Differential Allocation of Male Mating Effort in a Gift-Giving Insect

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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University of Toronto

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2015

Abstract

Nuptial gifts, typically beneficial items given by a male to his mate, can be costly and thus can cause males to discriminate between mates. Female black horned tree crickets *Oecanthus nigricornis* (Orthoptera: Gryllidae) feed on secretions from male dorsal glands, providing an opportunity for males to favor higher quality females by feeding them more. I tested whether such preferential allocation exists in *O. nigricornis*, when a male encounters multiple receptive females prior to mating and is thus expected to become choosier. Feeding duration, proportion of male-solicited remounts, and singing rates were used as measures of male post-copulatory mate choice. No significant effects were found (perhaps due to low power of the tests) except for an unexpected increase in the singing rate towards high condition females when males encountered multiple males prior to mating. This may be due to males perceiving increased male-male competition on high condition females.
Acknowledgments

First and foremost, I would like to express my utmost gratitude to Darryl Gwynne, who was my thesis supervisor for the two years of my Masters project. He was the best supervisor a graduate student could ask for: he was approachable, insightful, inspiring, and extremely patient with me for the entire duration of my journey. I cannot thank him enough for all the helpful advice and discussions he has offered me, and for the astounding length and depth of revisions he has provided for this very manuscript. In addition, the many talks we have had over coffee stimulated me to think about not only my own research but also many other fascinating and interesting topics, both scientific and general issues.

For providing additional insights into my project, I thank the members of my thesis advisory committee: Helen Rodd and Andrew Mason. Their advices helped greatly in shaping my vague ideas into a more concrete project. I would like to thank Andrew Mason especially for making the trip from the Scarborough campus to St. George or even UTM every time there was a meeting, and Helen Rodd for not only her help as a committee member but also on administrative issues of being a graduate student.

I also owe a great debt to my fellow students who worked with me in the lab: Kyla Ercit, Crystal Vincent, Ana Marija Malenica, Muhammed Ali Kizilbash, and Edianne Monique Carpio. In addition, I should include Lindsay Coome from the Monks lab here as she has essentially been a part of the lab for the better part of 2015. They have all helped me greatly, either through giving me advice, providing feedback on my presentations, helping me catch and keep animals,
conducting trials, collecting data, or even just being good friends. I could not have made it this far without them.

During my project I have received aid from numerous members of the faculty, and I thank the entire department of biology in UTM and ecology and evolutionary biology in St. George for all of their support. I thank Nick Collins and Helene Wagner in particular as I have approached them numerous times for their advice on the statistical side of my project. In addition, Glenn Morris has been a great help on providing feedback on my project, especially on keeping and conducting experiments with my study animals. I also thank the Natural Sciences and Engineering Research Council and the department of biology itself for funding my research.

The two years of being a teaching assistant for BIO318 Animal Behaviour was a wonderful experience, and I thank the people of the course for making it as such. The lab technicians Hana Otta and Susan Dixon worked tirelessly so that the classes could run smoothly, and taught me much about the animals that we used in the lab. The other TA, Samantha Mahabir, I only have the greatest respect for teaching me about my work as a TA and also organizing much of the labs. I should also thank the professors of the course, John Ratcliffe and Karen Williams, for being there during the TA strike of winter 2015. I was lucky to have professors who were so considerate of their TAs. Also, the students of the course themselves should not be forgotten, as they were a vital part of what made the experience so great. They have taught me as much as I have taught them during the course of two years I have been their TA.

To my friends, both within my academic life as well as outside of it, I extend my most sincere gratitude for being there for me to talk to me and also help me relieve stress over drinks or
sessions of gaming. They helped me get through my most difficult times and gave me strength to go on.

