Density-dependent parasitism in *Centropogon solanifolius* (Campanulaceae) and changes in floral and sex-phase longevity following infestation

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ABSTRACT

Centropogon solanifolius flowers (Campanulaceae) are parasitized by the fly larvae of *Zygothrica neolinea* (Drosophilidae). The larvae burrow into the anther of the developing flower bud and eat the pollen, which changes the longevity dynamics among the infested, protandrous flowers. This study investigated whether the flies were parasitizing in a density dependent manner. The study also explored strategies that flowers used to cope with the high infestation rates (50% in a previous study and documented here as well). To find answers to these questions, 83 flowers were located, their infestation status noted, and nearest neighbor distance measured. Additionally, 78 other flowers were bagged and tagged to look for changes in overall flower lifetime, as well as the longevities of the male and female phases. The results show that group dependence parasitism is operating, with 33% more grouped flowers parasitized (n = 83) (^{2} = 5.55, df = 1, P < 0.05). Parasitism based on distance to nearest neighbor is also observed (t = 2.261, P = 0.0266). To cope with parasitism and the loss of available pollen in the system, infested flowers shorten their male phase by approximately two days, from 4.2 ± 2.4 to 2.5 ± 2.1 days (t = 2.26, P < 0.21), while experiencing no significant difference in overall flower longevity (t = 0.55, P < 0.59). This results in an implied lengthening of the female phase in infected flowers, increasing the chances of pollination in the pollen-depleted system.

RESUMEN

Las flores de *Centropogon solanifolius* (Campanulaceae) son parasitadas por las larvas de mosca de *Zygothrica neolinea* (Drosophilidae). Las larvas hacen una madriguera en la antera del botón de la flor y comen el polen, esto cambia la dinámica de la longevidad entre las flores protandricas infestadas. Este estudio investigó si las moscas parasitadas dependiendo de la densidad de las flores. El estudio exploró también las estrategias que las flores utilizaron para enfrentarse con los altos valores de infestación (=50 % en un estudio previo y documentado aquí también) Para encontrar las respuestas a estas preguntas, 83 flores se localizaron, y se midio la distancia a su vecino mas cerca. Adicionalmente, otras 78 flores se empaquetaron y marcaron para buscar los cambios en la vida general de flor, así como las longevidades de las fases masculinas y femeninas. Los resultados muestran ese parasitismo depende del grupo, con 33% más de parasitismo en flores agropadas (n = 83) (^{2} = 5.55, df = 1, P < 0.05). El nivel de parasitismo se basó también en la distancia al vecino mas cercano (t = 2.261, P = 0.027). Para enfrentarse con el parasitismo y la pérdida de polen disponible en el sistema, las flores infestadas acortan su fase masculina por aproximadamente dos días, de 4,2 ± 2,4 a 2,5 ± 2,1 días (t = 2.26,

P < 0.213), al experimentar ninguna diferencia significativa en la longevidad general de flor (t = 0.55, P < 0.59). Esto tiene como resultado un alargamiento implicado a la fase femenina, aumentando las oportunidades de polinización en el sistema que tiene el polen agotado o escaso.

INTRODUCTION

The protandrous flowers of *Centropogon solanifolius* (Campanulaceae), a hummingbird-pollinated, understory plant in the Monteverde Cloud Forest Reserve (Zuchowski 2005), are parasitized by the fly *Zygothrica neolinea* (Drosophilidae) (Weiss 2000). *Zygothrica neolinea* deposits its eggs into the corolla of a developing bud and larvae subsequently burrow into the anther and eat the pollen, effectively lowering male reproductive success (Weiss 2000). Infestation rates by the fly are very high in Monteverde, Costa Rica; in surveys in 1987 and 1988, between 50-71% of the flowers were infested (Weiss 1996). *Centropogon solanifolius* can be found in groups or solitarily, which raises the question of whether the parasitism by *Z. neolinea* is density dependent. This question has not been explored previously for *C. solanifolius* and was raised as an important area of future research in past studies (Weiss 1996).

