

**SEXUAL SELECTION AND PATERNAL
CARE IN *RHYNOCORIS TRISTIS*
(HEMIPTERA:REDUVIIDAE)**

by

Christy Ann Beal

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Entomology and Wildlife Ecology

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DEDICATION

To my daughter Niobe and my husband John for everything ... and then some,
and
to my Grandfather, Thomas Mercer, who would have been amused that I have spent
several years of my life with tiny assassins.

TABLE OF CONTENTS

LIST OF TABLES.....	vii
LIST OF FIGURES	viii
ABSTRACT	ix
INTRODUCTION	1
THE STUDY SPECIES: <i>RHYNOCORIS TRISTIS</i> (STÅL 1855).....	7
Family Reduviidae.....	7
Study Species Description	7
Reproductive Biology.....	8
Paternal Care in <i>Rhynocoris</i>	9
PROJECT I: PATERNAL CARE	12
Rationale.....	12
Methods	16
General Methods	16
Project I.1 Size Preference.....	16
Project I.2 Site Preference	17
Project I.3 Preference for Communal Oviposition	18
Project I.4 Preference for Guarding Male.....	18
Results	21
General Life History Data	21
Project I.1: Size Preference	21
Project I.2: Site Preference	22
Project I.3: Preference for Communal Oviposition.....	22
Project I.4: Preference for Guarding Male	22
Discussion.....	23
PROJECT II: AMPHISEXUAL CARE	25
Rationale.....	25
Methods:.....	26
Project II.1	26
Project II.2	28
Results	28
Project II.1	28
Project II.2	29
Discussion.....	29
DISCUSSION.....	31
REFERENCES	34

LIST OF TABLES

Table 1: Summary of Project I Null and Alternative Hypotheses.	15
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LIST OF FIGURES

- Figure 1: Male *Rhynocoris tristis* guarding several egg masses..... 11
- Figure 2: Diagram of general cage for *R. tristis*. Dental wick was refreshed daily with a solution of sugar water. Unless otherwise specified, *Stylosanthes* sprigs were replaced weekly. 20

ABSTRACT

Exclusive paternal care is the rarest form of care in the animal kingdom. Since Triver's (1972) landmark paper, several hypotheses have been proposed to explain why male care occurs at all. *Rhynocoris tristis* (Stål 1855) is a small, African reduviid exhibiting exclusive male-care of multiple adjoining clutches of eggs. Its small size, relatively short life cycle, and close phylogenetic relationship to the maternally caring *R. carmelita* make *R. tristis* an excellent candidate for studying the existence of exclusive male care.

A laboratory colony of *R. tristis*, derived from stock collected at Namulonge Agricultural and Animal Production Research Institute near Kampala, Uganda and housed at the University of Delaware, was manipulated to examine behavioral characteristics leading to male care. Females showed no egg-laying preference for sites near food sources. Nor did females show any preference for larger or smaller males.

Females did show a significant preference for laying eggs adjacent to other females egg masses. Additional female choice experiments were abandoned when the colony died prior to their completion. With the death of the colony, I was unable to definitively determine if male guarding is driven by natural or sexual selection or by some combination.

This thesis also provides the first record of amphisexual care in an insect. Female *R. tristis* were observed to take up a guarding stance over their clutch of eggs if the guarding male died or abandoned the clutch. Both males and females were tested

for guarding tenacity with a simulated predator. Males and females showed no significant difference between the number of attacks by the predator that they tolerated while guarding an egg mass. Non-guarding males and females quickly departed when attacked. This suggests that both sexes can provide care for the eggs, but the female will only do so if there is no male present. The existence of amphisexual care in *R. tristis* suggests that male egg-guarding behavior in this species could be dependant on a particular set of circumstances, and not a fixed behavior.

Chapter 1

INTRODUCTION

The evolution of exclusive parental care from a state of noncaring has been seen as a fitness trade-off by one parent to insure the survival of the current brood of offspring to the detriment of future broods (Zeh & Smith 1985, Clutton-Brock 1991). Males and females experience this “trade-off” in different ways, possibly because the females make a higher initial investment in large gametes and are therefore most likely to be the care-giving sex (Queller 1997).

This hypothesis is supported by Bateman’s classic 1948 paper on *Drosophila melanogaster*. Bateman showed that females are limited by the number of eggs that they can produce, whereas the number of offspring that a male can produce is only limited by the number of fertilizations that he can achieve (Bateman 1948). This phenomenon led Bateman to the conclusion that the potential reproductive fitness of a male can increase exponentially *ad infinitum* with an ever-increasing number of matings, whereas a female is limited by the number of eggs that she can produce regardless of the number of matings that she can obtain (Bateman 1948, Arnold & Duvall 1994). Bateman’s predictions have been further supported by a series of experiments on the sex-role-reversed pipefish, where it is expected, from Bateman’s hypotheses, that the pouch-brooding males will be the more limited sex. Measurements on male and female *Syngnathus typhle* show that the number of offspring for female pipefish increases with the number of matings while male

reproductive success is limited by the number of progeny that can be brooded in his pouch (Jones et al. 2000, Jones & Avise 2001).

Bateman's predictions have also been supported in experiments on the rough-skinned newt (*Taricha granulosa*), a species with typical sex roles. Within a single population, male newts showed greater variation in sexually selected characters, such as tail length and body size, as well as a greater difference between males in mating success and number of progeny. Females, alternatively, showed no discernable sexual selection for physical characteristics, and a consistent reproductive rate (Jones et al. 2002).