Lastly, my family has been nothing short of supportive and accommodating for the entirety of my research. I thank them all for their emotional support, and apologize for not being able to spend as much time with them due to my schedule. To my father I am grateful for being my legs for much of the duration of this project, especially when I had broken my ankle in the spring of 2014. Without my family’s support none of this would have been possible, and I dedicate this thesis to them.
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Differential allocation of male mating efforts in black-horned tree cricket *Oecanthus nigricornis* (Orthoptera: Gryllidae)

Yurak Jeong

1.1 Introduction

In most mating systems, females exert mate choice (Trivers, 1972). Male reproductive success is mostly limited by the number of matings, and thus rejection of females and the resulting decrease in mating rate will lower male fitness (Barry and Kokko, 2010). Despite this, male mate choice is observed in many species (Bonduriansky, 2001). Males are expected to show mate choice when the benefits of choice outweigh the costs of decreased mating rate via a couple of factors (Gwynne and Simmons, 1990; Bonduriansky, 2001; Edward and Chapman, 2011). First, large variation in female quality, such as fecundity, minimizes the relative cost of rejecting lower quality females while increasing male fitness through mating with females of higher fecundity (Gwynne, 1991; Servedio, 2007; Nandy et al., 2012). Second, an increase in the number of sexually receptive females should decrease male effort in finding additional mates (Barry and Kokko, 2010). An increase in the number of receptive females relative to males (known as the operational sex ratio: OSR) can occur when males invest in costly mating behavior, which includes mate feeding (nuptial gifts), mate guarding (see Bonduriansky, 2001 for a review), and costly courtship rituals (Byrne and Rice, 2006; Heinig et al., 2014). These services can limit male mating rate via his ability to provide such investments rather than via the number of mates he can acquire (Emlen and Oring, 1977; Gwynne and Simmons, 1990).
In addition to these types of male mating efforts, which give males an advantage over their rivals in acquiring mates (Kokko and Jennions, 2008), parental effort can increase a male’s fitness by increasing the number, survival, and fitness of his offspring (Low, 1978). As care is often costly, species whose males make investments such as protecting eggs against predators (e.g. Itzkowitz et al., 1998), nest-building and rearing of young (e.g. Rowland, 1989) or even male incubation and provisioning of the embryo (e.g. a placenta-like structure in male-brooding pipefish: Kvarnemo et al., 2011) can show male choice. The intensity of male choice in species where males provide these investments is affected by OSR (Berglund, 1995), such that males in high condition or in populations with more receptive females tend to show higher levels of mate choice.

Female size is the most common trait assessed by males in taxa such as some insects, fish, reptiles, and amphibians, where female fecundity can vary greatly and larger body size is often associated with higher fecundity (Verrell, 1989; Rowland, 1989; Olsson, 1993; Itzkowitz et al., 1998; Bonduriansky, 2001; Herdman et al. 2004). Thus, males will almost always prefer larger females in a female-biased OSR (Shelly and Bailey, 1992; Allen and Bailey, 1994; Kvarnemo and Simmons, 1999), although there are some studies showing that males prefer smaller females, presumably to avoid increased sperm competition as higher condition females tend to mate more frequently (Simmons and Kvarnemo, 1997; Bateman and Ferguson, 2004). Males in a few species also choose females based on color and other conspicuous ornaments (Bussière et al. 2008; South and Arnqvist, 2011; Tigreros et al., 2014; Westerman et al. 2014), although exaggerated secondary sexual characteristics are quite rare in females as they likely exact a cost to fecundity (Fitzpatrick et al., 1995). Males may also detect and discriminate against diseased
females (Wittman and Fedorka, 2015), and can show preferences for dominant females in social species (Zhang et al, 2010).

One form of male reproductive effort often seen in insects is the nuptial gift, defined by Lewis et al. (2014) as “…material (beyond the obligatory gametes) provided by a donor to a recipient during courtship or copulation in order to improve donor fitness”. Gifts can be a captured prey, secretion from an external gland, a nutritious attachment to a spermatophore, part of the seminal fluid, or even the male’s own body parts (reviewed in Vahed, 1998; Gwynne, 2008; Lewis et al., 2014). These contributions can increase the fitness of the donor male, the receiving female, or fitness of one sex can be increased at a cost to the other (Gwynne, 2008). Nuptial gifts can increase male fitness by (i) delivering chemicals that lengthen a female’s refractory period and consequently delay mating with a rival male (e.g. Thornhill, 1976; Simmons and Gwynne, 1991; Arnqvist and Nilsson, 2000), (ii) increasing the production of his offspring by the female (e.g. Arnqvist and Nilsson, 2000; Chapman, 2008), or (iii) increasing survival of his offspring (e.g. Sandvik et al., 2000). Female fitness can also be increased by nuptial gifts through increased fecundity, better survival of offspring, and increase in female longevity (e.g. Boggs, 1990; Gwynne, 1993; Brown, 1994). Thus, nuptial gifts function as both mating effort and parental effort.