Although prevalent, density dependent predation is not ubiquitous in all biological systems. In one study, 16 of the 32 separate studies that were compiled to evaluate mathematical models of density dependence parasitism were found to have a positive relationship between parasitism and density (Lessells 1985). At the same time, another study only found a positive density dependence in 25% of the 171 insect host-parasitoid interactions that were analyzed, with the remaining 75% having independence from density or an inverse relationship with it (Stiling 1987). Given the variation in the prevalence of density dependent parasitism, it was important to establish whether *Z. neolinea* was parasitizing the flowers in a density dependent manner and with what frequency the parasitism was occurring.

Regardless of how the parasite chooses which flowers to lay its eggs in, *C. solanifolius* flowers are negatively affected by the fly parasite. Due to its pollen being eaten, its genes are not available to be passed on and the plant suffers decreased reproductive success (Weiss 1996). Because the plants are unable to control their proximity to each other, and therefore potential density dependent effects, it can cope with being parasitized by using one of three logical response strategies. First, it can drop the flower before it opens, or decrease the floral longevity, effectively lowering its energy investment in the flower. Second, it can increase the longevity of the flower, allowing for a longer female phase without affecting male phase longevity. Third, the overall flower longevity can stay the same, but it can shorten the male phase and lengthen the female phase, which would increase its chances of receiving pollen in the pollen depleted system.

The purpose of this study is to investigate which of the coping strategies is used by *C. solanifolius* in response to parasitism. While previous studies have shown that the population-wide average male longevity decreases in heavily parasitized populations, the longevity of parasitized vs. unparasitized flowers has not been documented (Weiss 1996). By investigating the longevity of the male and female phase in nonparasitized and parasitized flowers, this study will provide novel information to allow biologists to discriminate between possible response strategies of *C. solanifolius*. Further, this study will evaluate whether density dependent parasitism occurs. If it does, and is coupled with a shortening of the male phase in these dense areas, as expected, uninfested flowers in the area can increase fitness by having a higher ratio of their pollen in the system. Similarly, density dependent predation could decrease the amount of

pollen in close proximity for the females, thus favoring a lengthening of the female-sex phase, which would increase the chances of being pollinated.

METHODS

The study was conducted in the forest adjacent to Estación Biológica in the lower montane cloud forests of Monteverde, Costa Rica, between April 11 and May 7, 2007. The flowers of *C. solanifolius* were located by walking on Sendero División, as well as on Sendero Mirador. All of the plants were located a few meters from the main trail and were between 1700-1800 meters in elevation.

For the density dependence data, 83 flowers were located. These flowers had to be open flowers because this was the most reliable state to assess infestation. When the larvae leave the flower, (usually right before anthesis), they chew a small exit hole near the base of the corolla (Weiss 2000).
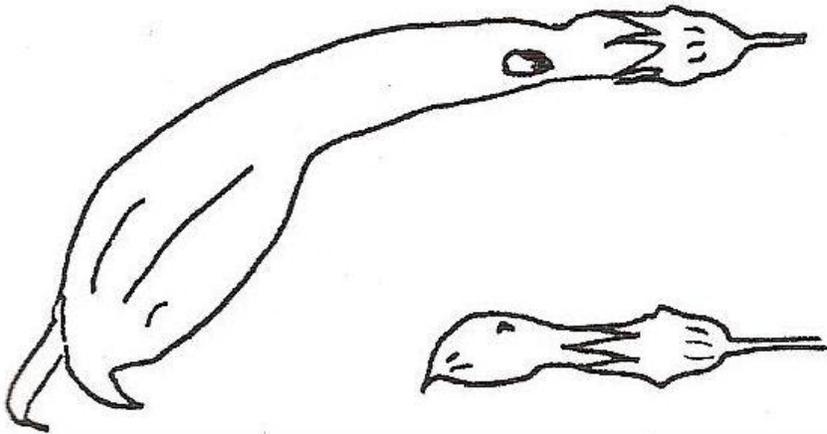