The difference between the potential reproductive rates of males and females has been cited as the reason that females are more likely to provide care. Because of her high initial investment in producing eggs (Clutton-Brock 1991), females are more committed to additional investment in the young and have more to lose from abandoning the young (Trivers 1972, Thornhill 1983). In addition, females have more to gain from providing additional care (Queller 1997). Even in species that show cooperative brood care (burying beetles, dung beetles, many birds), the female's investment is considerably higher than the male's (Fetherston et al. 1990). At any time, the parent that has the lower investment in young benefits from desertion if there are opportunities for additional reproduction. The male deserter loses less than his partner if no young survive, and the additional investment by the remaining partner will only benefit the deserter. The deserter, then, has the opportunity to re-mate and start another brood, thereby increasing his overall reproductive success (Trivers 1972). Male care is therefore predicted to be rare in animals, since females have the higher

initial investment, and males only increase their offspring by obtaining new mates (Trivers 1972) .

Males gain less by guarding young and lose less by abandoning a brood than females; yet, sometimes males do provide extensive parental care to eggs and offspring. Male care is rare in most taxa, but it is the predominant type of parental care in most fishes and frogs, and is known to have evolved at least 16 times in arthropods (Clutton-Brock 1991, Tallamy 2000).

Trivers (1972) predicts that paternal care is much more likely to evolve when the male is assured of paternity. He predicts that male animals that have external fertilization not only have a higher confidence of paternity, but because such males are associated with the eggs when they are laid, the opportunity to care is also present. Externally-fertilized fish and frogs exhibit many instances of exclusively paternal care (Bisazza & Marconato 1988, Knapp & Sargent 1989, Sikkel 1989, Goulet 1998). Among internally fertilized insects, parental care has been observed in 19 orders of insects (Tallamy & Wood 1986). Of those, only Hemiptera in the families Coreidae, Reduviidae and Belastomatidae, and Thysanoptera in the Phlaeothripidae are known to exhibit exclusive male care (Kaitala & Mappes 1992, Tallamy 2001). The reasons that paternal care occurs in so few insect taxa are not known, and until recently they have been given only cursory study (Tallamy 2000).

One hypothesis describing the evolution of male care predicts that male care will be advantageous when it does not interfere with opportunities for additional matings and when it increases the survival of the young (Trivers 1972, Maynard Smith 1982, Clutton-Brock 1991). In the case of males that are able to simultaneously provide care for multiple broods, the cost of male-guarding should decrease with the

number of broods that are defended; in this scenario, male care is likely to be evolutionarily stable (Zeh & Smith 1985).

The evolution of male egg-guarding has recently been modeled in detail by Manica and Johnstone (2004). They examined rates of care for males and females in two species of assassin bugs in the genus *Rhynocoris* (Reduviidae), one exclusively male-guarding (*R. tristis*) and one exclusively female-guarding (*R. carmelita*). Their analysis predicts that high density populations are more likely to support the evolution of male care, because a male will continue to encounter a high number of females even if he remains with the egg mass. Thus, when densities are high, male *R. tristis* suffer no loss of promiscuity (Manica & Johnstone 2004).

Male care provides a direct fitness benefit for the female by allowing her the freedom to forage and therefore to produce additional eggs (Zeh & Smith 1985). Females may thus favor mates willing to provide care (Thornhill 1976, Zeh & Smith 1985). Females benefit from being choosy by either a direct increase in their fitness or by an indirect improvement in offspring fitness (Andersson 1994, Møller & Jennions 2001). Since males unwilling or unable to demonstrate the intention of providing paternal care are less likely to mate (Thomas 1994), female choice seems to play a role in maintaining guarding behavior. In essence, female choice exercised prior to copulation may force the males to maintain or increase paternal investment (Trivers 1972).

Parental Care in Rhynocoris

Males of *Rhynocoris albopilosus*, *R. albopunctatus* and *R. tristis* guard multiple masses of eggs at a time from predators and parasitoids (Bequaert 1912, 1913, Nyiira 1970, Thomas 1994). Bequaert (1912, 1913) was the first to record male

egg-guarding in *Rhynocoris*. His observations include a description of male *R. albopilosus* standing guard over an egg mass. The males are described as exhibiting classic guarding behavior, where the rostrum and front legs are extended when the male is disturbed. Males that were chased from the egg mass returned a short time later to continue guarding. Odhiambo (1959) completed an extensive life-history study of *R. albopilosus*, that included observations of guarding males from the time the eggs were deposited until after hatching. Males only left their egg mass if threatened, and several remained guarding egg masses that were being transported by hand or in bottles for an hour or more.

Thomas (1994) compiled an extensive dissertation examining paternal care in *R. tristis* and maternal care in *R. carmelita*. In both the laboratory and the field in Uganda, she compared life histories, behavior and population densities of the two species. She found that unguarded broods suffered significant mortality in the field, and, in the case of *R. tristis*, total mortality if the eggs were unguarded. Thomas (1994: 69-70) suggests offspring survival is contingent on parental guarding. This does not demonstrate that paternal, or maternal, care arose solely from the need to protect the eggs from predation. Although she suggests that there may be alternative reasons for the egg-guarding behavior, she did not examine other possible causes. Thomas (1994: 99-117) determined female preference for an egg-guarding male by comparing the mating success of males guarding an egg mass to males without an egg mass. Using the sequential-choice design of Ridley and Rechten (1981), she paired females with either a guarding (+) or non-guarding (-) male for 4.5 hours and then immediately moved to a second pairing, either + or - for another 4.5 hours. Her results

show significantly more females laying for guarding males in the -/+ choice than in the -/- choice.

