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Regulation of Fig Wasps Entry and Egress: The Role of Ostiole of Ficus microcarpa L.【Research report】

正榕 (Ficus microcarpa L.) 榕果小孔在控制榕果小蜂鑽入與鑽出時的重要性【研究報告】

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Abstract

Ficus microcarpa L. and its pollinator, Eupristina verticillata Waterston, are obligate mutualists. The ostiole, the only way to connect inside and outside of figs, is very important to regulate the timing of both pollinator entry and all fig wasps egress. Field experiments were conducted on the campus of National Taiwan University in northern Taiwan to assess the effect of the ostiole on the number of pollinators that entered and flew out of figs of different phases. Wings remaining on ostiole at B- and C-phase figs were counted, and controlled experiments at B- and D-phase figs were conducted. Of all figs with wing-remains in the ostioles, 97.5% of early B-phase figs had pollinators inside. Thus, wings remained on ostioles are a good indicator of pollinators in B-phase figs. However, only 63.9% early C-phase figs contained pollinators, the result indicated pollinators probably re-emerge from figs. In the experiment with B-phase figs, significant differences were found between treatments. On average, 95.50 ± 0.73 % of bagged figs dropped from the tree, 76.05 ± 2.98 % of figs with a sealed ostiole dropped and 56.07 ± 3.08 % of control figs dropped. Figs in the ostiole-sealed treatment that remained on the tree were occupied by Odontofroggatia spp. and Walkerella kurandensis Boucek. The oviposition of these non-pollinators did not depend on other wasp species and they caused florets to grow into seed-like structures. These fig wasps prevented fig dropping, thus they are gall-makers. Only 4.95 ± 1.24 fig wasps flew out of D-phase figs in the ostiole-sealed treatment, whereas 57.20 ± 4.06 fig wasps emerged from figs in the ostiole-opened treatment and 38.55 ± 3.65 fig wasps flew out of control figs. The greater number of wasps emerging from opened figs than that of control figs indicates the ostiole is a barrier to egress and causes about 10 % - 20 % mortality of fig wasps.

摘要

正榕 (Ficus microcarpa L.) 與其授粉蜂 (Eupristina verticillata Waterston) 為互利共生之關係。榕果小孔是正榕對外聯繫的唯一管道。由於榕果小孔的開啟時間十分短暫，又是授粉蜂進駐榕果與所有榕果小蜂鑽出時的重要門戶，因此榕果小孔的開閤對榕果小蜂有決定性的影響。本試驗每週於樣區中35株正榕植株上，取樣B、C期榕果，檢視小孔上是否有授粉蜂之遺翅，並於榕果B期及D期時設計控制試驗以證實榕果小孔對其榕果小蜂之重要性。在所有具遺翅的榕果中，97.5 % 的B期榕果具有小蜂於其內，而僅63.9 % 的C期榕果具有小蜂，顯示B期榕果上之小蜂遺翅是代表授粉蜂於榕果內之良好指標，而授粉蜂在C期時無法偵測，可能是其在產卵後有再鑽出，或是屍體夾於小花中無法偵測到。B期控制實驗中三處理具顯著差異，套袋、封口、對照三組落果率分別為95.50 ± 0.73 %，76.05 ± 2.98 % 及56.07 ± 3.08 %，顯示若授粉蜂未進駐，榕果大部分會掉落。套袋及封口組未掉落的榕果中發現有Walkerella kurandensis Boucek 及Odontofroggatia spp.， 顯示此等小蜂不需依賴其他蜂類，即能阻止榕果掉落，為獨立造癭蜂。於D期榕果控制實驗中封口、剝半及對照三組中飛出的榕果小蜂各為4.95 ± 1.24、57.20 ± 4.06及38.55 ± 3.65隻，具顯著差異，由此推知正榕榕果的榕果小孔對於榕果小蜂的鑽出具有決定性的影響，而剝半及對照組之間的差異顯示榕果小蜂自榕果小孔鑽出時約有10 % - 20 % 的死亡率存在。

Key words: Ficus microcarpa, fig wasps, ostiole, controlled experiments, plant-insect interaction.

關鍵詞: 正榕、榕果小蜂、榕果小孔、控制試驗、動植物關係

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Regulation of Fig Wasps Entry and Egress: The Role of Ostiole of *Ficus microcarpa* L.