FIGURE 1: Illustrations of a *Centropogon solanifolius* (Campanulaceae) male phase flower (Top) and flower bud (Bottom) parasitized by the fly *Zygothrica neolinea* (Drosophilidae). Top: An exit hole has been made at the base of the corolla by the larvae as it leaves the flower, which happens just before anthesis; Bottom: A bud with a latex droplet that was left by the mother during egg deposition. *Modified from Weiss 2000.*

Latex droplets are sometimes left by the mother when she lays eggs in the flower (Weiss 1996), but because these were never observed during this study, the only way to assess infestation status was by looking for exit holes in open flowers. Once a flower was found, the distance to its nearest open-flower neighbor was measured (in cm) and the infestation status of both flowers was noted.

For analyzing the density dependence results, I discriminated between group and solitary flowers. To do so, the neighbor distances were added up for all flowers and the average was taken. Any flowers that were closer to their neighbor than the average value were considered group flowers; those further away than the average distance were considered solitary flowers.

For the flower phase longevity data, 33 plants and 78 small flower buds were used in the study. Forty-four of the buds were tagged with an individual number and enclosed in fine mesh bags, with the bottom of the bag being fastened shut with flagging tape. These were chosen as potentially unparasitized buds, but their status was definitively confirmed once anthesis occurred. For this subset, small buds were chosen because they are less likely to have parasites when very young (Weiss 2000). Buds were covered with a mesh bag to prevent parasitism, and when there were multiple flowers in close proximity, a single bag was used to cover all of them. The other 34 flower buds in the study were simply tagged with labeled flagging tape to indicate that this subset would be left exposed to parasites. Because I wanted these flowers to be parasitized, finding them early in their development was not as critical.

These flowers were visited five times a week, and the dates when they opened, as well what sex phase they were expressing, was noted. When there was a day lag between data collection, notes were made as to the likelihood of a flower opening or changing sex during the day without observation. I had high confidence in my ability to predict correctly if a flower would open during a day that I was not going to visit, based on visual cues (such as small cracks starting at the apex of the flower, as well as its size). Additionally, if a flower changed from a male phase to a female phase during the non-observation day, it was easy to determine which day the change occurred. As *C. solanifolius* flowers change from the male to female phase, the stigma barely protrudes from the anther on the first female phase day and continues to grow out of the corolla afterwards. The day of sex-change was easily inferred based on how far the female was protruding from the anther.

The infestation status of the flower was also checked each day, and only if a hole was present, the flower was marked as infested. The female stage was considered complete once either the whole flower or the stigma fell off.

RESULTS

Density Dependence
Among the flowers used in the density dependence data, the frequency of floral infestation was 60% (n = 83). There was no correlation between group size and infestation rate (R = 0.090, P > 0.05, n = 41). Additionally, there was no relationship between one flower's infestation status and the infestation status of its neighbor (χ^2 = 3.08, df = 1, P > 0.05). The percentage of flowers in patches being infested, 66% (n = 68), was higher than the percentage of solitary flowers that were infested, 33% (n = 15). Thus, solitary flowers are much less likely than grouped flowers to be infested by their parasites (χ^2 = 5.55, df = 1, P < 0.05). Additionally, the flower's distance to its neighbor was significantly lower for infested flowers than for uninfested flowers (t = 2.26, P = 0.027).