Thomas (1994: 103) also conducted a simultaneous choice experiment where a mating pair, with the male riding on the female's back, was introduced into a cage with an established male guarding a clutch of eggs. In ten out of twelve cases, the guarding male and the riding male fought over the egg clutch. In eight of those cases, the guarding male won the fight and then mated with the female prior to oviposition. Her results do not support female choice for a guarding male, since the female appeared to be a passive participant in male-male competition for the egg mass and the female.

Chapter 2

THE STUDY SPECIES: *RHYNOCORIS TRISTIS* (STÅL 1855)

Family Reduviidae

Assassin bugs (Hemiptera: Heteroptera) are a large and diverse family that is found world-wide, with most of the 5000+ species occurring in the tropics (Miller 1971). They are ancient insects with fossil records suggesting that the predatory forms evolved from plant-eating Hemiptera some 230 million years ago in the Permian/Triassic periods (Wootton 1981). The Reduviidae occupy largely tropical habitats in Africa, Asia and the Americas and exhibit a variety of feeding habits, including haematophagy in the Triatominae and various methods of predation (Haridass et. al., 1987).

Study Species Description

Rhynocoris tristis is a small reduviid, approximately 1 centimeter long. Individuals are primarily black with bands of orange along the abdomen. The orange bands are more noticeable in the female, particularly when the abdomen is swollen with eggs. Females are larger and heavier than males (mean male weight N=48, 0.02g; mean female weight N=38, 0.05g unpaired t-test $t_{84}=12.32$, $P=0.0001$) (Thomas 1994), and the sexes can be easily distinguished visually by their size difference and from the swelling of the abdomen that occurs in gravid females.

Like most assassin bugs, *R. tristis* is a visually oriented predator. Prey is approached with the forelegs held outstretched and the rostrum extended. Prey is grabbed with the forelegs and quickly paralyzed by an injection of saliva from the rostrum. The victim is then slowly digested while the exoskeleton remains impaled on the rostrum. *R. tristis* primarily hunt small Hymenoptera, but are opportunistic and will take any available prey (see compiled list of prey taken in Thomas 1994: 29). In the laboratory *R. tristis* have been successfully reared on *Drosophila* and sweep net contents.

Reproductive Biology

During mating, the male will ride the female for several hours, during which they copulate repeatedly. Just prior to oviposition, the male dismounts and the female ejects the spermatophore. Females lay clutches of 3-37 eggs each (19.6 ± 1.0 ; $N = 68$) (Thomas 1994), gluing them to the stems of *Stylosanthes guianensis*. There is nothing obvious that distinguishes the sites chosen for oviposition. In laboratory choice/no choice tests, females do not prefer to oviposit near sugar water food sources (exact binomial goodness of fit test $P = 0.36$ $N = 19$ see pg 22). There is therefore no evidence that females choose oviposition sites for physical advantages associated with a particular area. After ovipositing, females leave the site to feed and produce their next clutch (interclutch interval = 4.6 ± 0.5 days; $N = 30$). Females lay multiple clutches, often in multiple males' egg masses. Individual females often return to the same site to oviposit each time an additional clutch is mature. Furthermore, 1-5 females may oviposit at the same site (1.8 ± 0.2 ; $N = 44$ broods) (Thomas 1994). This results in an aggregation of up to 20 clutches in a single brood (30 to 514 eggs)

(Thomas 1994), each physically touching (or nearly touching) but easily distinguished from other clutches.

After the clutch has been laid, the male assumes a characteristic guarding position over the eggs, where he generally remains until the eggs hatch. The male does not move from his stance over the eggs unless mating with a newly arrived female or when he moves to the side to allow the female to deposit new eggs. Eggs hatch 16-17 days after oviposition at 37°C. Once the nymphs hatch, the caring males do not provide food or any other form of care to the nymphs. The nymphs remain around the hatch site until the second molt and then begin to wander. The tiny black nymphs complete five instars in an average of 50 days. In the lab, mortality was high, with greater than 50% of hatching nymphs failing to reach adulthood.

Paternal Care in *Rhynocoris*

Male-caring species display striking host-plant fidelity in contrast to the more generalized non-caring and maternal-caring species (Tallamy 2001). *Rhynocoris tristis* spends its entire life on *Stylosanthes guianensis*, an herbaceous legumes that is common throughout tropical Africa (Thomas 1994). The other two male-caring species in the genus also live exclusively on *Stylosanthes* species; *R. albopilosus* on *S. mucronata* (Odhiambo 1959) and *R. albopunctatus* on *S. gracilis* (Nyiira 1970). This host-plant fidelity may be a prerequisite for the evolution of male care. By localizing the population on one species, densities of *Rhynocoris* remain high in comparison to non-caring and maternally caring species (Thomas 1994). Males may be more likely to encounter females and thus suffer no loss in mating opportunities (Tallamy 2001).

Although male care occurs within *Rhynocoris*, Thomas (1994) suggests that there is no sex role reversal in *R. tristis* because the potential reproductive rates

(PRR) were skewed in favor of the males. That is, male reproduction is limited by the number of eggs sired, not by the number of offspring reared. Because egg clutches are active mating sites, males will aggressively compete for control of clutches regardless of the parentage of the eggs (Odhiambo 1959, Thomas 1994). Although this may seem to be contrary to natural selection, the male apparently does gain future matings by possessing an egg mass. Females are more likely to mate with a male that is already guarding a clutch, and each time females were observed adding eggs to an existing clutch, they mated with the guarding male first (Thomas 1994). This suggests that the primary method of selection is by sexual rather than by natural selection. In addition, males are more likely to encounter females while guarding, and thus may suffer no loss in mating opportunities during periods of care (Thomas & Manica 2005).