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ABSTRACT

Ficus microcarpa L. and its pollinator, *Eupristina verticillata* Waterston, are obligate mutualists. The ostiole, the only way to connect inside and outside of figs, is very important to regulate the timing of both pollinator entry and all fig wasps egress. Field experiments were conducted on the campus of National Taiwan University in northern Taiwan to assess the effect of the ostiole on the number of pollinators that entered and flew out of figs of different phases. Wings remaining on ostiole at B- and C-phase figs were counted, and controlled experiments at B- and D-phase figs were conducted. Of all figs with wing-remains in the ostioles, 97.5% of early B-phase figs had pollinators inside. Thus, wings remained on ostioles are a good indicator of pollinators in B-phase figs. However, only 63.9% early C-phase figs contained pollinators, the result indicated pollinators probably re-emerge from figs. In the experiment with B-phase figs, significant differences were found between treatments. On average, 95.50 ± 0.73 % of bagged figs dropped from the tree, 76.05 ± 2.98 % of figs with a sealed ostiole dropped and 56.07 ± 3.08 % of control figs dropped. Figs in the ostiole-sealed treatment that remained on the tree were occupied by *Odontofroggatia* spp. and *Walkerella kurandensis* Boucek. The oviposition of these non-pollinators did not depend on other wasp species and they caused florets to grow into seed-like structures. These fig wasps prevented fig dropping, thus they are gall-makers. Only 4.95 ± 1.24 fig wasps flew out of D-phase figs in the ostiole-sealed treatment, whereas 57.20 ± 4.06 fig wasps emerged from figs in the ostiole-opened treatment and 38.55 ± 3.65 fig wasps flew out of control figs. The greater number of wasps emerging from opened figs than that of control figs indicates the ostiole is a barrier to egress and causes about 10 % - 20 % mortality of fig wasps.

Key words: *Ficus microcarpa*, fig wasps, ostiole, controlled experiments, plant-insect interaction.

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Introduction

An obligate mutualism exists between pollinators (Agaoninae, Agaonidae) and their host fig trees (*Ficus*, Moraceae), each needing the other for reproduction (Galil, 1977; Janzen, 1979). Pollinators and their host fig trees exhibit an almost universal one-to-one host-specificity (Ramirez, 1970; Wiebes, 1986; Berg and Wiebes, 1992; Compton *et al*., 1996). The intra-tree synchrony and inter-tree asynchrony of fig crop production allow pollinators to fly out of male-phase (D-phase) figs and into female-phase (B-phase) figs where they oviposit and pollinate (Ramirez, 1970; Janzen, 1979; Kjellberg and Maurice, 1989; Chen, 1994). Pollinators home in on host tree-specific, volatile compounds that are produced in female-phase figs, and emanate from the ostiole. The pollinator of *Ficus* species must enter the figs to oviposit. The ostiole may be one of the mechanisms that maintain pollinator host specificity (Ramirez, 1974; Galil, 1977; Janzen, 1979). When a fig becomes to B-phase, the fig wall grows up from the base of stem, and the expanded wall results on ostiole loosen for about 1-3 days, the tissue surrounding the ostiole later matures, then the ostiole is closed again. Thus, the short period of ostiole opening is critical for the timing of pollinators entering the figs.

Beside the short period of time for pollinators entering, the ostiole prevents wasps that are not adapted to the ostiolar morphology of a particular *Ficus* species from gaining entry into the fig (Janzen, 1979; Verkerke, 1987, 1989). Fig ostioles have remarkable morphological adaptations to facilitate pollinator entry and clearly exert strong selective pressure on fig wasps that enter the fig to oviposit. The heads of females and males of 18 Agaonine and 28 Sycoecine species that pollinate 26 African *Ficus* species were measured by van Noort and Compton (1996). Ostiole length was the key factor correlated with pollinator head shape. An elongated head appears to be an adaptation to a long ostiole, but it is clearly not necessary to negotiate the short ostiole of small figs. The head of female *Eupristina verticillata* Waterston is flat and depressed, like a shovel, and the mandible is saw-shaped. This morphology helps *E. verticillata* females burrow through the ostiole (Hsieh, 1992).

Based on the short opening period and particular morphology, ostioles may be an important selective force to pollinator foundresses. Except pollinators, there are many non-pollinators living in figs, they oviposit eggs through fig walls directly. In these non-pollinators, there are gall-makers, parasitoids and inquilines. If pollinators are prevented from entering by sealing the ostiole, which kind of non-pollinators will be affected? We try to conduct controlled experiments with the ostioles of *Ficus microcarpa* L. B-phase figs to assess their role in regulating the entrance of pollinators.

Female pollinators work their way through the ostiole using their forelegs and antennae. Their wings are shed at the ostiole entrance as they enter the fig. Is this a meaningful of this behavior of pollinators? Furthermore, if the frequency of wings remaining on ostioles is high, we may use the wing-remains on ostioles to predict whether pollinators have been inside the figs. However, wings will remain for 1-2 weeks (unpub. data), so they will stay on ostioles till figs become to C-phase. When is the best time to examine *F. microcarpa* figs with wing-remains on the ostiole for pollinators? Some female pollinators burrow out of B-phase figs in which they have oviposited, and search for another fig to enter (Gibernau *et al*., 1996; Yao, 1998; Chen, 2000). Does *E. verticillata* behave this way? Will the wings drop when they burrow out of figs? We analyzed the correlation between wing-remains on ostioles and pollinator foundresses inside the figs at B- and C-phases.