Sex-Phase and Overall Flower Longevity
The frequency of infestation for the flowers used in the longevity portion of the study was 51% (n = 78). Seventy percent of the plants had at least one infested flower (n = 33). Among the infested and uninfested flowers, there was no significant difference between abortion rates (χ^2= 0.06, df = 1, P > 0.05). There was also no significant difference in the overall flower longevity, or the length of the female phase, for the infested and uninfested flowers (t = 0.55, P < 0.59 and t = 1.14, P < 0.26). The overall flower lifetimes were 13.1 ± 3.1 days for infested and 12.5 ± 3.7 days for uninfested (see figure 2). The average infested female lived for 10 ± 2.8 days, while the

uninfested females lived 8.9 ± 3.6 days (see figure 2). The male phase was significantly shorter in infested flowers, with the average being 2.5 ± 2.1 days for infested and 4.2 ± 2.4 days for uninfested (t = 2.26, P < 0.022) (see figure 2).
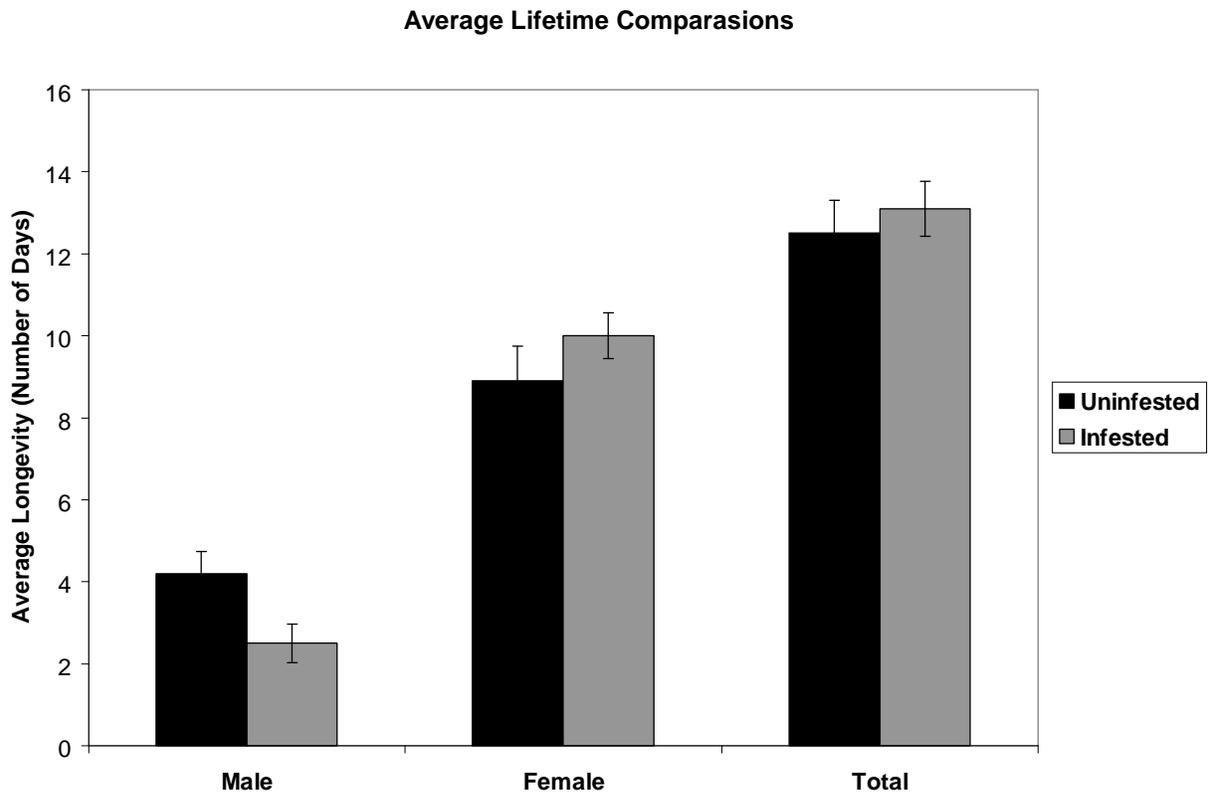
Average Lifetime Comparasions

FIGURE 2: This bar graph shows the average lifetimes of infested and uninfested flowers, separated into male, female and total. Error bars are included. The lifetimes, in days, are as follows from left to Right: Uninfested male, 4.2; infested male, 2.5; uninfested females, 8.9; infested female 10.0; uninfested total, 12.5; infested total, 13.1.