Figure 1: Male *Rhynocoris tristis* guarding several egg masses

Chapter 3

PROJECT I: PATERNAL CARE

DO FEMALE *R. TRISTIS* CHOOSE MALES BASED ON THEIR DISPLAY OF PATERNAL CARE?

Rationale

Sexual selection was defined by Darwin (1871) as occurring in two forms: (1) the competition between members of the same sex for the opposite sex and (2) the preference of members of one sex for traits in the other. He defined sexual selection as separate from the more commonly referenced natural selection by the critical difference of what was providing the selection pressure. Natural selection is caused by selection agents outside the species (Darwin 1859), either biotic (predators, food sources or diseases) or abiotic (asteroids, weather etc.). Sexual selection is caused by pressure exerted by members of a species on individuals within the *same* species, and acts differently on males and females. Sexual selection is credited with creating bizarre morphologies and behaviors, such as a male peacock's tail or a bowerbird's elaborate bower and a penchant for the color blue. Sexual selection pressure can also be directed towards the behavior of either the male or the female of a species. In the case of *Rhynocoris tristis* I attempted to distinguish whether the egg-guarding behavior of the male is reinforced through sexual selection by the female, or if it arose through natural selection.

Females of *R. tristis* tend to mate and lay egg masses with males who are guarding existing egg clutches (Thomas 1994). Determining female preference for demonstrated male caring is important in order to target sexual selection as the driving force in the development or at least the maintenance of exclusive male care. Female choice for better paternal care has been demonstrated in several vertebrate taxa (Östlund & Ahnesjö 1998, Kolm 2001). However, there are several scenarios that could lead to females choosing males with established egg clutches, and not all of these are driven by sexual selection. Are the females selecting a male based on his demonstrated caring ability, the proximity of food sources, the benefits of the dilution effect, the security of the actual egg laying location, or some combination of these? If males vary in their quality as guardians, females should choose the male that is providing an honest signal of paternal care (i.e. egg ownership). Thomas (1994) shows that males vary in their guarding duration and that females are not choosy in the absence of demonstrated paternal intent. She did not clearly demonstrate, however, whether females are selecting traits of the existing egg mass, the oviposition site, or the male-guarding an oviposition site. If females are truly “choosing” males based on their apparent ability to provide protection to the eggs, then this is indeed a case of the female exerting sexual selection pressure on the male. Since there is a total loss of unattended egg masses to predators and parasitoids in the field (Thomas 1994), an effective male-guarding trait could quickly become dominant at the population level. Project I is an attempt to tease apart the impact of sexual and natural selection on male guarding behavior once male egg-guarding has developed. See table 1 for a summary of the experiments and hypotheses in Project I. I recognize that these hypotheses are not mutually exclusive, nor are they all of the possible combinations.

The following possibilities exist:

1. Sexual Selection. Female is choosing to oviposit her eggs with a male demonstrating the willingness to care for the eggs (Ridley 1978, Ridley & Rechten 1981, Hoelzer 1989, Knapp & Sargent 1989, Kraak & Groothuis 1994) or;

2. Natural Selection. Female is choosing a favorable oviposition site and the male is at that site. *Rhynocoris tristis* females could select oviposition sites for their proximity to food for hatchlings. Females might also seek to oviposit in existing egg masses to reduce predation or parasitism on eggs, through the dilution effect (Hamilton 1971). In these cases then female choice of the site may not focus on the male at all. This scenario could lead to many females finding the site, regardless of male “attractiveness”. (Sargent 1988, Kraak & Groothuis 1994, Kraak & Weissing 1996).

Table 1: Summary of Project I Null and Alternative Hypotheses.

Experiment	Title	Null	Alternative
I.1	Size Preference	Females do not have a size preference for males	Females prefer either large or small males.
I.2	Site Preference	Female oviposits eggs randomly when compared to sugar water	Male is in possession of food source. Female chooses males based on his proximity to food or water regardless of his possession of eggs
I.3	Preference for Communal Oviposition	Female egg placement is random in relation to other females egg mass.	Female prefers to oviposit eggs with an existing clutch
I.4	Preference for Guarding Male	Female mates and lays egg with first male she encounters, regardless of whether he owns an egg mass	Female prefers male with egg clutch

Methods

General Methods

Three pairs and two egg masses of *R. tristis* were collected at the Namulonge Agricultural and Animal Production Research Institute near Kampala, Uganda to start the Delaware colony. Males and females were separated at adulthood. Adults and young were provided with *Drosophila* and either sciarid fungus flies or sweep net contents one time each day. Many adults were maintained as a breeding population. *Stylosanthes guianensis* was raised from seed and grown in the greenhouse in large pots.

Project I.1 Size Preference

I first needed to determine whether females prefer certain traits, such as size, in males that do not own an egg mass. One virgin female was placed in a 0.5L container with a clear plastic lid. The container contained two sticks (< 3mm thick) and a dental wick moistened with sugar water. Two virgin males were randomly marked with a dot of White Out™ for identification and then weighed prior to placement in the container. Males were weighed after being marked to consider any added weight that the White Out™ might contribute. The container was small enough for the female to easily encounter both males. The adults were provided with *Drosophila* and fungus flies in abundance.

