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The effect of pedicel length in regulating seed and wasp production in the fig-wasp oblígate mutualism of Ficus tuerkheimii (Moraceae) and Agaonidae.

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Department of Biology, University of Wisconsin-Madison

Abstract

Pedicel and style lengths of fig flowers were measured in order to determine their role in regulating seed and wasp production within a *Ficus tuerkheimii* fig. Figs were collected in two groups at two different stages in development. Foundress number, foundress ovipositor length, pedicel lengths, and style lengths were measured (to the nearest micrometer) in the younger figs. Flower pedicel lengths were measured (to the nearest micrometer) and flower occupancy was recorded in mature, but unripe figs. Young figs: In a sample size of 10 young figs, 60% had two foundresses, 20% had one, and 20% had three. The average ovipositor length in 20 foundresses was 10.16 micrometers. Average style length was about 11 micrometers. Based on style length frequency, the predicted wasp: seed ratio is 44%: 55% respectively. Mature figs: the observed wasp: seed ratio is 45.9% female, 4.3% male, 37.35% vacant, and 12.45% seeds. A significant negative correlation was found between style lengths and pedicel lengths ($r^2 = .495$, $P < .0001$). A simple regression analysis between the number of flowers at each pedicel length and between the proportion of female wasps, male wasps, seeds, and vacancies. There is a significant negative correlation between flower number and percent females ($n = 118$, $r^2 = .081$, $P = .0376$). There are not enough male wasps to draw a significant correlation ($n = 11$, $r^2 = .02$, $P = .3103$) between flower number, pedicel length, and percent males, even though the graph does show a less defined, but very similar negative trend for the male wasps. There are also not enough seeds to draw a significant correlation ($n = 32$, $r^2 = .009$, $P = .4926$) between the amount of flowers and pedicel length, however the graph shows a slight positive correlation. There is however a strong positive correlation between percent vacancies and flower number ($n = 96$, $r^2 = .134$, $P = .0066$). As flower number increases, there are more flowers with short pedicels and long styles which produce a high number of vacancies. In conclusion, this study demonstrates that pedicel length and thus style length do play a major role in regulating seed and wasp production and are therefore important factors in maintaining the mutualism; however, many other factors such as foundress number, ovipositor length, egg limitation, and pollen limitation are confounded with style length and must be taken into consideration. Currently, in *F. tuerkheimii*, wasps have the upper hand.

Resumen

Pedicel y las longitudes del estilo de las flores del higo fueron medidos para determinar su papel en la producción de regulación de la semilla y de la avispa dentro de un higo del tuerkheimii de Ficus. Los higos fueron recogidos en dos grupos en dos diversas etapas en el desarrollo. El número de la fundadora, la longitud del ovipositor de la fundadora, las longitudes del pedicel, y las longitudes del estilo fueron medidos (al micrómetro más cercano) en los higos más jóvenes. Las longitudes del pedicel de la flor fueron medidas (al micrómetro más cercano) y la ocupación de la flor fue registrada en los higos maduros, pero inmaduros. Higos jóvenes: En un tamaño de muestra de 10 higos jóvenes, el 60% tenían dos fundadoras, el 20% tenían uno, y el 20% tenían tres. La longitud media del ovipositor en 20 fundadoras era 10.16 micrómetros. La longitud media del estilo era cerca de 11 micrómetros. De acuerdo con frecuencia de la longitud del estilo, la avispa predicha: el cociente de la semilla es el 44%: el 55% respectivamente. Higos maduros: la avispa observada: el cociente de la semilla es 45.9% la hembra, 4.3 % varón, 37.35% vacantes, y 12.45% semillas. Una correlación negativa significativa fue encontrada entre las longitudes del estilo y las longitudes del pedicel (r^2 = el 495, P < .0001). Un análisis de regresión simple entre el número de flores en cada longitud del pedicel y entre la proporción de avispas femeninas, de avispas masculinas, de semillas, y de vacantes. Hay una correlación negativa significativa entre el número de la flor y las hembras de los por ciento (n = 118, r^2 = 081, P = 0376). No hay bastantes avispas masculinas para dibujar una correlación significativa (n = 11, r^2 = 02, P = 3103) entre el número de la flor, la longitud del pedicel, y los varones de los por ciento, aunque el gráfico demuestra haber definido menos, solamente la tendencia negativa muy similar para las avispas masculinas. No hay también bastantes semillas para dibujar una correlación significativa (n = 32, r^2 = 009, P = 4926) entre la cantidad de flores y la longitud del pedicel, no obstante el gráfico demuestra una correlación positiva leve. Hay sin embargo una correlación positiva fuerte entre las vacantes de los por ciento y el número de la flor (n = 96, r^2 = 134, P = 0066). Pues el número de la flor aumenta, hay más flores con los pediceles cortos y los estilos largos que producen un alto número de vacantes. En la conclusión, este estudio demuestra esa longitud del pedicel y labra así longitud desempeña un papel importante en la producción de regulación de la semilla y de la avispa y es factores por consiguiente importantes en mantener el mutualismo; sin embargo, muchos otros factores tales como número de la fundadora, longitud del ovipositor, limitación del huevo, y limitación del polen se confunden con longitud del estilo y se deben tomar en la consideración. Actualmente, en tuerkheimii del F., las avispas tienen la mano superior.