Pollinators lay eggs in florets and die

in the fig. The offspring pupate as the figs enter the male phase (Galil and Eisikowitch, 1968). Males emerge from galls before females. They search for galls with female pollinators and mate with females while they are still inside their gall. Then males burrow a tunnel through the ostiole creating an open passage to the outside (Galil *et al*., 1973; Frank, 1984). All fig wasps except wingless males leave through the ostiole and fly off to look for female-phase figs (Hsieh, 1992). Thus, in *F. microcarpa*, the ostiole regulates fig wasp entry during female-phase, and is an essential exit for females to leave male-phase fig. In some dioecious species of *Ficus*, the ostiole automatically opens at male-phase, and male florets grow surround the ostiole. Fig wasps will be stuck pollens while flying off. This is the strategy of passive pollination (Yao, 1998). However, male florets grow dispersedly in a fig of *F. microcarpa*, monoecious species, pollinators have to get pollens by themselves. Thus, must pollinators leave male-phase figs through ostioles? Do they burrow through fig walls directly?

The ostiole of *F. microcarpa* figs is a very important physical barrier to fig wasps as they enter or leave figs. We can see evolutionary traits between fig and fig wasps by observing the behavior of fig wasps passing through ostioles. Remaining wings of pollinators on ostioles at B-phase is not a passive behavior, we believe there are some evolutionary significance of such a particular behavior. If pollinators do not enter figs on time, will all figs drop? Or are there another effects by non-pollinators? Besides, is the ostiole the only way that offers all fig wasps out at D-phase? Is there any exception to this situation? If all fig wasps can only fly off through ostioles, the ostiole must evolve a particular physiological character to determine the behavior of fig wasps. In this study, we determined the effect of the ostiole on fig wasps of *F. microcarpa* L.

Materials and Methods

Study field was on the campus of National Taiwan University in Taipei, figs on 35 trees of *F. microcarpa* were observed. A number of experiments were performed to elucidate the relationship between figs, their pollinators and non-pollinators. Our preliminary test showed that when bracts of an ostiole are loosened, a needle can be inserted from the ostiole entrance to the central cavity inside the fig. The needle cannot be inserted into the cavity of figs if the ostiole is closed.

1. Index of wing remains

Figs on 35 trees of *F. microcarpa* in the study field were observed weekly from August 1992 to December 1998 for six and half years. Approximately 5-10 figs on each tree were collected randomly if we observed B- and C-phase sampled trees. For each fig the (1) phase, (2) presence of pollinator wings in the ostioles, and (3) presence of pollinators inside were recorded. χ^2-test was used to determine when wing remains in the ostiole and pollinators in the fig are most closely correlated.

2. The role of the ostiole in pollinator entry and fig retention

Crops were randomly chosen in 35 sampled trees. In this experiment, the ostiole was modified to three treatments for examining its effect on pollinator entry and determing the effect of pollinator occupation on fig retention. (1) Bagging treatment--- Net bags were used to enclose 50-70 A-phase figs on 3-5 small branches, to prevent all wasps entering the figs. (2) Ostiole-sealed treatment--- Resin was used to seal the ostiole of 50-70 late A-phase figs that had not yet been occupied by pollinators, to prevent only pollinators from entering the figs. (3) Control---50-70 A-phase figs on branches close to those harboring the figs in (1) and (2) were marked and left undisturbed. When figs in each treatment reached the male phase, about one month or more, they were collected and taken back to laboratory. To determine which species of fig wasps in galls, the figs were dried in an oven.

3. Role of the ostiole in wasp emergence from figs

Our preliminary test revealed that if figs were picked from a tree, they developed normally in 1-2 days. D-phase figs will suddenly expand, and the ostiole was loosened again by the needle test. The ostiole entry is slightly reddish and the figs become softer. The morphological changes indicate that fig wasps are going to emerge in 1-2 days (Hsieh, 1992). Comparing figs in the field to carried figs at this time, there were no significant differences in the number of emerging fig wasps between figs in the field and laboratory. This result allowed us to take figs into the laboratory to perform some simple treatments.