Encounters between the sexes were monitored. Attempts to mate by males, duration of copulation, and post-copulatory riding was recorded. Aggressive interactions between the two males were recorded, noting the aggressor and the winner

of the contest. Although the second male was placed into the container immediately following the first, the order that the males were placed in the container was recorded and compared to female preference to determine if the female simply chose the first male that was placed in the container.

Last-male sperm precedence is assumed. The last male with whom the female mated prior to oviposition was recorded as the successful male. Number of eggs oviposited was recorded. After oviposition the female was removed from the container. The experiment was repeated with new players 10 times.

Project I.2 Site Preference

Rhynocoris tristis have been observed drinking from stems of *Stylosanthes* (Thomas, 1995). I needed to determine if females are choosing to oviposit eggs based on proximity to a food source rather than a guarding male. To accomplish this, sugar water location within containers was manipulated.

A virgin female and a virgin male were placed in a 0.5L container with a clear lid. The container contained two sticks (< 3mm thick) marked in 2 cm intervals and did not contain a stem of *Stylosanthes*. The insects were provided with *Drosophila* and fungus flies *ad libitum*. One dental wick moistened with sugar water was placed at the top of one stick. A second dental wick, that remained dry, was attached to the top of the second stick. After oviposition, I measured the distance between the eggs and the moistened dental wick and compared by an exact binomial goodness of fit test with random oviposition on the two sticks (N=20). Random oviposition was defined as the probability of the egg mass being oviposited within any of the 2cm markings along

the sticks. Length of time for the female to oviposit (from her initial introduction to the container) and the number of eggs in her first clutch were also recorded (N=20).

Project I.3 Preference for Communal Oviposition

For egg guarding to be a sexually selected trait, the female needs to choose mates based on the males' intent to guard, rather than the protection that is afforded her eggs by a large egg mass. Males and females were all of approximately the same age to control for mating preferences that may be related to age. A female was permitted to mate, and was then separated from the male (N=20). The gravid female was then placed in a 0.5L container with a clear plastic lid. The container contained two sticks (< 3mm thick) and a dental wick moistened with sugar water. One stick contained an egg mass previously deposited by a different female that was no longer present. The sticks were marked in 2cm intervals. The location of the new egg mass was recorded. The distance separating the new eggs from the existing egg mass was measured and compared by X^2 with random distribution of eggs on the two choices. Random oviposition was defined as the probability of the egg mass being oviposited within any of the 2cm markings along the sticks.

Project I.4 Preference for Guarding Male

If egg guarding is a sexually selected trait, females should prefer guarding males over non-guarding males. The sequential choice design of this experiment follows Ridley and Rechten's (1981) investigation of parental choice in the threespine

stickleback, and Thomas' (1994) design testing female *R. tristis* preference for males with eggs.

Male with clutch vs. male without clutch: One gravid female was randomly assigned to either a non-guarding male (-) or a guarding male with one egg clutch (+). A gravid female was used in order to shorten the time between her introduction to the container and oviposition. Although egg clutches previously laid by each female were used in this experiment, care was taken to prevent any female from being tested with her own eggs. The pairs were placed in a 0.5L container with a clear plastic lid and no food. Pairs remained together for 4.5 hours. Females were then reassigned to either a guarding (+) or non-guarding (-) male's container. Females in the process of ovipositing at the end of 4.5 hours were permitted to finish. Males riding females at the end of 4.5 hours were separated from the female. Ten different females were used in each of the four pair combinations (--, ++, -+, +-). Results were analyzed using the GENMOD procedure for repeated measures logistical regression using SAS. Both the relationship between males with and without egg masses and the relationship of female choice between trial one and trial two were examined.

Male with clutch vs. clutch without male: Experiment I.4 was originally designed to consist of two separate comparisons to determine whether the female chooses a guarding male or an attractive egg-laying site. If the site itself was attractive to the female, and the male was simply sitting at the egg mass to gain additional matings, a lone clutch should attract a gravid female as often as a clutch that is guarded by a male. The design for the second half of this experiment was identical to the male with clutch vs. male without clutch experiment, except that the females were given a choice of either a egg clutch without a male (-) or a guarding male with

one egg clutch (+). Unfortunately, the Delaware colony died prior to the completion and only the first half of experiment I.4 was completed.

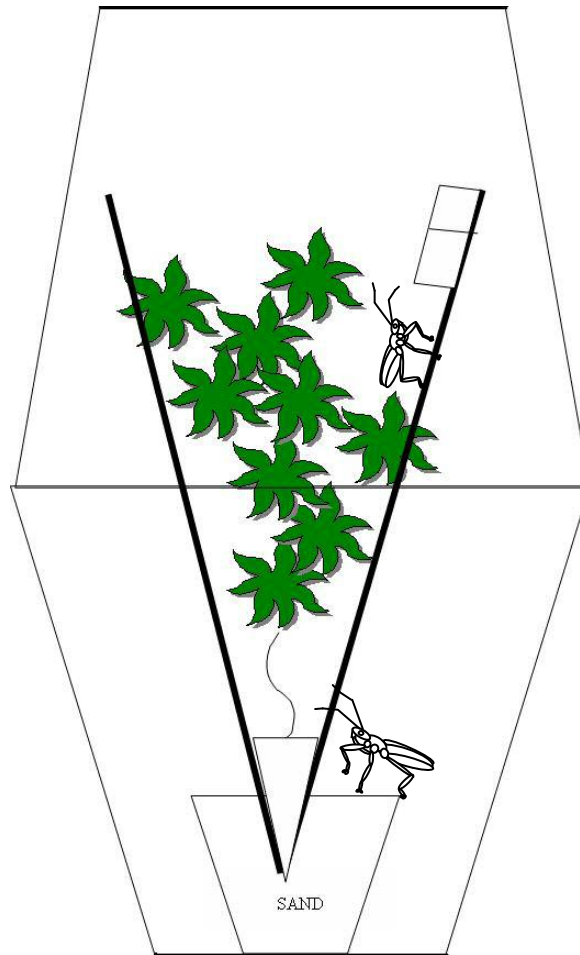


Figure 2: Diagram of general cage for *R. tristis*. Dental wick was refreshed daily with a solution of sugar water. Unless otherwise specified, *Stylosanthes* sprigs were replaced weekly.