Introduction

Fig trees and their wasp pollinators are found in tropical and subtropical areas around the world. There are about 900 known fig species worldwide, and sixty of those are found in Costa Rica, most commonly found at an elevation of 2,500m. The fig tree, a keystone species, is a valuable member of its community. One special characteristic of the fig tree that allows it to remain a keystone species is its fruiting capabilities. It provides fruit for a large variety of species at staggered intervals throughout the year when other food sources are scarce. The obligate mutualism between figs (Ficus spp., Moraceae) and their pollinator wasps (Hymenoptera: Chalcidoidea: Agaonidae: Agaoninae) is thus extremely important and one of the most unique interspecific interactions found in nature. (M. Nadkarni and T. Wheelwright, 2000).

Each species of fig is pollinated by a unique species of Agaonidae fig wasp and no other insect. This is a unique characteristic that ensures the fig trees survival by limiting pollinator competition (J.C. Nefdt and G. Compton, 1996). The most fit pollinator for the job will not be threatened by other pollinators who do not necessarily always pollinate fig trees since figs are not crucial for their survival. Pollination occurs when a female wasp with pollen and squeezes through the fig ostiole, or small opening, to reach the enclosed flowers of the syconium (inflorescence, fig 1). This process shears the wings off the wasp. The ostiole closes shortly after her arrival and she is trapped inside the syconium. Inside the fig, the wasp pollinates the stigmas while she lays eggs in the styles of the female flowers which provide ovaries to feed the offspring (J.C. Nefdt and G. Compton, 1996). After approximately one month, wingless male agaonid offspring crawl out of the flowers, inseminate the females still in the florets, and then chew an opening out of the fig to escape. The mated females emerge from the florets and leave the fig via the chewed opening without damaging their wings. As the female leaves, she takes with her pollen from the anthers on the male florets and will continue the cycle by pollinating another receptive fig tree (Janzen 1979, Fig 1).

The mystery lies within the flowers. It is uncertain which flowers become seeds and which becomes wasps (and why). The fate of the flowers however reflects the conflict within the mutualism: both the fig and the wasp must utilized the (same number of) available flowers to ensure its own survival, and both species would like to utilize more flowers than the other. Janzen, 1979 argues that the number of seeds and wasps within a fig is controlled by the plant itself. He states that there are distinct long and short styled flower types within a fig, used as a mechanism to avoid complete flower utilization by pollinator wasps, and thus ensure fig survival. He explains that wasps will preferably lay their eggs when their ovipositor touches the ovule at the bottom of the style since the ovule is a food source for her offspring. He theorizes that a wasp's ovipositor length, in addition to the flower's style length dichotomy, will determine flower utilization. Short styles will produce wasps and long styles will produce seeds, resolving the conflict within the mutualism.

However further studies post Janzen find a unimodal frequency distribution of style lengths showing no distinct groupings of short styles and long styles in fig flowers, but a wide variety of lengths (Bronstein 1988b). Further comparing ovipositor length to style length, (indicates that) many species may produce only wasps. Bronstein, 1988b found that on average 82% of all styles per fig in *Ficus pertusa* were shorter than or equal to the foundress wasp's average ovipositor length. If style length was the only factor in wasp and seed production, 82% of the fig would be expected to produce wasps and 18% would be expected to produce seeds, creating a slight disadvantage to the fig tree. Bronstein (1988b) observes that this theoretical model does not hold true in *F. pertusa*. Most *F. pertusa* figs actually contain 23% wasps, 24% seeds, and the remaining 53% vacant, with neither wasps nor seeds, even though wasp ovipositors were long enough to reach 82% of the styles. Bronstein concludes that for *F. pertusa*, perhaps there is a limitation of eggs carried in by the wasp. Egg limitation is possibly a result of foundress limitation. Perhaps figs in *F. pertusa* restrict foundress number entries (through earlier ostiole closure) in order to prevent wasp eggs from killing every flower.

A similar study (Nefdt and Compton 1996), investigating the role of egg limitation in 11 species of African figs discovered that when more pollinator foundresses were experimentally introduced per fig, more styles (%? Exact amount is not recorded) were utilized for egg deposition regardless of style length. (Observations of the artificially increased ovipositing wasps under the microscope showed that the wasps would first utilize the short-styled flowers, but would move on to the longer-styled flowers when none of the former were available). They concluded that foundress number restriction maintains mutualism stability, not style length, as females are egg limited.

While most figs contain only one foundress, such as 77% of *F. burttdavyi* figs in Nefdt and Compton's 1996 African fig study, *F. tuerkheimii* in the Monteverde and La Cruz area of Costa Rica contain multiple foundresses as a rule. Twenty-five figs in a sample size of 30 figs contained four foundresses and 16 figs of another 30 fig sample size contained three foundresses (Hogan 1994, student project). If the artificially increased foundress number in the African species increased wasp number per fig, while lowering seed number, perhaps this could be happening on a regular basis for *F. tuerkheimii* which naturally contain more foundresses per fig. If so, what are the other factors controlling seed set to ensure that at least some flowers become seeds?

This study will examine possible factors that ensure a sufficient amount of seeds within *Ficus tuerkheimii*, despite the high foundress number. Factors under investigation include the relationship between pedicel length and style length in fig flower utilization, in addition to factors that contribute to egg limitation such as foundress number and ovipositor length. Both pedicel length and style length are predicted to determine the 'fate' of the fig ovule: wasps should reside in the styles their ovipositors can reach, (shorter styles with long pedicels), and seeds should reside in the longer styles with short pedicels where average ovipositors cannot reach.

