a parasitoid on a pollinator. The presence of *Philotrypesis* sp. reduced the number of pollinators through parasitism. The analysis also showed that this parasitic wasp had a medium effect on increasing the number of gallers (*Apocryptophagus* sp., P = 0.31, Figure 3). A possible explanation may concern the energy allocation to each flower. A decrease of pollinator larvae resulting from *Philotrypesis* sp. might increase the energy allocation to a flower containing *Apocryptophagus* larva, and then increased the survival rate of this species. However, *Philotrypesis* sp. also exhibited an indirect effect on *C. vetustus* through the *Apocryptophagus* sp. with the path coefficient of *Philotrypesis* sp. on *Apocryptophagus* sp. at 0.31, and *Apocryptophagus* sp. on *C. vetustus* at -0.32. Therefore, the total effect of the *Philotrypesis* sp. on *C. vetustus* was at the path coefficient of -0.38.

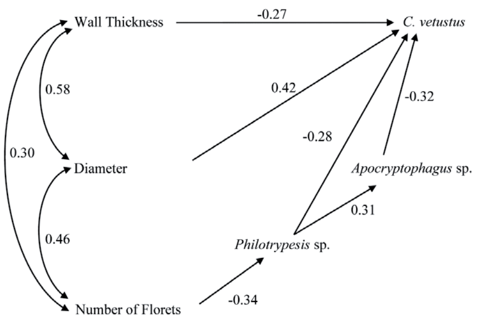

Figure 3. The 'Over identified model' in a path diagram of the significant direct and indirect effects of causal variables on *C. vetustus.* Path and correlation coefficients are shown in single-headed arrows and double-headed arrows respectively. The solid lines represent a significant effect on the variables.

In addition, the diameter, the number of florets and the wall thickness had a significant positive correlation with each other in the path analysis (Figure 3). The correlation as estimated from a Pearson analysis showed the same trend, in which the diameter had a positive correlation to the wall thickness (r = 0.68, n = 59, $p < 0.001$) and to the number of florets per fig (r = 0.40, n = 59, $p < 0.001$). Moreover, the fig diameter and the wall thickness had a direct effect on the pollinator at the path coefficients of 0.42, and -0.27, respectively (Table 1). The wall thickness and the diameter were probably associated with ostiole characteristics that, in turn, played an important role as a selective filter to the pollinator [31]. The thickness of the ostiole is represented by the wall thickness that may have a direct effect on the ability of the pollinator to enter a fig's cavity. The number of florets showed a positive indirect effect on the pollinator (*C. vetustus*) by providing more available resources for egg-laying through two distinct pathways that were associated with the non-pollinators (*Philotrypesis* sp. and *Apocryptophagus* sp.) at path coefficients of 0.13 (Table 1). In general, the higher number of florets in the fig supports more pollinator numbers. Nevertheless, the result from path analysis showed that the effect of the number of florets was lower than the diameter. This low coefficient is probably because the number of florets used in this study based on number of both galled and undeveloped florets.

Table 1. Summary of the effect of variables in the 'over identified model' with 95% confidence intervals.

Variables1	Variables2	Direct Effect	Indirect Effect	Total Effect (direct + indirect effects)
C. vetustus	*Apocryptophagus* sp.	-0.32	-	-0.32
	Philotrypesis sp.	-0.28	(0.31 x -0.32)= -0.10	-0.38
	Wall thickness	-0.27	-	-0.27
	Diameter	0.42	-	0.42
	Number of florets	-	(-0.34 x -0.28) + (-0.34 x 0.31 x -0.32) = 0.13	0.13
Apocryptophagus sp.	*Philotrypesis* sp.	0.31	-	0.31
	Number of florets	-	(-0.34 x 0.31) = -0.11	-0.11
Philotrypesis sp.	Number of florets	-0.34	-	-0.34

4. CONCLUSIONS

This study is the first to report on a detailed set of investigations on the effect of each of the non-pollinator wasp species on the pollinator population. The functional model demonstrated interspecies interaction in the fig wasp community on *F. schwarzii*. The pollinator was affected by *Apocryptophagus* sp. and *Philotrypesis* sp. through a competitive niche and parasitism, respectively. *Apocrypta* sp. was not included in the analysis due to less relationship with its host as this species was considered to be a generalist. Nevertheless, future study with more evidences, including ecological and histological experiments, is needed to fulfill the knowledge of the interaction between species. Three measured traits of *F. schwarzii* (diameter, wall thickness, and number of florets) were proposed as factors that maintained the stability of mutualism between the fig and its pollinator.

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REFERENCES

[1] Weiblen G.D., Yu D.W. and West S.A., *Proc. R. Soc. Lond. Ser. B.*, 2001; **268**: 651-659. DOI 10.1098/rspb.2000.1389.

[2] Weiblen G.D, *Annu. Rev. Entomol.*, 2002; **47**: 299-330. DOI 10.1146/annurev.ento.47.091201.145213.

[3] Dunn D.W., Segar S.T., Ridley J., Chan R., Crozier R.H., Yu D.W. and Cook J.M., *PLoS Biol.*, 2008; **6(3)**: e59. DOI 10.1371/journal.pbio.0060059.

[4] Cook J.M. and Segar S., *Ecol. Entomol.*, 2010; **35**: 54-66. DOI 10.1111/j.1365-2311.2009.01148.x.