Results

General Life History Data

Of the three pairs of *R. tristis* that were collected in Uganda to start the Delaware colony, only one pair mated and produced eggs in the lab. This pair eventually produced six adult females and six adult males. The two egg masses that were collected produced 22 females and 50 males with 57% of the eggs successfully hatching. Females took an average of 24 days after their initial set-up in the containers to deposit their first clutch of eggs (range = 1-81, N=38). The number of eggs laid in a female's first clutch also varied widely, from as few as 3 to as many as 40 with an average of 17 (N=38). Eggs took an average of 13 days to hatch in the lab (N=30). In the greenhouse, which was considerably warmer, eggs took an average of five days to hatch (N=5).

Project I.1: Size Preference

Females did not show significant preference for the smaller males (N = 10, exact binomial goodness of fit $P=0.623$). Males did not vary markedly in size with a range of 0.0195 to 0.0332 g, average male weight was 0.0256 g (N=20). Within each replicate, the heavier males were an average of 0.0037 g heavier than the lighter male. There was no significant effect on female choice of the order that the males were placed into the container (N=10 Chi-squared $P=0.8266$). In only one case did the males fight over the female; in this case, the lighter of the two males was the eventual winner of the contest.

Project I.2: Site Preference

There was no difference between the frequency of egg masses deposited on the stick containing the sugar water or on the dry stick. In one case eggs were oviposited on the bottom of the container, and therefore that datum was removed from the analysis. Results were compared using an exact binomial goodness of fit ($P=0.36$, $N=19$). Females preferred to lay egg clutches within the top 4 centimeters of the stick (exact binomial goodness of fit: $P= 0.0044$, $N=19$).

Project I.3: Preference for Communal Oviposition

Females preferred to deposit eggs within previously laid egg masses. All females in the study ($N=20$) deposited their eggs adjoining the previously laid clutches of eggs.

Project I.4: Preference for Guarding Male

The results for Project I.4 were not statistically significant, but they do suggest two interesting trends that need to be further examined. Regardless of which order the females were introduced to a guarding male, she was marginally more likely to lay eggs with a male who possessed eggs in the time period provided (repeated measures logistical regression, GENMOD $P=0.1117$). A female was more likely to lay eggs in the second trial, regardless of whether the male was in possession of an egg mass (repeated measures logistical regression, GENMOD $P=0.1216$).

Discussion

In general, the life history data collected as part of Project I corroborates that reported by (Thomas 1994). The high rate of mortality coupled with the long time to maturity and egg laying made conducting experiments with large populations very difficult. Although every attempt to cross-breed the offspring was made, the small founding population meant that there were only three grandsires for the entire population and, at most, five granddames. This was very likely one of the contributing factors in the slow growth and eventual death of the colony. Nevertheless, the experiments that were completed with our colony permitted me to expand on our basic understanding of the possible reasons for paternal care in *R. tristis*.

Females did not prefer lighter males, as in Thomas' (1994) mating experiment. The weight differences between the males were very slight, averaging 0.0037g. A greater difference in weights should give more definitive results. There also was no preference for an egg-laying site in the proximity of sugar water. Females laid egg clutches arbitrarily between the two sticks.

There was a trend for females to lay eggs with males in possession of an egg mass. Unfortunately, the colony died prior to the completion of this experiment. Ideally, I would have been able to include a second set of tests in which a female would be offered sequential choices of an egg mass without a male, or a male without an egg mass. This manipulation would have helped to differentiate between whether male guarding, or a preference for laying eggs communally are the primary reasons for female preference to lay eggs with a guarding male.

Females preferred to lay eggs with existing egg masses, which typically are guarded by males. This suggests that both natural and sexual selection may be

factors in the evolution of male guarding. The female preference for depositing her eggs with an existing egg mass suggests that the dilution effect (Hamilton 1971) is playing a part in the egg laying choices of females. Eggs that are laid in large, communal masses limit the number of offspring that a single female loses to predators and parasitoids. One possibility is that the behavior of females laying eggs in communal masses may have preceded the advent of male care in the *Rhynocoris* lineage. In this case a male may have used communal egg masses to increase his mating opportunities. Since all non-guarded eggs are parasitized in the wild (Thomas 1994), a trait where males provide care for the eggs while waiting for additional gravid females would quickly become pervasive in the population.

A second series of population level experiments was begun in the greenhouse to determine if males were more likely to abandon egg masses in very small populations. For these experiments a lone male was introduced into a 1m by 1m cage containing a *Stylosanthes* plant. Males were randomly selected to receive from one to three females. One female simulated the encounter rate likely to be experienced by males in a small, founder population and three females simulated a large population encounter rate. Gravid females were introduced every two days until a maximum of three females were added. This experiment had to be cut short because the extremely high temperature in the greenhouse was causing the life cycle of the bugs to move too quickly to be properly observed. In addition, the absence of the native parasitoids to *R. tristis* limited the usefulness of the experiment.