Materials and Methods

Fig collections

Figs were collected at two different stages in development from two randomly selected Ficus tuerkheimii trees at the Sapo Dorado hotel in the lower mountain wet forest of Monteverde at 1400m, in Costa Rica. Two data sets were assembled from the collections. The first consisted of larger, softer figs that entered the maturation period in which the foundress had died and larval development began. The second set was collected from the same tree, and another randomly selected tree nearby. This set consisted of smaller, harder figs, right before the maturation period of larval development just after the 'receptive stage' of the fig.

Foundress number and ovipositor length versus style length comparisons

Ten figs in the receptive stage were first sliced in vertical thirds. The middle third was left intact to preserve the foundress (es) located in the center. The foundress(es) were extracted with a pin and ovipositor length was measured to the nearest micrometer under a dissecting microscope. All three fig pieces were halved and ten flowers were randomly selected. Style lengths were measured while flowers remained attached to fig wall. Mean style lengths can vary slightly in individual figs (Compton & Nefdt 1989). Comparisons of average ovipositor length with the frequency distribution of style lengths estimated the proportion of flowers potentially accessible to the wasp, or the proportion of flowers that will become wasps. Potentially accessible flowers had equal or shorter style lengths than the average ovipositor length. Number of foundresses per fig were recorded for this data set.

Pedicel and style length comparisons

Ten figs just past the receptive stage were sliced vertically in half. Each half was sliced in fourths, careful to leave styles intact. Ten flowers were randomly selected. Both style length and pedicel length were measured while still attached to fig wall. Measurements were taken to the nearest micrometer under a dissecting microscope and recorded in two columns. Each fig used for flower measurements was recorded in a third column. Comparisons between the pedicel length and the style length determined if the two were (negatively) correlated, or if pedicel length could also determine the outcome of the fig by controlling which flowers were accessible to the wasps ovipositor and which flowers were not accessible.

Observed pedicel length and flower utilization
Eleven mature figs (containing wasp pupae) were sliced in vertical halves. Twenty pedicel lengths were measured in tact along the inner edge of the open cross section. (In previous studies, flowers were scraped out from their original position on the inner wall, possibly contributing to inaccurate measurements relative to the actual length within the fig; Bronstein 1988). Each pedicel was measured from the base on the inner wall to the base of the ovary. Measurements were taken to the nearest 0.5 micrometer under a dissecting microscope. Ovary occupant (female wasp, male wasp, fig seed, or vacancy) was recorded for each pedicel length to determine the relationship between pedicel length and flower utilization.

Results

Foundress number, ovipositor length, and style length
In ten figs observed at the 'receptive stage' (before larvae development) for foundress number, 60% of the figs contained two foundresses, 20% contained one foundress and another 20% contained three foundresses. Twenty total foundresses were extracted from ten figs (foundress number frequency distribution, Fig 2).

In the same ten figs, the observed frequency distribution of style lengths showed a normal distribution. All style lengths were represented and therefore distinct groupings of long and short styles do not exist for (Fig 3). The calculated mean ovipositor length from the 20 foundresses was 10.16 micrometers. This average ovipositor length demonstrates that most wasps should be able to lay their eggs in styles that are 10.16 micrometers long or less, indicated by the arrow in fig 3. This estimation gives a theoretical proportion of how many flowers will become wasps and how many will become seeds…

Style length versus pedicel length
To determine the effect of individual fig on style length and pedicel length two one-way ANOVA tests were run; one for number of individual figs and style length, and the other for number of individual figs and pedicel length. Style lengths and pedicel lengths did not differ significantly between samples measured from different individual figs (respectively, ANOVA, $F = 1.054$, $P = .4048$, ANOVA, $F = .885$, $P = .5423$). Therefore, all data were combined for further analysis.

A correlation test was run to establish the relationship between style length and pedicel length so pedicel measurements could be used in data collection in place of style length measurements since most styles are damaged or gone after oviposition in mature figs. A significant correlation was found between the style lengths and the pedicel lengths (Fig 4, $r^2 = .495$, $P < .0001$). This is a negative correlation indicating that as pedicel lengths increase, style lengths decrease and vice versa.

Expected versus observed flower utilization by wasps

Based on the observed style length frequency and average ovipositor length, wasps are expected to make up the 44% of flowers in a fig whose styles are shorter, and fig seeds are expected to make up 55% of the flowers in a fig, whose styles are longer. This is only a theoretical estimate if style length alone determined the fate of the flowers inside the fig (Fig 5a).

A Chi-squared test determined a significant difference between the expected outcome of the fig and the actual outcome of the fig ($X^2 = 89.2$, $P < .05$). While in theory 44 flower ovaries have short enough styles to be accessible to the wasp, and 55 flower styles are too long and should become seeds, wasps actually occupied 50% of the fig, seeds only occupied 12.5% of the fig, and the remaining 37.4% of flowers were vacant (had neither seeds nor wasps) (Fig 5b). Thus, there were slightly more wasps than expected, but much fewer seeds and many vacancies.