[5] Cruaud A., Rønsted N., Chantarasuwan B., Chou L.S., Clement W.L., Couloux A., Cousins

S., Genson G., Harrison R.D., Hanson P.E., Hossaert-McKey M., Jabbour-Zahab R., Jousselin E., Kerdelhué C., Kjellberg F., Lopez-Vaamonde C., Peebles J., Peng Y.Q., Pereira R.A.S., Schramm T., Ubaidillah R., Van Noort S., Weiblen G.D., Yang D.R., Yodpinyanee A, Libeskind-Hadas R., Cook J.M., Rasplus J.Y. and Savolainen V., *Syst Biol.*, 2012; **61**: 1029-1047. DOI 10.1093/sysbio/sys068.

[6] Bronstein J.L., *Oikos*, 1991; **61(2)**: 175-186. DOI 10.2307/3545335.

[7] Kerdelhué C. and Rasplus J.Y., *Oikos*, 1996; **75(1)**: 3-14. DOI 10.2307/3546315.

[8] West S.A., Herre E.A., Windsor D.M. and Green P.R.S., *J. Biogeogr.*, 1996; **23**: 447-458. DOI 10.1111/j.1365-2699.1996.tb00006.x.

[9] Patel A., *Biotropica*, 1998; **30(3)**: 474-480. DOI 10.1111/j.1744-7429.1998.tb00082.x.

[10] Karunaratne I., *Cey. J. Sci. (Biol. Sci.)*, 2009; **38(2)**: 67-73. DOI 10.4038/cjsbs.v38i2.1860.

[11] Peng Y.Q., Yang D.R. and Wang Q.Y., *Ecol. Entomol.*, 2005; **30**: 70-77. DOI 10.1111/j.0307-6946.2005.00650.x.

[12] Harrison R.D., *Popul. Ecol.*, 2001; **43**: 63-75. DOI 10.1007/PL00012017.

[13] Clement W. and Ushihara A., *Proceeding of the International Field Biology Course*, Sarawak, Malaysia, 15 July-14 August 2004; 63-71.

[14] Berg C.C. and Corner E.J.H., *Flora of Malesiana. Series I, Volume 17/ Part 2*, National Herbarium Nederland, Universiteit Leiden branch, The Netherlands, 2005.

[15] Whitmore T.C., *Tropical Rain Forests of the Far East*, 2nd Edn., Oxford University Press, Oxford, 1984.

[16] Grison-Pigé L., Bessiere J.M. and Hossaert-McKey M., *J. Chem. Ecol.*, 2002; **28**: 283-295. DOI 10.1023/A:1017930023741.

[17] Proffit M., Schatz B., Borges R.M. and Hossaert-McKey M., *J. Anim. Ecol.*, 2007; **76**: 296-303. DOI 10.1111/j.1365-2656.2007.01213.x.

[18] Elias L.G., Menezes A.O. and Pereira R.A.S., *Symbiosis*, 2008; **45(1-3)**: 107-111.

[19] Lleras C., Path analysis; in Kempf-Leonard K., ed., *Encyclopedia of Social Measurement*, Academy Press, New York, 2005: 25-30.

[20] Kerdelhué C., Rossi J.P. and Rasplus J.Y., *Ecology*, 2000; **81(10)**: 2832-2849. DOI 10.1890/0012-9658(2000)081 [2832:CCESOO]2.0.CO;2.

[21] Suhr D., Step your way through path analysis; Available at: http://www.wuss.org/ proceedings 08/08WUSS%20Proceedings/papers/pos/pos04.pdf

[22] Ullman J.B., *J. Pers. Assess.*, 2006; **87(1)**: 35-50. DOI 10.1207/s15327752jpa8701_03.

[23] Yimkaew J. and Satasook C., *Proceedings of the International Bioscience Conference and the 5th International PSU-UNS Bioscience Conference (IBSC 2014),* Phuket, Thailand, 29-30 September 2014; 44-47.

[24] Cruaud A., Jabbour-Zahab R., Genson G., Kjellberg F., Kobmoo N., Van Noort S., Da-Rong Y., Yan-Qiong P., Ubaidillah R., Hanson P.E., Santos-Mattos O., Farache F.H.A., Pereira R.A.S., Kerdelue C. and Rasplus J.Y., *BMC Evol. Biol.*, 2011; **11**: 178-192. DOI 10.1186/1471-2148-11-178.

[25] Peng Y.Q., Yang D.R. and Duang Z.B., *J. Trop. Ecol.*, 2005; **21**: 581-584. DOI 10.1017/S0266467405002634.

[26] Ranganathan Y., Ghara M. and Borges R.M., *Entomol. Exp. Appl.*, 2010; **137**: 50-61. DOI 10.1111/ j.1570-7458.2010.01038.x.

[27] Hossaert-McKey M., Soler C., Schatz B. and Proffit M., *Chemoecology*, 2010; **20**: 75-88. DOI 10.1007/s00049-010-0043-5.

[28] Harrison R.D. and Shanahan M., Seventy-seven Ways to be a Fig: Overview of a Diverse Plant Assemblage; in Roubik D.W., Sakai S. and Karim, A.A.H., eds., *Pollination Ecology and The Rain Forest: Sarawak Studies*, Springer, New York, 2005: 111-127.

[29] Compton S.G. and Robertson H.G., *Ecology*, 1988; **69(4)**: 1302-1305. DOI 10.2307/1941288.

[30] Chen H.H., Yang, D.R., Gu D., Compton S.G. and Peng, Y.Q., *Ecol. Entomol.*, 2013; **38(4)**: 381-389. DOI 10.1111/een.12030.

[31] Liu C., Yang D.R. and Peng Y.Q., *Entomol. Exp. Appl.*, 2011; **138**: 249-255. DOI 10.1111/j.1570-7458.2011.01096.x.