To determine how the ostiole affects fig wasp emergence from figs, 40 D-phase figs were assigned to each of three treatments: (1) The ostiole of each fig was sealed with glue to prevent fig wasps from leaving the fig by the normal route; (2) Each fig was pulled apart into two pieces by hand to allow fig wasps to leave the fig without the constraints imposed by the ostiole; (3) Control figs. Figs were collected 1-2 days before females were ready to leave the fig and each was placed in a bottle following treatment. After 3 days, when all fig wasps had flown off in the bottles, we counted the number of emergent fig wasps and the number of tunnels made in the walls of each fig by fig wasps. The number of fig wasps in each treatment was compared using Fisher's least significant difference (LSD) multiple range tests.

Results

1. The opening time of ostioles and wing-remain index

Generally speaking, the B-phase lasts 1 week but the ostiole is open for only the first 1-3 days actually in summer-fall seasons. That is, the ostiole of figs is open at early B-phase (Fig. 1a). Most pollinators that attempt to enter early B-phase figs are able to do so, while those that attempt to enter late B-phase figs are seldom successful. If pollinators did not enter, B-phase figs will drop after one week in summer-fall seasons. However, the situation is different in winter-spring seasons. If pollinators did not enter the figs, the ostiole would keep loosening for longer time for over one week. Interestingly, the length of time the ostiole is open depends, in part, on how long it takes pollinators to enter and pollinate the fig. The ostiole is finally closed in 1-2 weeks if no pollinators entering and the diameter of the fig is larger than normal size. The figs staying on trees for over 2 weeks look yellow-greenish, like dropped figs, and all dropped in several days with higher temperature. The situation occurred in winter-spring seasons when pollinators population is very low. However, the ostiole is soon closed in half to one day after the first pollinator has pollinated the fig in any seasons. Pollinators that attempt to enter after this time may be stuck between the bracts of closing ostiole.

Pollinator wings were found in the ostioles of early B- and early C-phase figs (Fig. 1b). Pollinators were found in 94.5% of the early B-phase figs and 63.9% of the early C-phase figs that had wing in the ostiole. In early B-phase figs, the presence of wing and pollinators were highly correlated (χ^2-test, $p < 0.01$) (Table 1). However, it is interesting to note that 36.1% early C-phase figs with wings on ostioles had no pollinators inside. The florets in all figs with wing-remains on ostioles will become to galls and seeds, the result proved that these figs have been oviposited and pollinated. Pollinators oviposit in some florets and pollinate florets they climb, finally they die inside the fig and the bodies are broken down in 2 weeks. The wings will last for about 2 weeks then disappear.