Observed pedicel length and flower utilization

Pedicel length impacted which flowers were most likely to contain female, male, seeds, and which produced nothing at all. Most flowers observed in the study have shorter pedicel lengths and thus longer styles. Four simple regression analyses showed the relationship between the number of flowers within each pedicel length and percent occupancy (Fig 6): as the flower number increases, there is a greater amount of longer styled flowers present, and thus less female wasps. There is a significant negative correlation between flower number and percent females ($n = 118$, $r^2 = .081$, $P = .0376$). There are not enough male wasps to draw a significant correlation ($n = 11$, $r^2 = .02$, $P = .3103$) between flower number, pedicel length, and percent males, even though the graph does show a less defined, but very similar negative trend for the male wasps. There are also not enough seeds to draw a significant correlation ($n = 32$, $r^2 = .009$, $P = .4926$) between the amount of flowers and pedicel length, however the graph shows a slight positive correlation. There is however a strong positive correlation between percent vacancies and flower number ($n = 96$, $r^2 = .134$, $P = .0066$). As flower number increases, there are more flowers with short pedicels and long styles which produce a high number of vacancies. These trends indicate that wasp number does not increase, but in fact decreases as flower number increases, showing that pedicel and style lengths significantly impact seed: wasp ratios.

Figure 7 shows both number of females, males, seeds, and vacant flowers by pedicel length and the relative proportion of each. The few flowers with very long pedicels, 20-24.9 microm, hence shortest styles, were >55% female, males making up 30%, and seeds make up the remaining 15%. Females showed a unimodal distribution, however, there was a preference for pedicels of intermediate length, 12-15.9 microm, which had 95% female occupancy, the remaining 5% were males. Males followed the females and were present in all pedicel lengths (at 10% or less) except for the shortest pedicels (longest styles), and the longest pedicels with 30%. The shortest pediceled flowers, 0-3.9 microm, which had no males, only had 10% females in comparison to 20% seeds and 70% vacant. Both seeds and vacancies continued to decrease as pedicel length increased (style length decreased), and completely disappeared in the intermediate length categories. Vacancies went from 35% to 10% in second and third length categories, while seeds went from 5% to 10%. Seeds reappeared at about 15%

in the longest pedicel length category. Based on average ovipositor length, falling at 8.4 on the graph, indicates the shortest a pedicel length can be, in order for the style to remain accessible. Any flower with a pedicel length shorter than 8.4 indicates that the styles are longer than average ovipositor length, theoretically remaining inaccessible. This is only a theoretical prediction proven false by the occupancy of the female wasp in both of the shortest pediceled length (longest style length) categories.

Discussion

The existence of the fig-wasp mutualism depends upon an inherent conflict: pollinator wasps are the only insect responsible for the fig's pollination, yet at the same time are also predators of the fig flowers. What keeps the wasps from eating all of the flowers? Laying her eggs in all possible flower styles would kill the fig and eventually, in an extreme scenario, the fig species could become extinct. Obviously, this has not happened for extant figs. This suggests a mechanism to keep wasps in check, allowing at least some flowers to produce seeds.

The purpose of this study was to determine the effect of pedicel length, hence style length, on wasp and seed production for a species with multiple foundresses. Previous studies (Bronstein 1988) suggest that other fig: agaonid mutualisms are maintained, in the past, through variation in style lengths in relation to the average length of the pollinator's ovipositor coupled with egg limitations.

This study predicted that pedicel length (style length) will impact wasp: seed ratios where longer style lengths than the average ovipositor length should produce seeds. In addition, higher foundress number would favor additional wasp production.

The results of this study show that length does, at least to a large extent, determine a flower's fate. Short styled flowers (below average ovipositor length) with long pedicels were mostly occupied by female wasps, though female wasps also appeared in long styles. The male wasps were found only in the flowers with short styles (and long pedicels). Seeds and vacancies were mostly located in the flowers with longer styles and shorter pedicels. These results demonstrate that flowers with short styles seem preferable for wasp utilization but the rest are not necessarily left to pollination. (If egg production is limiting Nefd and Compton (1996) suggest that the wasps might prefer shorter styles, regardless of ovipositor length and probing ability, because probing of longer styles takes more time and results in lower ovipositing success rates due to extended contact or friction between the ovipositor and style canal). Remaining long styles become seeds just 10% of the time; most contain neither seeds nor wasps.

Clearly, style length is not the only factor determining a flower fate. Other possible factors regulating the fig wasp mutualism (which are confounded with the variation in pedicel and style lengths) must be considered due to the fact that some of the results do not completely support the hypothesis and instead show wasps in some long styled flowers, some seeds in the shorter styled flowers, and many vacancies (in the long styled flowers). To resolve wasp occupancy of longer styles, perhaps actual ovipositor length of the agaonine wasp is longer than I previously measured. Perhaps most of the ovipositor is hidden within the wasp and only a fraction of its length is showing; however during oviposition, the ovipositor is thrust out and can drop eggs into longer styles. Nefdt and Compton (1996) support this observation after finding specific fig wasp species that have ovipositors long enough to reach 99% of the ovaries, and in six out of eleven species studied, 80% of the ovaries were accessible.

Perhaps it is the multiple foundress factor, observed in *tuerkheimii*, forces wasps to utilize more of the flowers in general. In order to maximize their fitness, laying eggs in longer styles is better than not laying eggs at all. This however does not account for all the vacancies within the long styled flowers.