Glandular gifts are common in orthopteran insects, and the costs of these can be high, such as in some tettigonids where a male secretes a nutritious nuptial gift that can be up to 30% of his body mass (Wedell, 1993; Vahed and Gilbert, 1996). In some of these species, the OSR becomes female-biased when food resources are scarce as females seek additional gifts while males’ ability to provide the gifts decreases (Gwynne, 1984; Gwynne and Simmons, 1990; Simmons and Bailey, 1990; Shelly and Bailey, 1992). Consequently, males of these tettigonid species
display choice before mating by rejecting small females. Gift giving males can also selectively court certain mates prior to copulation as a form of mate choice, as is seen in cabbage butterflies that give seminal gifts to females (e.g. Tigreros et al., 2014).

However, nuptial gifts are not always beneficial to the female. While some nuptial gifts provide substantial nutritional benefits to the recipient (e.g. Voigt et al, 2008), others can increase female refractory period and lead to suboptimal mating rates (Arnqvist and Nilsson, 2000; Chapman, 2008), or only contain phagostimulatory chemicals that make the gift attractive to the female but with no real nutritional value (Sakaluk, 2000; Vahed, 2007). In such cases, there will be selection against females from being coerced into mating with useless gifts, and so provisioning of the nuptial gift can only be maintained by a strong selection to preserve the gifting behavior in males resulting from the benefits the gifts provide to the male (Gwynne, 2008).

A major benefit a male gains from a transfer of a nuptial gift, whether it is nutritious or not, is that female consumption of the gift increases the duration of mating and ultimately the size of ejaculate transferred to the female (Sakaluk 1984; Wedell and Arak, 1989; Simmons, 1995). The larger the gift, the longer it takes for the female to consume it, and thus more sperm is transferred in the additional copulation (or spermatophore attachment) time. Increased allocation of nuptial gifts can also increase the amount of chemicals that induce the refractory behaviour and consequently increase the refractory period of the female, which can give the mating male an advantage over his rivals in sperm competition (Arnqvist and Nilsson, 2000). In addition, male behaviours that prolong mating duration can serve similar functions to nuptial gifts. For example, mate guarding in many gryllids can delay a female from mating with another mate and physically prevent her from terminating sperm transfer prematurely (Alexander and Otte, 1967; Loher & Dambach, 1989; Hockham and Vahed, 1997; Bateman and MacFadyen, 1999). Thus,
allocating more nuptial gift to females increases paternity. However, male resources are limited, and glandular nuptial gifts can become depleted after multiple successive matings (Verrell, 1989; Brown and Kuns, 2000; Nandy et al., 2012). This means that male fitness can be maximized by reducing donations to low condition females while allocating more to high condition females if male cost is high and female condition is sufficiently variable (Edward and Chapman, 2011).

Selectively investing more into matings with high condition females can be considered a form of post-copulatory mate choice (Engqvist and Sauer, 2001), a parallel to the post-copulatory choice exerted by females that for example bias paternity through differential use of sperm from different mates (Eberhard, 1996). A gift giving male can display post-copulatory choice either by giving greater amount of ejaculate to females with preferred traits, which benefits the male by increasing the amount of sperm transferred to those females (Simmons and Kvarnemo, 1997), or changing the content of the gift so that higher condition females receive more nutritious gifts that can increase female and offspring fitness (Jarridge et al., 2013). Like pre-copulatory choice, post-copulatory male choice should occur when males experience a female-biased OSR; more receptive females relative to males will cause males to become more resource limited (Bonduriansky, 2001).

Male black horned tree crickets, *Oecanthus nigricornis* (Walker), provide a secretion produced from a nuptial gland at the base of the male’s forewings, which a female continually feed upon during mating while she is mounted on top of the male (Bell, 1980). In ensiferan Orthoptera, transfer of sperm occurs through a spermatophore (Alexander and Otte, 1967), and nuptial feeding in tree crickets can continue extensively after the spermatophore has also been transferred through copulation. Following copulation, a female stops feeding from the gland and frequently dismounts from the male, which causes him to sing and follow her with his gland
exposed which can entice her to return and continue feeding (Fulton, 1915). Male glandular secretions are mutually beneficial, as longer post-copulatory feeding duration, which corresponds to more gift transferred, results in both more sperm transferred as well as increased female lifespan (Brown, 1997b). However, a male only has a limited amount of resources he can allocate to creating gifts, as his nuptial gland can become depleted in multiple matings (Brown and Kuns, 2000). Due to this, males in this species vary gifting adaptively; they reduce the duration of post-copulatory feeding when fed a low protein diet (Brown, 2011) or when they interact with more females before mating (Bussière et al., 2005).