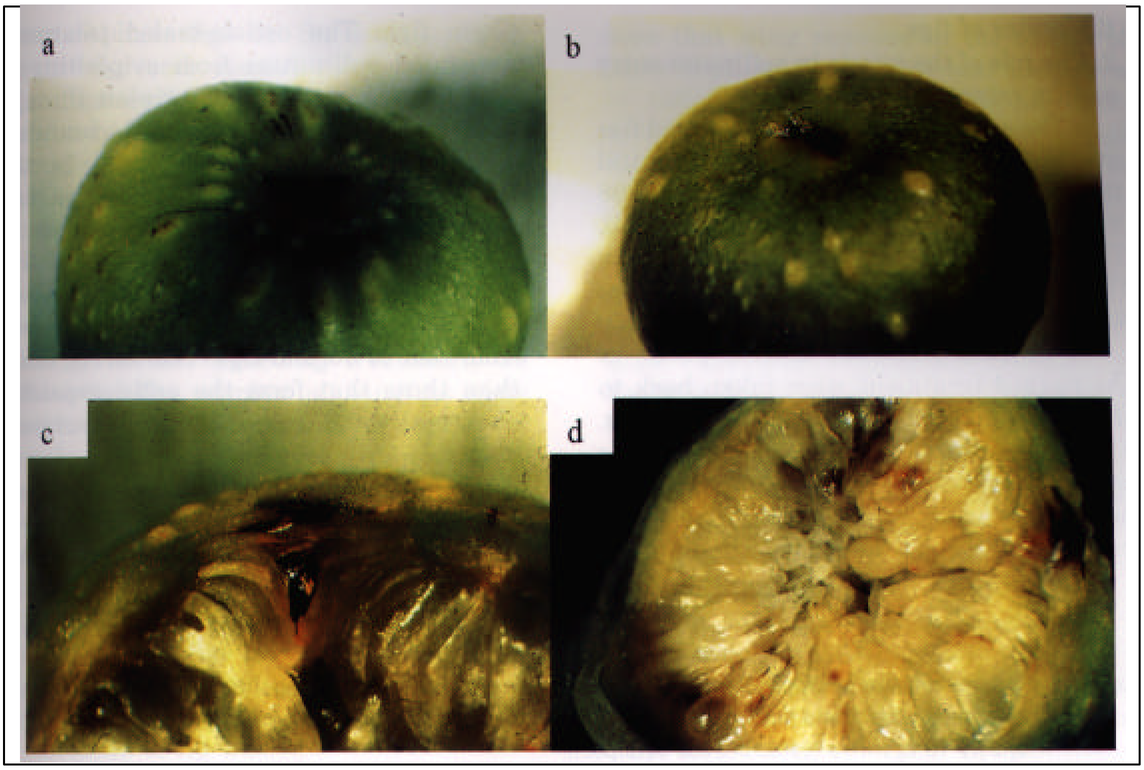

Fig. 1. Ostiole morphology of figs at early B- and C-phase. (a) The ostiole of figs is loosened at early B-phase, three bracts on the ostiole are not overlapped and like " Y" shape from top view of the ostiole. (b) Wings of pollinators remain on the ostiole while pollinators entering at early B-phase. (c) A pollinator was stuck between bracts of ostioles at early C-phase figs, its head direction is toward to outside the fig. Three bracts on the ostiole are overlapped. (d) The cavity of fig center disappeared at C-phase.

Table 1. Wing remains and pollinators in B- and C-phase figs. Wing remains and pollinators were highly correlated in B-phase figs (χ^2- test, $p < 0.001$)

Phase	Wing-remains	Figs with pollinators	Figs without pollinators
B	With wings	155	9
	Without wings	3	274
C	With wings	69	39
	Without wings	16	219

When we checked the wing remaining on early C-phase figs, we found a lot of pollinators died between bracts of the ostiole. With the 137 early C-phase figs with only one pollinator inside, there were 58.4% figs in which pollinators died between the bracts of ostioles, another 41.6% figs in which pollinators died in the center of figs. Some pollinators died between bracts were facing the outside (Fig. 1c). This result shows that pollinators probably were trying to re-emerge from the figs. The cavity in the fig center finally disappears at C-phase (Fig. 1d), the bodies of pollinators inside figs are broken up and cannot be found when florets grow up to fill the center of figs.

2. The role of the ostiole in pollinator entry and fig retention

The average percentage of dropped figs in the bagged, ostiole-sealed and control treatments was 95.50 ± 0.73 %, 76.05 ± 2.98 % and 56.07 ± 3.08 %, respectively (Table 2). The rates of fig drop among B-phase figs in the bagged, sealed and control treatments were significantly different.

Figs that remained until D-phase in the bagged treatment were taken back to the laboratory for drying and dissecting. These figs were occupied by *Walkerella kurandensis* Boucek. Because the figs were bagged during early B-phase, *W. kurandensis* must had oviposited at A-phase figs. The ostiole-sealed treatment prevented pollinators from ovipositing in figs, but other fig wasps oviposit through the walls. Figs that stayed on the branches were collected during D-phase. In the laboratory these figs were dissected and *Odontofroggatia* spp. were found inside.

The morphology of the florets of figs occupied by the offspring of *W. kurandensis* and *Odontofroggatia* spp. was different from that of normal figs. The florets, other than those that form the galls, expanded abnormally and looked like seeds, but were empty.

3. Role of the ostiole in wasp emergence from figs

The number of fig wasps emerging

Table 2. The percentage of dropped figs (%) in bagged, ostiole-sealed and control treatments. No significant difference were found among the three crops. Means of each treatment in total three crops are listed and 50-73 figs were tested in each data point.

Crop duration	Bagged (%) (*n*)	Sealed (%) (*n*)	Controlled (%) (*n*)
First crop[1]			
1	90.5 (63)	62.5 (64)	62.5 (64)
2	94.1 (68)	87.5 (64)	48.3 (58)
3	97.1 (69)	84.6 (65)	62.7 (59)
4	94.4 (72)	61.5 (65)	47.1 (68)
5	94.4 (54)	86.4 (59)	69.6 (69)
Mean ± SE	94.10 ± 1.05c*	76.50 ± 5.94b	58.04 ± 4.41a
Second crop[2]			
1	98.5 (63)	77.1 (55)	54.7 (53)
2	94.2 (68)	62.8 (64)	42.3 (53)
3	97.4 (73)	78.1 (68)	48.5 (71)
Mean ± SE	96.70 ± 1.29c	72.67 ± 4.94b	48.50 ± 3.58a
Third crop[3]			
1	97.1 (69)	84.6 (65)	72.7 (59)
2	94.4 (72)	73.2 (56)	47.1 (68)
3	98.4 (54)	78.3 (50)	61.3 (62)
Mean ± SE	96.63 ± 1.18c	78.70 ± 3.30b	60.37 ± 7.40a
Total mean ± SE	95.50 ± 0.73c	76.05 ± 2.98b	56.07 ± 3.08a

* Means in the same row followed by the different letter are significantly different by Fisher significant difference (LSD) multiple range tests ($p < 0.05$).

