A Comparison of Co-Occurring Sexual Versus Gynogenetic Reproductive Strategies in the Sycamore Leaf Beetle, *Neochlamisus platani*

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BRIEF. A comparison between sexual and gynogenetic *Neochlamisus platani* leaf beetles was conducted through feeding/mating choice tests.

ABSTRACT. The enigmatic leaf beetle, Neochlamisus platani, feeds, mates, and lays its eggs exclusively on the sycamore tree and expresses gynogenesis in its populations. This form of asexuality in which females must mate with males to produce offspring, without a genetic contribution from the male, yields genetically identical daughter beetles. The sexual strategy of field collected mothers were determined based on the sex(es) of their offspring. These offspring were reared and compared by host preference, mate-choice, and observational tests to understand how sexuals and gynogens differ ecologically considering particular hypotheses. For example, it was expected that males would prefer to mate with sexual females to pass on their genes. It was also expected that gynogens would more readily feed on non-sycamore leaves than sexuals because Gynogens are triploids, with an extra set of chromosomes believed to derive from hybridization between sexual N. platani and another Neochlamisus species that may influence the behavior of the gynogens to be less specialized. The results from the feeding choice tests revealed a significant difference between levels of specialization of gynogens versus sexuals on sycamore foliage, with gynogens feeding 8% less on sycamore compared to alder foliage supporting the hypothesis that gynogens are less specialized.

INTRODUCTION.

Gynogenesis is a rare phenomenon in the realm of offspring-producing sexual strategies. Mixing aspects of both sexuality and asexuality, gynogenetic organisms produce offspring clonally while still requiring copulation with a sexual male (Figure 1). Even though gynogenesis is rare, in populations of Neochlamisus leaf beetles, gynogenesis is often present, meaning both asexual and sexual beetles cohabitate the trees that they are specialized to [1]. This system is confusing because there isn't an obvious advantage for gynogenesis to exist as a sexual strategy, nor are there any advantages for sexual males to mate with gynogenetic females. Gynogens have the negative aspects of sexual reproduction because they must utilize time and energy to search out mates in order to produce offspring [2]. They also have the fatal flaw of asexual populations because they tend to accumulate deleterious mutations since their offspring are not results of recombination of the DNA from two separate organisms [2]. However, the short term advantages of asexuality would seem to have the potential to cause a population to become exclusively gynogenetic since asexual individuals can essentially produce offspring twice as efficiently considering all offspring are daughter producing females. If gynogenetic individuals were to outcompete their sexual counterparts and drive them from the habitat then gynogens would then lack the ability to reproduce. They require sexual females to produce sexual males, and they require sexual males to reproduce.

We know because of previous studies that gynogenetic Neochlamisus females are triploid, meaning they have an extra set of chromosomes which the sexual beetles don't have [2]. This physical difference, brought about by hybrid origin, or the offspring production by a mating between two different species earlier on in history, seems to cause gynogen females to have the tendency to be larger. This polyploidy also has the capability to affect other aspects of the gynogen's biology as well. To study this, we performed experiments studying host plant preference and mate preference in order to discover whether gynogens and sexuals differ in three of the most important aspects of animal life, eating, mating, and decision making. We expected that gynogenetic females would be more willing to eat foliage from a plant other than the American sycamore. Their genes influence them to become specialized to their host plant, but because they have an extra set of chromosomes due to hybrid origin, gynogens may be predisposed to be less specialized than their sexual counterparts since they have other genes as well. This extra set of chromosomes also has the capability of interrupting their plant-associated decision making processes since they aren't so specialized to one plant. We also expect gynogenetic females to be less picky about their mates since they don't actually use the genes of the male beetles, they only require copulation. These potential differences could give insight into a strange phenomenon that doesn't have a solid reason for its presence in populations of different organisms.

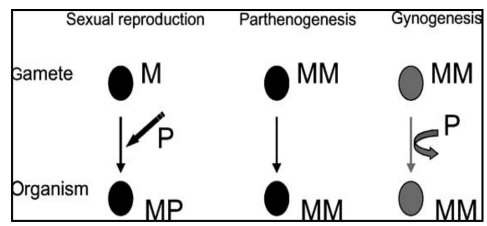

Figure 1. Gynogenesis combines aspects of sexual and asexual reproduction, with *M* referring to female genetic contribution and *P* referring to male genetic contribution [2].

MATERIALS AND METHODS.

Field Collection of Beetles.

Previous studies by Chris Brown have revealed Natchez Trace National Park in Nashville, TN to be an excellent breeding ground for Neochlamisus platani due to the concentration of American sycamore trees (Figure S4) [3]. Roughly sixty beetles were collected by hand and stored in tubes for future setting-up and sexing under a microscope. For each collected beetle, an indication of specific collection site and time is present in order to distinguish each individual.

Set-up of Adult Beetles.