Although the population crash prohibited definitive conclusions, the series of experiments in Project I did provide some additional, and much needed insight into the basic paternal behavior of *Rhynocoris*.

Chapter 4

PROJECT II: AMPHISEXUAL CARE

AMPHISEXUAL MATERNAL CARE IN AN INSECT WITH EXCLUSIVE PATERNAL CARE

Rationale

Models describing the origins of parental care (Maynard Smith 1977, Balshine-Earn & Earn 1997, Wade & Shuster 2002, Manica & Johnstone 2004) propose three resolutions to the evolutionary conflict over which parent provides care: neither parent cares; both parents care, or one parent compensates for lack of care by the other. The third option, uniparental care, can be provided exclusively by either the mother or the father, but the costs associated with care usually favor maternal over paternal care (Queller 1997). Nevertheless, many fish, frogs, salamanders, and a few birds and arthropods have evolved exclusive paternal care (Ridley 1978, Wells 1981, Gross & Sargent 1985, Tallamy 2001). In a scattering of these species, females will guard eggs and young if the male does not (Clutton-Brock 1991, Machado and Oliveira 1998). One striking example is found in the South American terrestrial frog, *Eleutherodactylus johnstonei*, where the male typically cares for eggs. If, however, a male fails to provide care, or abandons the eggs within 72 hours, the female will return to care for the brood (Bourne 1998). The converse has also been recognized in the opilionid harvestmen *Goniosoma longipes* where females guard eggs on cave walls. If

a female is removed from her eggs, the resident male takes over guarding duties (Machado & Oliveira 1998).

In the vast majority of subsocial insects, it is the female that provides post-copulatory care of offspring (Tallamy & Wood 1986) however, exclusive paternal care has evolved in at least six lineages of Heteroptera (Coreidae, Reduviidae and Belastomatidae), and Thysanoptera (Phlaeothripidae) (Zeh & Smith 1985, Kaitala & Mappes 1992, Tallamy 2001). Assassin bugs in the genus *Rhynocoris* are among the best known exhibitors of exclusive paternal care and have served as a model system for investigating the evolution of male care for decades (Odhiambo 1959, Thomas 1994, Thomas & Manica 2003, Manica & Johnstone 2004, Thomas & Manica 2005). Here I report evidence that *Rhynocoris tristis* females will facultatively care for eggs in the event that male care is withheld.

While studying the influence of male caring display on female choice in *R. tristis* I noticed that, under laboratory conditions, females stand guard over their eggs if males are removed or die while guarding. To determine whether females were actually protecting the eggs in the absence of a male, or whether they passively remained near an oviposition site to await the approach of a new male, I measured parental aggression in both sexes when faced with repeated attacks by a simulated predator.

Methods:

Project II.1

A virgin pair (N=10) was placed in a 0.5L Styrofoam™ container with an inverted clear plastic cup serving as a lid providing a space 30cm high with a

maximum diameter of 12cm for the bug habitat. The container contained two dowels (< 3mm thick) attached together at the bottom to form a large “V”. A dental wick moistened with a 10% sugar solution was attached to one dowel with thread and refreshed daily. Each pair was provided with *Drosophila*, sciarid fungus flies, and an assortment of wild-caught Heteroptera and Hymenoptera *ad libitum*. The pair was permitted to mate and oviposit one clutch of 13-23 eggs. After the male began guarding the clutch, his guarding tenacity and aggression was quantified by approaching him with a simulated predator (a dead coccinellid beetle (*Hippodamia convergens*) glued to a thin wire 15cm long) repeatedly until he abandoned the clutch (i.e. retreating more than 2cm from the nearest egg). Aggressive behavior was defined as the bug raising its front pair of legs and extending the rostrum in the threatening manner typical of assassin bugs. Although our observations all occurred under laboratory conditions, there was no mistaking the female behavior I observed for random wanderings near the eggs. After the males were removed from the arenas, females moved to within 1 cm of the clutch or stood over the eggs themselves until the eggs hatched, a period of 16-17 days. This is something that the females never did if the male remained in the arena except during the short period where she returned to an egg mass to deposit additional eggs. To simulate an attacking predator, the beetle was slowly moved to within 1cm of the egg mass along the dowel. As soon as the male raised its forelegs to threaten the predator, the predator was slowly moved backward along the dowel. After 30 seconds the predator was again made to approach the male. The male was not permitted to grab or touch the coccinellid, and the beetle was backed up slowly from the male as soon as the male assumed an aggressive stance, each approach and retreat of the predator was counted as a single guarding event. Once the

male had retreated from the eggs, he was removed from the container and the time required for the female to move to the eggs was recorded. The female was then confronted with the same simulated predator, and guarding tenacity was recorded in the same manner as for the male. Females that did not move to the eggs within 72 hours were categorized as non-guarding.

Project II.2

To test whether the aggressive response seen was egg guarding behavior or a general reaction to the approach of the simulated predator, both males and females were tested when they were not guarding a clutch of eggs. The non-guarding bugs were approached in the same manner as the guarding individuals. Lone males were approached without the presence of a female or egg mass in the same container (N=10). Lone females (N=10) were approached by the simulated predator while a male stood guard over her egg mass.

Results

Project II.1

There was no difference between the frequency and intensity of caregiving between male and female *R. tristis*. Males that guarded did so as soon as the female completed ovipositing. In one case, a male never guarded and the female immediately assumed guarding duties after laying the eggs. Nine out of 10 females that moved to the eggs did so within 24 hours of the male's removal. Both males and

females raised their front legs in the same manner when confronted with the coccinellid. The number of attacks tolerated prior to abandonment ranged from 0 to 32 for females, and from 2 to 65 for males. The number of attacks tolerated did not differ significantly between sexes (Mann-Whitney U-test, $U= 52.5$, $P=0.8808$, $N=10$). The median number of attacks tolerated was 5.5 for males and 7.0 for females.