1. 1994.03.04.–04.28.
2. 1995.09.09.–10.27.
3. 1996.07.16.–08.21.

from D-phase figs in the ostiole-sealed, opened-fig and control treatments was significantly different. In the ostiole-sealed treatment, only 4.95 ± 1.24 fig wasps emerged from each fig, whereas 57.20 ± 4.06 and 38.55 ± 3.65 fig wasps emerged from each fig in the opened-fig and control treatments, respectively (Fig. 2). Because some wingless male wasps were found in the bottles with emergent females, especially in the opened-fig treatment, we subtracted the number of wingless male wasps in each treatment. The number of female pollinators emerging from each fig in the ostiole-sealed, opened-fig and control treatments was 4.85 ± 1.18, 48.35 ± 3.09 and 37.80 ± 3.62, respectively, and the differences between treatments were still significant. The greater number of wasps emerging from figs in the opened-fig than control treatment indicates the ostiole is a barrier of egress and causes about 10 % - 20 % mortality of fig wasps.

Discussion

Comparing the correlation of wings on ostioles and pollinators inside, we were sure that wing remains in the ostiole of early B-phase figs are the best indication of pollinators inside. This useful information can be used as a quick assessment the status of figs. We may record directly that as the percentages of figs occupied by pollinators on trees. By this way, the pollinator population in the field can be predicted as soon as possible. Wing remains on ostioles at B-phase are very good indicators of pollinator occupation. From a distance, pollinators locate trees with B-phase figs by following plumes of volatile chemicals emitted by the figs citation. At close range, fig wasps use vision to find B-phase figs (Hosseart-Mckey *et al*., 1994). Wings of pollinators at the entrance of the ostiole probably indicate that the fig has been occupied. There is no slit on wings according to the morphological illustration by Chou and Wong (1997), this suggests that it is not easy to shed their wings by pollinators themselves. The wings of pollinators were not broken up naturally. It is possible that wings were broken away by the tissue of ostioles, or by themselves when pollinators enter figs.

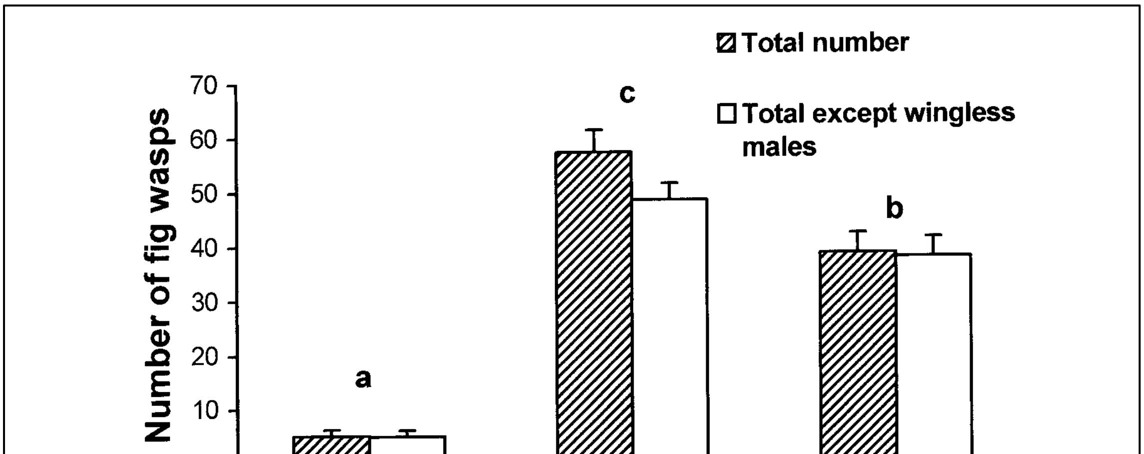

Fig. 2. The total number of fig wasps (hatched bars) and total number except wingless males (white bars) in D-phase figs in the ostiole-sealed, opened-fig and control treatments were significantly different (by Fisher least significant difference (LSD) multiple range test, $p < 0.05$). Sampled size was 40 figs in each treatment.

If the function of marking figs with wing remains is to encourage pollinator dispersal to occupy (and the truth is 1-2 pollinators occupy each fig on average), there must be some advantage, to the figs and/or pollinators, to dispersing. Herre (1985, 1987) considered that too many pollinators (e.g. more than 5 pollinators) occupying the same fig are disadvantageous to figs in producing seeds. For *Ficus*, seed production is directly related to fitness. By limiting the number of pollinators in each fig, seed production and fitness can be optimized (Herre, 1985, 1987). Closing the ostiole as soon as possible is one method *Ficus* uses to control excessive pollinators.