Adult beetles collected from sites were paired, one female beetle with one male beetle, for as many males as there were to pair with. Both paired and unpaired females were individually stored in plastic boxes, roughly shoebox sixed, with moistened paper towels lining the bottom to control humidity, sycamore sticks and leaves for the females to lay eggs on, and also lush sycamore foliage for them to feed on. The boxes were covered with tin foil to keep them relatively air-tight and they were kept in incubators at 24˚C with a 14:10 light-dark cycle. Every 5 days the foliage and paper towels were changed and all the eggs werecounted for each female. The eggs were then placed into a petri dish with dry filter paper and were incubated at the same conditions of the adults to promote larval hatching.

Set-up of New Larvae.

The egg dishes were checked every day for new hatchlings. The eggs generally hatched after 9 days of incubation and new larvae were weighed and then set up in petri dishes containing filter paper moistened with 200μl of water and a small piece of sycamore foliage. Filter paper and foliage were changed out for each individual beetle every two days. Larval condition and death rates were recorded for each larva at every check.

Set-up of New Pupae.

After a larva pupated, the new weight was recorded and it was moved into a separate petri dish with dry filter paper and no foliage. It was then placed in a different box with moistened paper towels on the bottom so that it could be quickly checked every day.

Set-up of Emerged Adults.

After 16-20 days of pupation, metamorphosis of larvae into adult beetles is generally complete. However, certain individuals were unable to cut out of their fecal cases because low humidity can cause the case to be too hard. At this time a small portion of the case is cut to observe the progress of the beetle and ensure a successful emergence. New adult beetles were weighed and sexed by observing key features on their bodies, such as the presence of a fovea on the underside of female individuals, and were set-up in petri dishes with moist filter paper and a small piece of foliage. They were kept like this until most of the beetles have emerged, at which point significant host preference and mating preference experiments could be performed. All tests were adapted from previous studies to suit the system being studied [4].

No Choice Feeding Tests.

After determining the sexual strategy of our beetles based on the male to female ratios in each brood, we can distinguish the gynogenetic beetles from the sexual beetles. All female broods with at least ten individuals give us a 99.9% certainty of the presence of gynogenesis and a single male proves sexual reproduction. Both types of beetles were subjected to 24 hour no-choice feeding tests in which the tested beetles were given either alder foliage, a plant that is fed on by other Neochlamisus species and can be fed on by N. platani, or sycamore foliage cut into discs with a hole-punch and were left alone for 24 hours. After this time had elapsed, the foliage cuttings were observed under a gridded microscope to measure the consumed foliage. This process was then repeated for each individual using the foliage not present in the first trial.

Observational (Foliage Choice) Tests.

Observational tests were conducted by placing leaf discs, two of alder and two of sycamore, in a crisscross pattern in a petri dish. These discs were placed with one sycamore disc at the top left and bottom right and one alder disc placed at the top right and bottom left for consistency. Beetles were then placed into these dishes for 2 hours and were checked at ten minute intervals. At each interval, the beetle's position in the dish was recorded (*i.e.*, top sycamore, bottom alder, *etc*...).

Mating Preference Tests.

Mating preference tests were conducted by randomly setting up males with females of each sexual strategy type in a petri dish and checking these individual pairs periodically for 2 hours. A classification system was set up for the type of behavior the beetle pairs exhibited, with the main two conditions being the copulation of the beetle pair versus the separation of a pair of beetles in a dish.

Statistical Analysis.

All data taken from the three experiments were analyzed using a t-test between information from the sexual and gynogenetic groups. P-values were assigned to these data and standard error was used to further demonstrate the relationship between feeding of the different groups.

RESULTS.

Preliminary Data.

Prior to metamorphosis of the beetles, the data taken in categories such as percent of eggs hatched from a given female, weight of pupae, and eggs laid per day by a female were plotted in an attempt to get an early indication of physiological differences between gynogens and sexuals. A bimodal distribution of values for these categories does not seem to be present (Figures S1-3), but for much of the data sample size was low and therefore could not be a clear representation of any such patterns.

No Choice Feeding Tests.

By conducting no choice feeding tests for the beetles, we determined that gynogenetic individuals do have a tendency greater than that of sexual individuals to feed on alternate host foliage. Gynogen beetles fed on sycamore foliage for 69.25% of the foliage consumed over the two no choice feeding trials, compared to 77.04% sycamore foliage consumed on average by the sexual beetles (Figure 2). This difference was proven to be a function of the difference between the two beetle types by a p-value < 0.01.

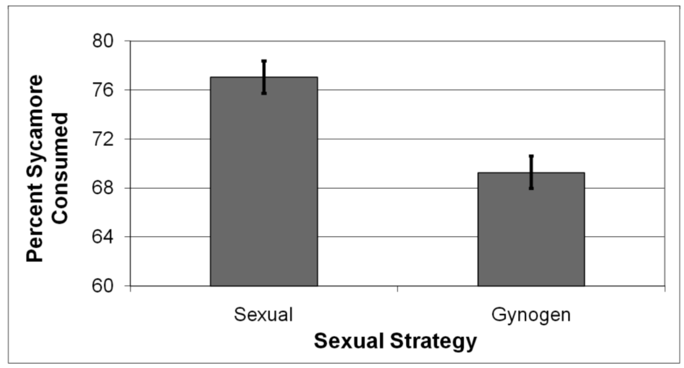

Figure 2. Percent sycamore consumed categorized by sexual strategy. Error bars help demonstrate statistical difference between two data sets for the gynogen and sexual groups in which sexual individuals are more specialized to feeding on sycamore foliage.