Additionally, fitness of *O. nigricornis* males can be further increased in proportion to their investment into gifts by showing mate choice when resources become sparse. In gift giving species, females seek males for the benefits of the nuptial gift when resources are scarce while males’ time away from the mating pool increases (Bonduriansky, 2001; Edward and Chapman, 2011). Also, because females feed from the nuptial gland both before and after copulation in *O. nigricornis* (Bell, 1980), it may be possible for males to better assess female quality and control the amount of secretion transferred as the mating progresses, similar to other species where post-copulatory feeding occurs (Edward and Chapman, 2011). In support of this hypothesis, Brown (2011) showed that males that were fed high protein diets provide gifts with higher protein content to higher condition females, which is a possible form of post-copulatory mate choice in this species. Gift delivery in tree crickets differs from gifts that are transferred in a single packet such as in katydids (Lehman, 2012) and scorpionflies (Thornhill, 1976), where the amount of gift allocated to a female cannot be adjusted once its transfer has been completed before or during copulation.
While Brown (2011) showed that male *O. nigricornis* display post-copulatory mate choice, he did so only with regard to adjusting gift content under varying resource conditions. In a study where OSR, but not diet, was varied, Bussière et al. (2005) showed that males in this species must be capable of assessing OSR and seem to become resource limited under female-biased OSR, as the gift size decreased when males encountered more females before mating. Additionally, males sing and pursue a female when she is dismounted after copulation, and such active male association with a female was shown to be a costly male investment in other insects (Simmons et al., 1992; Hoback and Wagner, 1997; Hockham and Vahed, 1997; Bateman and MacFadyen, 1999; Saeki et al., 2005) and is thought to prolong nuptial feeding (Fulton, 1915), which in turn increases the number of sperm transferred (Brown, 1997b). Thus, an increase in male singing and pursuit of females after copulation could be a form of male choice because ejaculate size is increased to high quality mates.

Using this system, I asked whether gift giving males show post-copulatory choice under female-biased OSR conditions by allocating more gift and mating efforts to high condition females. Female condition was varied by varying diet and thus female mass; larger females most often have higher fecundity, which is a trait that is commonly under male choice in insects (Bonduriansky, 2001). While OSR changes can occur in gift giving insects due to changes in food resource availability (Gwynne and Simmons, 1990; Simmons and Bailey, 1990), it can be useful to consider the effects of OSR in absence of varying food resources as sex ratio can also change greatly in other circumstances (e.g. with sex-specific mortality from a pathogen: Jiggins et al., 2000).

I hypothesized that male tree crickets would allocate greater amount of gift and mating efforts to high condition females compared to low condition females following exposure of males to a
female biased environment, whereas no choice should be present after exposure to a male-biased environment. I quantified the amount of gift allocated to a female during mating as the duration of nuptial feeding, and measured two mating efforts that appear to function in increasing ejaculate transfer; proportion of male-solicited remounts compared to the total number of remounts (male-solicited or not), and the singing rate during the post-copulatory feeding period. I predicted that males would show greater feeding duration, proportion of male-solicited remounts, and singing rate when mating with a high condition female compared to a low condition female, but this difference in allocation of male effort should be present only for males that interacted with many receptive females prior to mating compared to males that were exposed instead to other males. Given the results of Bussière et al. (2005), the size of gift given to females, regardless of female condition, may be greater in male-biased OSR compared to female-biased OSR. However, the relative amount of gift and mating effort allocated to high condition females should be greater in a female-biased OSR.