DISCUSSION

This study and a previous investigation into parasitism of *C. solanifolius* by *Z. neolinea,* show that infestation rates are high at Monteverde. In both studies, no less than 50% of the flowers in the population are infested by the parasite (Weiss 1996). The questions that I addressed were whether high infestation rates were density dependent, and whether flowers adaptively altered their phenology to try to maximize the likelihood of pollination under parasite-mediated population sex-ratio biases.

The most important finding related to the spatial pattern of parasitism of *C. solanifolius* by *Z. neolinea* is that flowers in groups were more likely to be parasitized than those that were solitary. That is to say, so long as the flowers were in a group of two or more, they were more likely to be parasitized; but a group of five was no more likely to be parasitized than a group of two. Although my study was unable to detect that larger groups were parasitized more heavily, I expect that it would be true with a larger sample size or with larger patches. My study had too

fine of a scale and too small of a range (I only had groups of one to five individuals) to detect a trend. I was, however, able to detect that individuals that were closer to their neighbor were more likely to be parasitized, which strengthens the argument for distance dependent parasitism.

Janzen (1970) showed that parasites can act in both density dependent and distance dependent manners, so my finding of distance dependent parasitism is not unique. The result of the distance dependent parasitism in Janzen's study was that trees in tropical forests were not likely to be found in great densities. These results were not possible for plants of *C. solanifolius*, as in my observations they seemed to congregate in well lit areas, such as tree fall gaps. These growing preferences push them towards congregations with high densities. Therefore, Janzen's result of having trees (or plants) found in lower densities is not expected for the plants of *C. solanifolius*. The plant's reactions to the distance and group density dependence will be explained momentarily, when response strategies are discussed.

I am able to conclude that distance to neighbor, and grouping status are both important factors in determining if a flower will be parasitized. With that in mind, coupled with the fact that the parasitism was 33% greater for grouped flowers, and 82% of the flowers in my study were grouped, it is obvious that the flowers need a strategy to cope with the parasite.

Pests parasitizing plants by using them as a food-source has been found in many studies. For instance, Thompson (1983) found that moths of the genus *Depressaria* feed mostly on the flowers and immature seeds of their host plants. Similarly, Needham (1948) found that *Bidens pilosa* was both the host and food-source to a variety of insect parasites, such as *Cyclorrhapous* flies and several moths, which fed on the heads of the flowers. Furthermore, and similar to the flowers of *C. solanifolius, B. pilosa* had three species of fly larvae, *Agromyza vivens, Paroxyna picciola* and *Xanthaciura insecta*, that eat the pollen, seed sap and flower heads, respectively, after boring into the base of the corolla. Nothing is known about a change in flowering phenology or flower structure of *B. pilosa* in response to this parasitism. Another example of pests eating plants was seen in this study of *C. solanifolius* when a beetle pest from the family Carabidae was found eating the corolla, anther and stigma of the study flowers (Appendix 1).

Parasitic relationships can push plants and trees to adapt, through natural selection, the timing of flowering and leaf flushing. One example is *Enterlobium cyclocarpum*, which has new leaf production four to eight weeks before the beginning of the rainy season. They do this in order to have tougher leaves at the onset of the rainy season, when most of the herbivore populations increase exponentially (Janzen 1983). The flowers of *C. solanifolius* are experiencing similar pressures by floral parasites and the results here show that they also adaptively respond with changes in phenology.

As previously discussed, *C. solanifolius* has three possible response strategies to the parasitism of its anthers. The first option is to decrease the longevity, or abort parasitized flowers before they open. My results indicated that neither of these options is employed. First, there was no difference in abortion rates between infested and uninfested flowers. Secondly, the floral longevity for infested and uninfested flowers was not statistically different.

The next option would be to increase the overall lifetime of the flower, passing the increased longevity onto the female phase. This would allow the female to be open longer and increase its chances of being pollinated, and thus increase the reproductive success of the plant. However, I found no evidence to support this, based on the fact that the female phase and the overall longevity were not significantly different for infested and uninfested flowers.