Because pollinator siblings mate in the fig, inbreeding, which may be important for retention a specific morphology, is common. Out-breeding occurs occasionally in figs occupied by two or more foundresses. However, it is difficult to determine whether all foundresses which occupy in a fig are from the same mother. Even the situation occurs, there is probably that the foundresses were siblings because *E. verticillata* is a weak flyer. Thus, pollinators of *F. microcarpa* seem to have an inbreeding strategy.

In addition, we were interested in the information of the C-phase figs with wing remains in the ostioles, 36.1% were not occupied by pollinators. It is reasonable that more pollinators are found inside figs than wings on ostioles. However, why there were wings on ostioles but pollinators were not detected? There are two possible explanations for this. (1) The bodies of pollinators are broken up and could not be detected. As the figs grow, florets expand and crowd the center of the fig. The bodies of foundresses will be broken up or stuck between florets, and are too hard to be observed. The situation was occurred in *Ficus microcarpa*, *Ficus pumila* and *Ficus irisana* in Taiwan (personal observation). (2) It is also possible that some pollinators re-emerge. Gibernau *et al.* (1996) found that some pollinators exit, or try to exit, from the fig after pollination and oviposition. Those that left were able to oviposit successively in two different figs. In Taiwan, pollinators of *Ficus pumila* and *Ficus irisana* frequently re-emerge after oviposition (Yao, 1998; Chen, 2000). We didn't observe *Eupristina verticillata* leaving *F. microcarpa* in this way directly, however, 36.1% C-phase figs with wing remains in the ostioles were not found the occupying pollinators although pollinators have been oviposited eggs and pollinated inside. In addition, of 137 figs with one dead pollinator inside, 58.4% pollinators stuck between bracts of ostioles, and the head direction in some of them oriented themselves toward to outside the figs. These results imply that pollinators probably re-emerge. Although we don know why they re-emerge from the figs, the probability is high that they would attempt to enter another figs.

The figs on one trees are at the same phase, a pollinator can easily find another fig closed to the first fig which she has pollinated. However, the ostioles are sooner closed after the first pollinators entering, these re-emerged pollinators are difficult to enter the second figs because the ostioles have been almost closed simultaneously. Almost all re-emerged pollinators failed to drop on the ground and we have never directly investigated any pollinator is going to enter the second fig, but we still cannot exclude the very low possibility that they may enter the second figs successfully.

Half of the control figs dropped. This treatment showed the normal percentages of dropped figs at the time of experiments. The three crops were in March 1994, September 1995 and July 1996 and there were no significant differences among them. The information shows that the percentages of dropped figs in nature are high, about 50% or higher. In bagged and sealed treatments, both preventing pollinator entry, the percentages of dropped figs were significantly higher. Unpollinated figs in these two treatments were occupied by *Walkerella kurandensis* or *Odontofroggatia* spp. *Walkerella kurandensis* oviposits eggs on figs at A-phase when we had not bagged figs, they are gall-makers who oviposit figs at the most early time. *Odontofroggatia* spp. were dissected in figs of sealed treatment, they mature in figs without other fig wasps and oviposit at B- or C-phase. These wasps can develop in figs independent of pollinators because they prevent figs dropping. The majority of florets, except for galls and blasted florets, were expanded, like seeds, but were empty inside. Gall-makers exploit the resources of figs but provide no benefit to the plant. Like pollinators, they parasitize some of the florets and so occupy the same feeding niche, these gall-makers and pollinators probably compete.

From the significant difference between two treatments, we infer the population size of *Walkerella kurandensis* is smaller than that of *Odontofroggatia* spp. It could be accepted because the frequency of appearance of *Odontofroggatia* spp. in the field is higher than that of *Walkerella kurandensis* (Chen, 1994). Furthermore, the number of dropped figs in bagged treatment is lower than sealed one, the result implied the population of *W. kurandensis* is lower than that of *Odontofroggatia* spp.

The fig ostiole is also critical during D-phase in *F. microcarpa*. The ostiole is the easiest means of egress for all fig wasps adults, even some fig wasps die before they escape. Opening of the ostiole during D-phase is critical for the fig wasps. In the ostiole-sealed treatment, most male pollinators burrow in, or very close to, the ostioles. With few exceptions, all the fig wasps in this treatment died in the figs. The difference between the number of female wasps emerging from figs in the controlled and opened-fig treatments indicates that fig wasps have some difficulty emerging from D-phase figs, and that some die in the process. Why must fig wasps exit through the ostiole in *F. microcarpa*? Why fig wasps of *F. microcarpa* did not emerge from D-phase figs directly through fig walls? From the developmental morphology of figs of *F. microcarpa*, we suggest two possibilities to explain why fig wasps have to exit only through ostioles:

Ostiole is loosen in 1-3 days The fig wall belongs to the tissue of receptacle. The receptacle grows up from the base nearby stem, the fig expands but the tissue surround the ostioles is the last place to grow up. Thus, the ostiole will loose for the first 1-3 days while growing up to the next phase because of developmental process, then closes. Figs at early D-phase, they expand suddenly and the ostioles lossen, the bracts in the ostiole are perhaps softer than the fig walls at this time to wingless males to burrow. This time is when wingless males start to burrow tunnels, they would chose the more convenient site, ositiole, to burrow out. Because of developmental process of ostiole, the ostiole is a quick way for all fig wasps to egress. In some dioecious *Ficus* species (e.g. *Ficus pumila*), the ostiole even opens automatically to facilitate the emergence of fig wasps (Lu *et al*., 1987).