Project II.2

None of the non-guarding males or females tested tolerated any attacks by the simulated predator. In every case, the bug backed away from the approaching beetle immediately. In one case a male took flight to avoid the approach of the beetle.

Discussion

Both male and female *R. tristis* aggressively guarded the egg mass when approached by the simulated predator. In contrast, non-guarding bugs immediately retreated when the simulated predator approached. The aggressive behavior that the bugs displayed towards the simulated predator only occurred while bugs were egg-guarding. Because solitary bugs did not display any aggressive behavior toward the coccinellid away from the egg mass, I believe the bugs reacted to the coccinellid as an egg predator rather than as potential prey.

Even in species with uniparental male care, females can gain an advantage from providing parental care, if the male abandons the clutch, because of her comparatively large investment in gametes (Williams 1966, Trivers 1972, Queller 1997). Large gametes have been said to place females that rely on male care in a “cruel

bind” if the male fails to deliver care (Trivers 1972, Dawkins & Carlisle 1976). Facultative maternal care is a mechanism that should be favored when the cost of egg production exceeds the cost of care. In *R. tristis*, egg production is a function of hunting success. Male care frees iteroparous females to hunt where prey densities are highest, rather than only near existing egg clutches. This enables females to generate additional clutches at an estimated eight times faster than if they were confined as guarders to an egg mass (Manica & Johnstone 2004). In the rare event that male care is not delivered, however, females provide the needed care for their first clutch themselves and thus are “willing” to absorb the reduction in future fecundity associated with care to provide the needed care for their first clutch themselves.

It is not as surprising as it may seem that facultative maternal care has gone unnoticed in an organism as well-studied as *R. tristis*. In populations that contain more than one male, both lab and field observations have shown that males compete for possession of egg masses (Thomas 1994). If a guarding male dies or does, in fact, abandon the eggs, another male typically replaces him at the eggs in short order. Thus, the need for females to take over caring duties from males is almost certainly quite rare in well-established populations, but may be important in small founding populations.

This is the first record of amphisexual care in a paternally caring insect. More than just an ecological curiosity, facultative maternal care is a powerful tool in studies of the evolution of parental care because it permits the quantification of the relative costs of exclusive maternal and exclusive paternal care within a single species. Such data are necessary to test recent models describing the evolution of paternal care unambiguously (Manica & Johnstone 2004).

Chapter 5

DISCUSSION

Exclusive male care is the rarest form of parental care. Of the 19 orders of Arthropods that exhibit some form of care, only two orders of insects possess exclusive male care. This thesis addresses why male care occurs in *Rhynocoris tristis*, and whether it evolved through sexual or natural selection.

The most likely explanation is a combination of both. Females in project I preferred to lay eggs with an existing egg mass. A female bias for communal egg laying may have preceded male care in this species. That bias, coupled with high population densities in *R. tristis*, makes it advantageous for a male to stand over a batch of eggs. By standing at a communal egg mass the male has more opportunities to mate with the multiple females that are coming to the clutch. By actively guarding the eggs from predators and parasitoids the male is guaranteeing that some of his offspring survive, therefore, increasing the number of possible offspring he has in two ways. While the male is guarding the eggs, the female has the opportunity to increase her fecundity through an increase in the freedom to forage while the male guards. For both sexes, male egg-guarding provides an increase in the potential number of offspring.

The experiments conducted here need to be expanded to determine if female preference is for egg-guarding males or if male guarding is secondary to

communal oviposition. Completing Experiment I.4 with a larger number of mating pairs would clarify what female *R. tristis* are actually choosing.

Amphisexual behavior in *R. tristis* suggests that male egg-guarding is not a fixed behavior. If male egg-guarding is population dependant, as Manica and Johnstone (2004) hypothesize, founder population females may need to guard their own egg masses in order for any of the offspring to survive. Only when the population becomes large enough for males to consistently encounter females will the males begin to guard the egg masses.

I was only able to complete a short experiment on the behavior verifying that the females were indeed displaying egg-guarding behavior in the absence of males with our small colony. I would like to expand this experiment in several directions, to not only verify that the behavior occurs in the wild, and under what circumstances, but if amphisexual care is also seen in the closely related male-caring species *R. albopilosus* and *R. albopunctatus*.

Evidence of amphisexual care in *R. tristis* (Beal and Tallamy 2006) provides a unique opportunity to test explicitly models of parental care. These models, such as Manica and Johnstone's (2004) model of the evolution of care based on population density and the cost of care, have, until now, needed to extrapolate data for one or the other sex in determining the costs of uniparental care. Expanding the experiments to include the other two male caring species, *R. albopilosus* and *R. albopunctatus*, in addition to *R. tristis*, as well as the maternally caring *R. carmelita*, will enable me to quantify for the first time the *actual* costs of both male and female care and the evolution of male care within several species in the same genus.

In order to develop a clear understanding of how male and female care arose in *Rhynocoris*, it will be essential to develop a phylogenetic hypothesis for the genus, or at minimum the species that are thought to be closely related to those that provide care. A phylogenetic hypothesis would clarify placement of the caring species within the largest and most opaque genus of the Reduviidae, and identify progenitors of caring lineages. Such information is required to test hypotheses regarding the evolutionary mechanisms that led to paternal care in *Rhynocoris*.

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