The last response strategy the plants could have is to keep the overall flower longevity the same, but decrease the male phase and therefore increase the female phase. My data lend support for this strategy. Evidence showed that infested males lived approximately two days less than the uninfested males and that the longevity of the infested and uninfested flowers was not significantly different. Although I was unable to obtain significant information on a change in the length of the female phase, simple logic would say that if the total floral longevity is similar in infested and uninfested flowers (approximately 13 days), and the infested male phase is about two days less than in the uninfested flowers, then the female phase would have to become longer in the infested flowers to compensate for the decrease in male phase longevity (figure 2). I expect that with a bigger sample size, I would have obtained this result of lengthening of the female phase and shortening of the male phase, with the total lifetime of the infested and uninfested flowers being the same.

Biologically speaking, the flower's response is the most energetically favorable and will afford the highest reproductive success. When pollen is consumed by larvae, it is not available to pollinate the females. Logically, the male phase would shorten, because the pollen depleted male has significantly decreased reproductive potential. At the same time, the parasitized flower can increase reproductive success by extending the expression of the female phase, thereby increasing its chances of receiving pollen in the pollen-depleted system. This way, the flower does not need to increase its overall energy input; instead it simply shifts the energy input for each sex phase.

In reality, the most reproductively successful option for an infested plant would be to completely skip the male phase and only express the female phase, effectively becoming an imperfect flower. In a dense patch that is being hit heavily by the larvae, the pollen availability will be even lower and thus using energy from the male phase to express the female phase for a extended period of time greatly increases likelihood of pollination. This phenomenon of completely skipping the male phase was actually seen in three of the infested flowers of my study, as well as some of the flowers in Weiss' study (Weiss 2000).

Likewise, if a flower is uninfested, it can increase its reproductive fitness by never expressing the female stage, especially if it is in a highly dense patch where other males have been parasitized. If there is less pollen in the system, it is reproductively favorable for a flower with a lot of pollen to put all of its energy into getting its pollen into the system. This phenomenon was seen in one uninfested flower in my study.

In conclusion, because the flowers are more commonly found in groups and therefore more likely to be parasitized, they must converge on a strategy that will allow similar or increased reproductive fitness compared to when they are unparasitized. The flowers of *C. solanifolius* seem to have evolved a strategy to cope with the parasitism by *Z. neolinea*. By shifting their energy input ratios for the parasitized flowers, they seem to be increasing their investment in the female phase at the expense of the male phase, while keeping the overall investment in a flower the same. This behavior increases the flower's chances of becoming pollinated in the pollen-depleted environment that the *Z. neolinea* has forced upon the plant populations.

Future studies should explore a possible increase in female phase longevity in parasitized flowers. I feel this could be accomplished with a larger sample size. Additionally, it would be important to investigate further possible density dependence of the parasitism. Distance and group dependent parasitism were shown, and with a larger scale and larger range of group sizes, I believe density dependence would also be seen. Additionally, it might be interesting to look for

the complete absence of male or female phases, which would suggest that the flowers may be moving towards the use of imperfect flowers. Finally, a search of the flowers for the Carabid beetle that was observed eating the flower parts, and further parasitizing the flower, may bring about more interesting floral longevity reactions.

ACKNOWLEDGEMENTS

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APPENDIX

Potential Further Parasitism of *Centropogon Solanifolius* by a Beetle of the Family Carabidae:

1) There were five flowers that had a lot of damage to their corolla, anther and stigma. I assume that this was done by a feeding beetle of the Carabidae family, because the beetles were found in three of the five flowers. They were small (8-10mm), flat and black. None of the five flowers were used in the statistical analyses of this study.

There are approximately 2200 species of Carabid beetles in Costa Rica. Most of the larvae are nocturnal and live among forest leaf-litter. Their diet normally consists of other insects but others are known to eat seeds, fruits and other vegetative products (Solis, 1999).