There are four possible reasons that vacant flowers exist and occupy longer styles: larval mortality, failed oviposition attempts (Bronstein 1988), over abundant oviposition sites (Bronstein 1988), or pollen limitation. Whether or not length is a factor in the wasps utilization of the long style, an egg laid in a style much longer than the ovipositor length will be deposited only part way down the style. If the egg does not hatch, or hatches but larva does not crawl down the style to the food source, the long style remains blockaded, and pollination will not occur; no seed will develop. The larva will die and no wasp will develop either. The long styled flower remains vacant. In this study, no dead larva were encountered within the long vacant styles, perhaps due to the mature stage of the fig.

Vacancies could possibly result from damaged styles due to multiple probing attempts to lay an egg in a style much longer than the ovipositor length, where the wasp is continually trying to feel for the ovary at the bottom of the style but cannot find it. Or perhaps, if there is an abundance of foundresses, eggs, or long average ovipositor length, a large egg load is laid, but a smaller number of larvae reach maturity than original egg abundance due to density dependent resource based competition. Un-hatched eggs leave vacancies where seeds cannot develop. This however, does not explain why the vacancies would dominate longer styles.

Pollen limitation of the ficus flower could explain the vacancies in the long styles. Wasps will lay their eggs in the shorter styles, and not enough pollen will come in to pollinate the long styles. Thus they are left vacant. Pollen limitation can explain why there is such a small percentage of seed outcomes in relation to wasps. Multiple foundresses may come in carrying very little pollen from the previous fig, allowing the wasps to dominate the fig.

The low seed count could possibly be due to the exceptionally large size of *F. tuerkheimii*. The larger ostiole may allow more female wasps with more eggs to enter the fig. This could account for the slightly larger than expected wasp occupancy and smaller than expected seed occupancy. In other fig species, increased wasp body size would not be very beneficial due to the smaller ostiole sizes. *Ficus pertusa* has a smaller foundress number than *tuerkheimii*, possibly due to ostiole diameter (Nefdt and Compton 1996).

In order for this unique mutualism to continue, both wasps and figs must co-evolve. To increase seed production, the fig should create smaller ostioles, close the ostiole sooner to limit foundresses that enter, supply larger pollen loads, and contain longer, inaccessible styles. In response, the wasp should find a way to become smaller, yet carry more eggs, have a longer ovipositor, and enter the fig with a smaller pollen load.

Future studies regarding unanswered questions about the seed: wasp ratio include counting individual pollen grains going into the fig to assess if there is a pollen limitation. To investigate egg limitation, the number of eggs can be collected from female wasps just before they enter the fig. To be certain about where the vacancies lie, and if dead larvae play a role, figs can be opened at different stages within their development.

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Literature Cited

Bronstein, J.L. 1988a. Limits to fruit production in a monoecious fig: consequences of an obligate mutualism. Ecology 69: 207-214.

Bronstein, J.L. 1988b. Mutualism, antagonism, and the fig-pollinator interaction. Ecology 69: 1298-1302.

Gilbert, L.E. 1983. *Blastophaga* and Other Agaonidae (Avispita del Higo, Fig Wasp). In: Costa Rican Natural History, D.H. Janzen, ed. The University of Chicago Press, Chicago, IL, pp. 696-700.

Hogan, Anne Marie. 1994. Frequency of foundress wasps in *Ficus tuerkheimii* in Monteverde and La Cruz. Independent project 154-60, CIEE Program, Monteverde, CR.

Nadkarni, N.M. and N.T. Wheelwright. 2000. Monteverde: Ecology and Conservation of a Tropical Cloud Forest. Oxford University Press, New York, page 271.

Nefdt, R.J.C., and S.G. Compton. 1996. Regulation of seed and pollinator production in the fig-fig wasp mutualism. Journal of Animal Ecology 65: 170-182.