Observational (Foliage Choice) Tests.

To quantify the data from the observational tests, the number of times a beetle switched between two different types of foliage was considered. A score of 0 was given to a beetle that never switched from one foliage disc to another. Gynogen individuals switched an average of 0.45 times compared to an average of 0.53 times for sexual individuals. This difference resulted in a p-value of 0.3195, meaning no significant difference between sexual and gynogenetic beetles.

Mating Preference Tests.

On average, gynogen females were 16% more likely to mate with a sexual male than were sexual females. This difference was proven to be significant with a p-value of 0.020. Individuals from each category who mated were also considered in two different ways. Values from 0-12 were used to measure both quickness to start mating and number of observations in which they were mating. As for the quickness to start mating, the value was gotten by taking the number 13 minus the number corresponding to observation when they first started to mate so that a higher number is assigned to a faster individual. For gynogens, the results were 9.12 and 8.56 respectively, and for sexuals they were 8.75 and 8.44 respectively. Neither of these values yielded a significant difference.

DISCUSSION.

Feeding Tests.

The results show that there is a significant difference between host specialization of gynogen and sexual beetles, with sexual beetles feeding on average eight percent more on sycamore foliage. Gynogenesis is considered to be a phenomena because it combines negative aspects of both sexuality and standard parthenogenesis [2], however these results show an advantage to the system. The capability to readily feed on an alternate host plant could prove useful for gynogenetic populations in the case of certain circumstances, such as extreme deforestation of their usual sycamore host plant. This could also give them access to an alternate source of male mates, because if they are more capable of tapping alternate resources, then they could also mate on these other resources. In theory there would be no disadvantage to this for gynogenetic beetles because mating with these other males would not produce offspring of low viability as would be the case with sexual individuals. Though gynogens have more apparent disadvantages than the sexual beetles, this is one way in which their physiology could prove to be a solid defense against habitat loss. That being said, sexual beetles were capable of feeding on alternate host foliage in a no choice situation, so further tests should be conducted to examine the effects of long term exposure of alternate host foliage consumption on the fitness of both beetles types.

Observational (Foliage Choice) Tests.

When comparing the decision making abilities of the two beetle types, gynogens aren't significantly different from sexuals. Even though gynogens have an extra set of chromosomes which could potentially skew their decision making process since genes in that third set could potentially make it more difficult to settle on a plant, both types of beetles have similar tendencies in choosing a host to reside on. If one beetle group was significantly less capable of making decisions, that would increase their susceptibility to predation and also cause them to use more energy than is necessary in settling on a host. This similarity could be a function of the natural tendency of an organism to avoid predation by keeping unnecessary movement to a minimum.

Mating Preference Tests.

The most basic element of the mate preference tests was the presence of mating between individuals. The tests showed that gynogens were less picky about who they mated with since they were on average about 14% more likely to mate. This makes sense considering the fact that they only require copulation and not the male's genetic contribution to reproduce. This is advantageous for gynogenetic individuals since they can produce healthy and fit offspring even when mating with either unfit males or males that would generate unfit or even sterile offspring. For the individuals from both groups that did mate, not much difference was observed in when they started mating and how much they mated. This suggests that individuals willing to mate are similar regardless of sexual strategy since at the point of copulation, they have already gotten past the mate choice stage.

CONCLUSION.

Gynogenetic individuals co-occurring in a population with sexual individuals is a strange and rare system that is still shrouded in mystery. Direct comparisons were used across three different types of tests to elucidate certain physiological and behavioral differences between these two distinct groups. Results showed that significant differences are present in important components of life such as eating and mating for the *Neochlamisus* leaf beetles, but their decision making may potentially be quite similar. Showing that these two groups of beetles differ is strong evidence that there are ways in which gynogenetic beetles may have an advantage over sexuals. Sample size for the mating preference tests was balanced for both beetles groups, but more tests are planned to increase resolution for this data. Tests similar to these with the introduction of closely related *Neochlamisus* species as potential mates are also planned. It is expected that gynogens will show less mating discrimination for these tests as well.

The reasons for the presence of gynogenesis still are not totally clear. This study succeeds in presenting data supporting certain advantages to being a gynogenetic individual, but does not provide explanation for why gynogenesis is present in beetle and other populations of organisms in the first place. Even proving that gynogens are more receptive to mating, which is interesting and important to the interactions between the groups, does not cover up the fact that, if they could clonally reproduce without copulation, they could thrive in their habitats. Other differences may be present in gynogen populations that further explain its persistence.

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SUPPORTING INFORMATION.

Figure S1. Percent Egg Hatch
Figure S2. Range of Pupal Weight
Figure S3. Eggs Laid Per Day
Figure S4. Map of Natchez Trace

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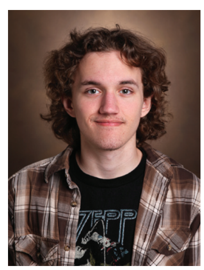

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