Fig wall is difficult to burrow out There are a lot of milky juice in the tissue of fig walls, wingless males are hard to burrow through fig walls successfully. They are probably glued by milky juiceand die. The same as the fig wasps who will leave from figs, they will also encounter the same question like wingless males. The mortality of fig wasps of burrowing through fig wall should be much higher than that through ostioles. The tissue of fig wall is not suitable to fig wasps to burrow and emerge successfully. These two possibilities can completely explain why fig wasps only chose the ostiole to emerge out.

The strategies of fig wasps emerging

are different among *Ficus* species. The ostioles of *F. pumila* open automatically, fig wasps fly out from ostioles easily. Ostioles of *F. virgata* are never opened at D-phase, figs wasps in figs emerge from thin fig wall directly (personal observation). In *F. microcarpa*, fig wasps have to dig tunnels through ostioles by themselves. 10-20% mortality occurred in this emerging process. However, even there was a mortality of fig wasps while emerging from figs, the mortality can be suffer because the risk of emerging from ostioles are less than that from fig walls. For evolutionary respects, the mortality of fig wasps has not become a selective force of affecting the long-term fitness of fig wasps. Thus, such fixed action behavioral traits of fig wasps emerging from ostioles are kept among generations.

The function of the fig ostiole is critical during B- and D-phases in *F. microcarpa*. It is a physical barrier that prevents too many pollinators from entering the fig, and it enhances pollination by trapping those pollinators that do enter, inside the fig. Sealing the ostiole prevents from pollination but does not stop gall-makers from ovipositing through the fig wall. During D-phase, the ostiole is evolved the only and easiest way of egress for all fig wasps, even some fig wasps will die in the process of emergence from figs. For *Ficus*, prevent too many pollinators entering and control all fig wasps emerging out by only one way, ostiole, can be easier to evolve.

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正榕 (*Ficus microcarpa* L.) 榕果小孔在控制榕果小蜂鑽入與鑽出時的重要性

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摘　　要

正榕 (*Ficus microcarpa* L.) **與其授粉蜂** (*Eupristina verticillata* Waterston) **為互利共生之關係。榕果小孔是正榕對外聯繫的唯一管道。由於榕果小孔的開啟時間十分短暫，又是授粉蜂進駐榕果與所有榕果小蜂鑽出時的重要門戶，因此榕果小孔的開闔對榕果小蜂有決定性的影響。本試驗每週於樣區中** 35 **株正榕植株上，取樣** B、C **期榕果，檢視小孔上是否有授粉蜂之遺翅，並於榕果** B **期及** D **期時設計控制試驗以證實榕果小孔對其榕果小蜂之重要性。在所有具遺翅的榕果中，**97.5 % **的** B **期榕果具有小蜂於其內，而僅** 63.9 % **的** C **期榕果具有小蜂，顯示** B **期榕果上之小蜂遺翅是代表授粉蜂於榕果內之良好指標，而授粉蜂在** C **期時無法偵測，可能是其在產卵後有再鑽出，或是屍體夾於小花中無法偵測到。**B **期控制實驗中三處理具顯著差異，套袋、封口、對照三組落果率分別為** 95.50 ± 0.73 %**，**76.05 ± 2.98 % **及** 56.07 ± 3.08 %**，顯示若授粉蜂未進駐，榕果大部分會掉落。套袋及封口組未掉落的榕果中發現有** *Walkerella kurandensis* Boucek **及** *Odontofroggatia* spp.**，顯示此等小蜂不需依賴其他蜂類，即能阻止榕果掉落，為獨立造癭蜂。於** D **期榕果控制實驗中封口、剝半及對照三組中飛出的榕果小蜂各為** 4.95 ± 1.24、57.20 ± 4.06 **及** 38.55 ± 3.65 **隻，具顯著差異，由此推知正榕榕果的榕果小孔對於榕果小蜂的鑽出具有決定性的影響，而剝半及對照組之間的差異顯示榕果小蜂自榕果小孔鑽出時約有** 10 % - 20 % **的死亡率存在。**

關鍵詞：正榕、榕果小蜂、榕果小孔、控制試驗、動植物關係。