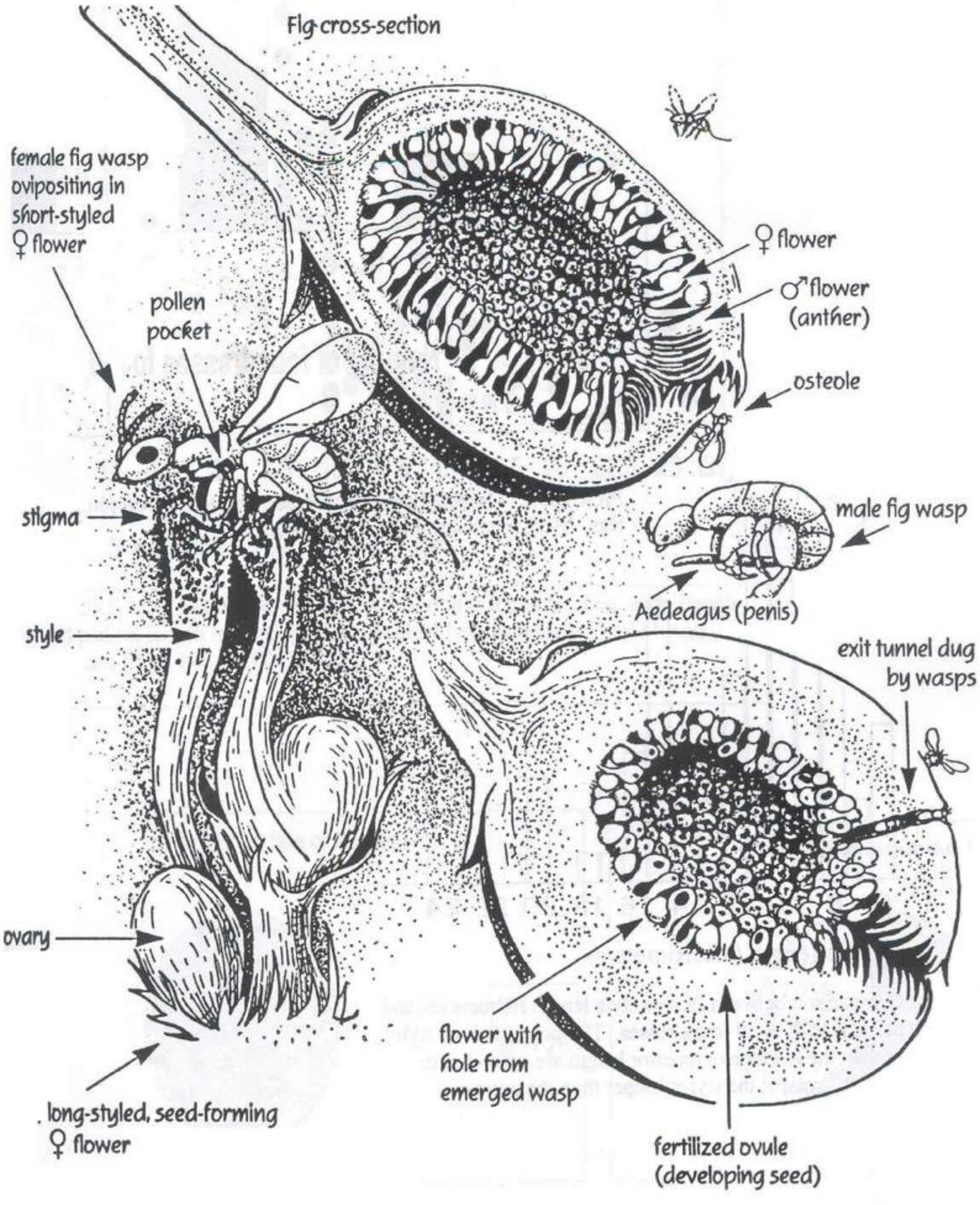


FIGURE 1. Each species of fig is pollinated by a unique species of Agaonidae fig wasp and no other insect. This is a unique characteristic that ensures the fig trees survival by limiting pollinator competition (J.C. Nefdt and G. Compton, 1996). The most fit pollinator for the job will not be threatened by other pollinators who do not necessarily always pollinate fig trees since figs are not crucial for their survival. Pollination occurs when a female wasp with pollen and squeezes through the fig ostiole, or small opening, to reach the enclosed flowers of the syconium (inflorescence, fig. 1). This process shears the wings off the wasp. The ostiole closes shortly after her arrival and she is trapped inside the syconium. Inside the fig, the wasp pollinates the stigmas while she lays eggs in the styles of the female flowers which provide ovaries to feed the offspring(J.C. Nefdt and G. Compton, 1996). After approximately one month, wingless male agaonid offspring crawl out of the flowers, inseminate the females still in the florets, and then chew an opening out of the fig to escape. The mated females emerge from the florets and leave the fig via the chewed opening without damaging their wings. As the female leaves, she takes with her pollen from the anthers on the male florets and will continue the cycle by pollinating another receptive fig tree (Janzen 1979).

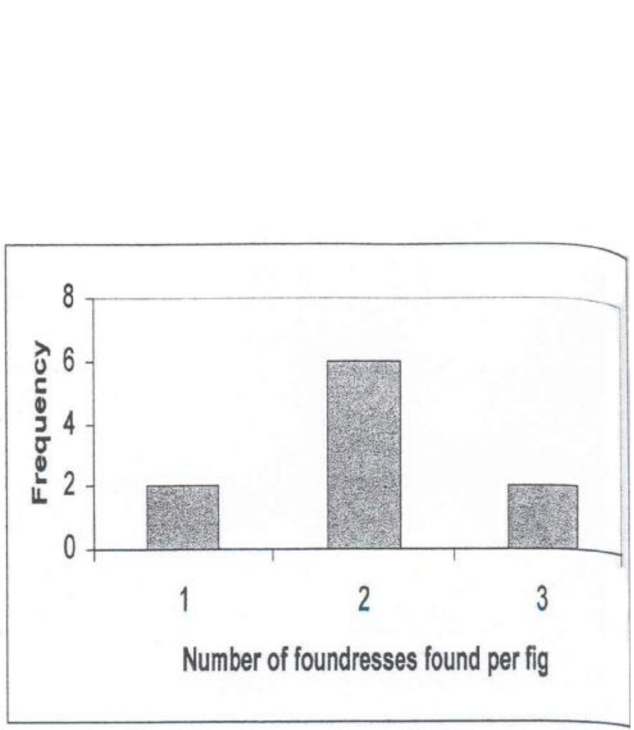


FIGURE 2. The number of foundresses found in a sampling of ten receptive figs. Most figs housed two foundresses.

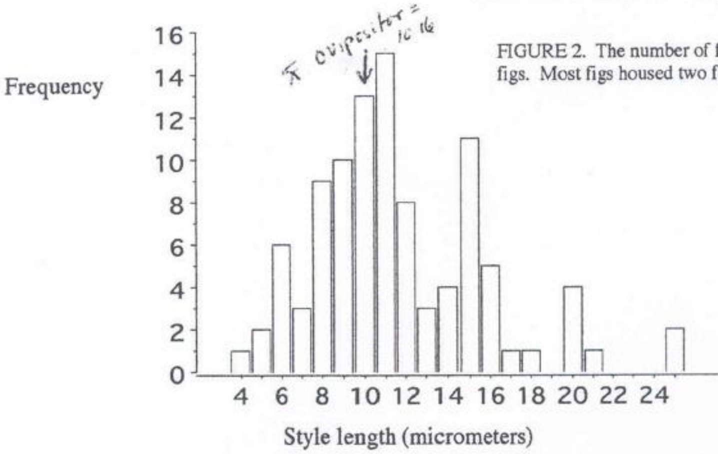


FIGURE 3. Comparison between flower style length frequencies and average ovipositor length of all foundresses. The proportion of style lengths shorter than the average ovipositor length are predicted to become flowers with wasps; the styles longer than the average

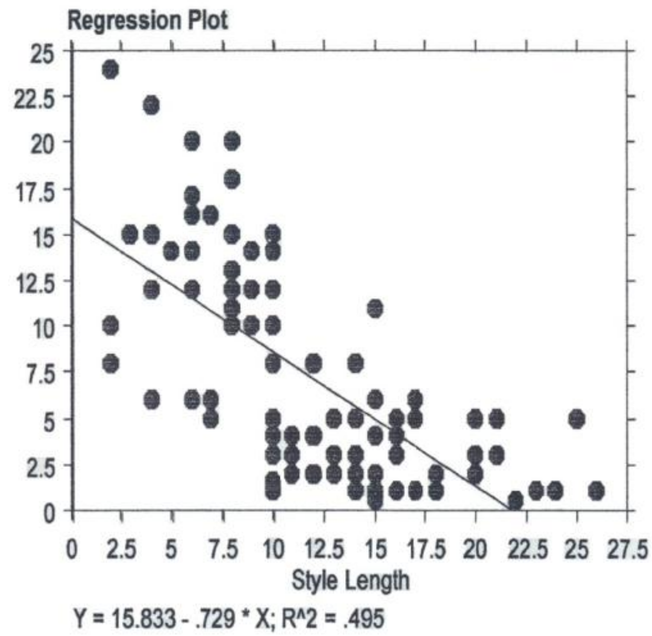


FIGURE 4. A significant negative correlation is found between style length and pedicel length indicating that as pedicel lengths increase, style lengths decrease ($r^2 = .495$, $P<.0001$).

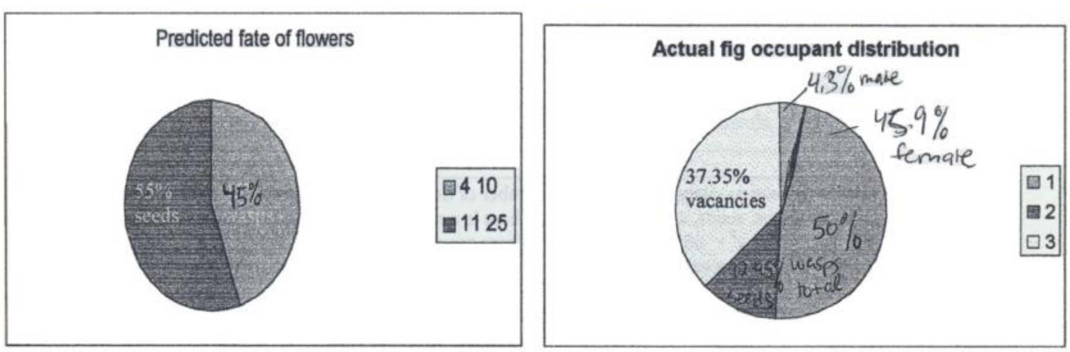


FIGURE 5. a) Predicted wasp and seed proportions within a single fig based on style length frequency and average ovipositor length. b) Actual occupancy frequencies within a single fig based on measurements and observations.

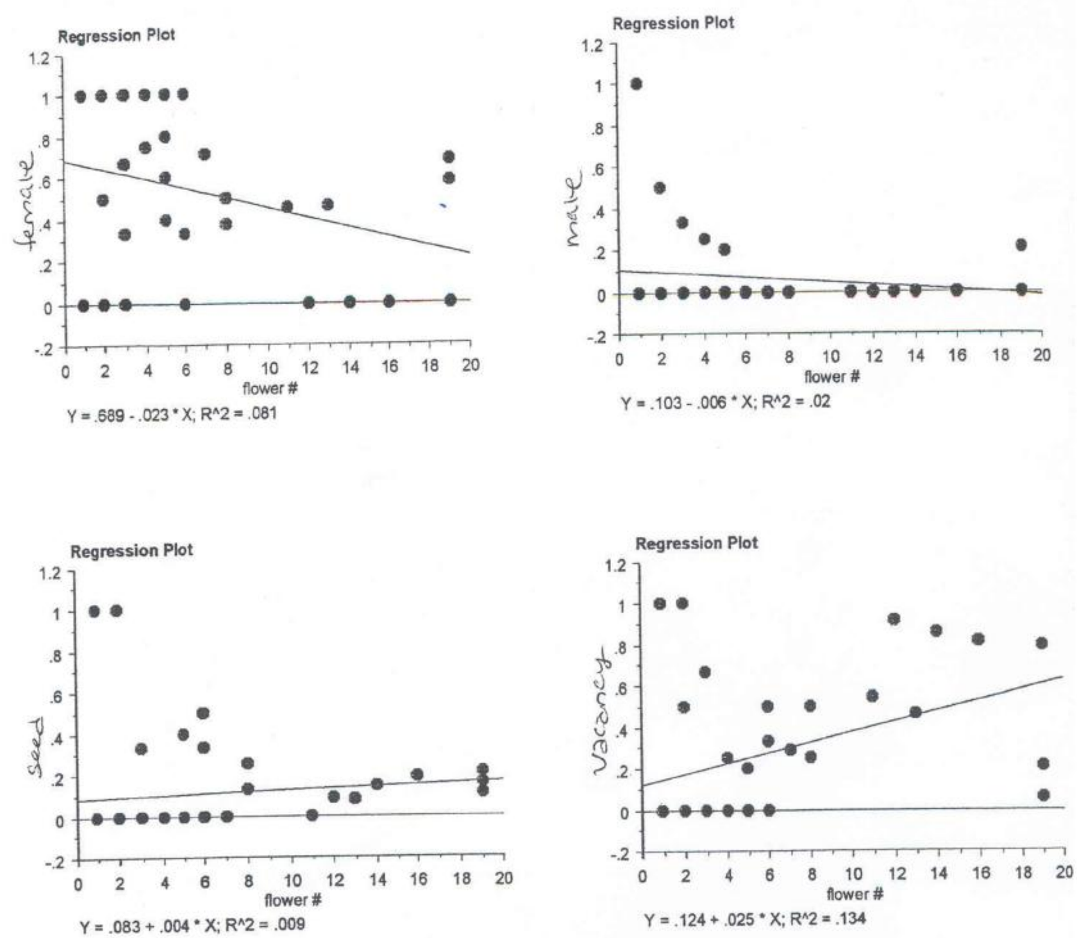
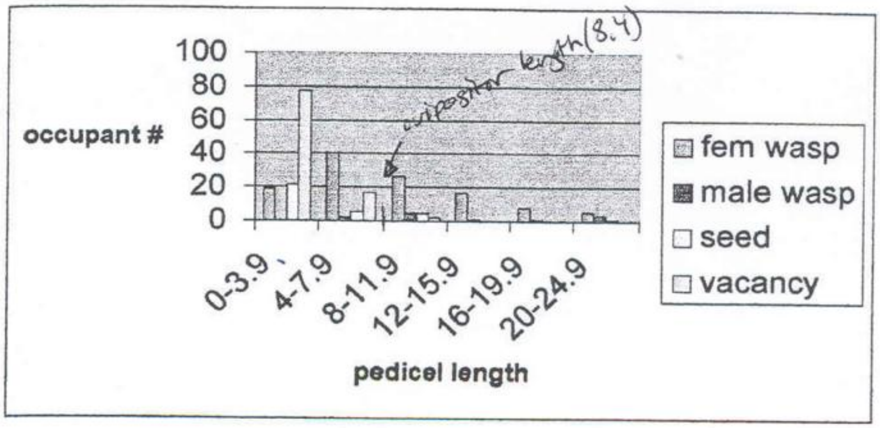


FIGURE 6. A simple regression analysis between the number of flowers at each pedicel length and between the proportion of female wasps, male wasps, seeds, and vacancies. (a) There is a significant negative correlation between flower number and percent females ($n = 118$, $r^2 = .081$, $P = .0376$). (b) There are not enough male wasps to draw a significant correlation ($n = 11$, $r^2 = .02$, $P = .3103$) between flower number, pedicel length, and percent males, even though the graph does show a less defined, but very similar negative trend for the male wasps. (c) There are also not enough seeds to draw a significant correlation ($n = 32$, $r^2 = .009$, $P = .4926$) between the amount of flowers and pedicel length, however the graph shows a slight positive correlation. (d) There is however a strong positive correlation between percent vacancies and flower number ($n = 96$, $r^2 = .134$, $P = .0066$).



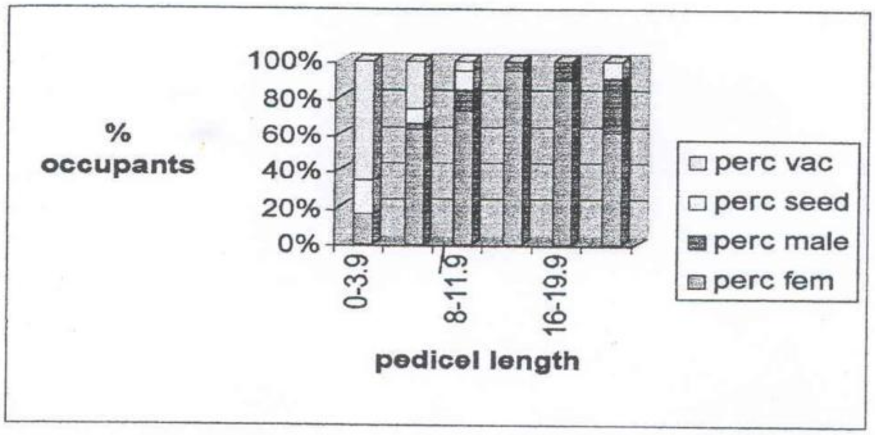


FIGURE 7. (a) The number of female wasps, male wasps, seeds, and vacancies per pedicel length increments of four. (average ovipositor length is at 8.4 and above). (b). Proportion of female wasps, male wasps, seeds, and vacancies per pedicel length interval. Largest proportion of total wasps is in intermediate length pedicels.