1.2 Methods

1.2.1 Biology of tree crickets

Oecanthus nigricornis are common in old field habitats of southern Ontario where goldenrod (Solidago spp.) is abundant. They oviposit in the stems of woody bushes (e.g. raspberry, Rubus spp.: Fig. 1.1), leaving a distinctive linear scar. Nymphs hatch in June and mature into adults starting in late August (Fulton, 1915). Adults remain active up into October, until they are killed by the frost. Eggs that have been exposed to a period of cold in winter can be brought into the lab and hatched following warming (Bell, 1979).
Males use forewing stridulation to both attract and court females. The female mounts the male and feeds on secretions from a metanotal gland beneath his forewings while he inserts a tube from a sperm-filled spermatophore into her genital chamber (Bell, 1980, Fig. 1.2). Increased gland feeding increases a female’s longevity (Brown, 1994), whereas the spermatophore, consumed after the pair separates, simply acts as a sperm transfer vessel with little nutritional value (Brown, 1997b).

1.2.2 Rearing

I collected eggs of black-horned tree crickets in the winter/spring of 2014 from an old field on the University of Toronto Mississauga campus. I placed sections of raspberry (Rubus spp.) stems containing O. nigricornis eggs by clipping the stems on each side of the oviposition scar and then placing stem sections in plastic containers with water at the bottom for hydration. Containers were kept in an environmental chamber with 12hr night and day cycle for light, and a day temperature of 30°C and a night temperature of 20°C. I changed the water for the stems every week, and checked the stems every day for hatching nymphs after two weeks.

I moved the hatched nymphs onto potted wheat grass (Triticum spp.) in three BugDorm™ plastic cages (30cm x 30cm x 30cm), with about 50 nymphs per cage. I placed water-filled centrifuge tubes stopped with cotton for water, and pollen for extra nutrition (such as protein) in each cage. Wheat grass was watered three times per week, and was replaced with a new pot when the plants wilted. The centrifuge tube was re-filled with water once a week, and the cage was also sprayed with water daily. When the nymphs moulted into fifth instars, I removed them from the communal cages and placed them into individual cylindrical plexiglass containers (10cm
diameter, 10cm height) covered at the top with fibreglass mesh screen. Containers also had fibreglass mesh liners as climbing and moulting substrate. Each container had pollen and ground rabbit/cat food, along with water filled centrifuge tubes.

1.2.3 Mating experiments

When female crickets became adults, I weighed and then randomly assigned them to either a high or low quality diet to produce high and low condition individuals. Similar to the methods used by Brown (2011), the low quality diet consisted of a slice of apple replaced every other day, whereas high quality diet consisted of a slice of apple along with added protein in pollen and ground rabbit/cat food.

I gave all adult crickets 7 to 14 days after their adult moults to reach sexual readiness. To vary the operational sex ratio, each focal male was consecutively placed with two non-focal receptive females (female-biased treatment) or two non-focal sexually active males (male-biased treatment) prior to an experimental mating. Thus, males in the female-biased treatment experienced 1:3 male to female sex ratio, whereas males in the male-biased treatment experienced 3:1 male to female sex ratio. I allowed each interaction with a non-focal individual to continue for 8 minutes (an adequate interaction interval: see Bussière et al., 2005) or until a non-focal female mounted the male, whichever occurred earlier. If a non-focal female mounted the male, they were quickly prevented from mating using a blade of grass (a natural disturbance) to gently push them out of the mounting position. For consistency in animal treatment, focal males and non-focal crickets that did not mount for the duration of their 8 minutes interactions were also brushed with a blade of grass before the interaction was terminated.
Following an experimental mating, a male was given 48 hours to replenish his metanotal glands, after which the trial was repeated with a female from the other diet treatment. Thus, each male mated with one high condition female and one low condition female in random order. Each focal male interacted with the same non-focal crickets for these two matings (OSR treatment), while each high and low condition female was reused once, thus mating with one focal male from the male-biased OSR treatment and one focal male from the female-biased OSR treatment per female in random order (Fig. 1.3). Thus each trial had focal males from a male biased and a female biased OSR conditions that interacted with a same set of non-focal crickets, reducing variation in the mating behaviour of the focal male that might be caused by variation in non-focal crickets. This consistency also allowed me to reduce the number of non-focal crickets needed for the experiment.

During mating trials, I measured three aspects of male reproductive effort. Brown (2011) used feeding duration as a proxy for the amount of nuptial gift transferred during mating. However, feeding duration by itself may be a poor indicator of male allocation of reproductive efforts to mates of different quality, as feeding duration is influenced by the latency to spermatophore consumption, which terminates nuptial feeding and is under female-control (Brown and Kuns, 2000). In fact I had noted in pilot mating trials that females terminated their gland feeding early when fed a high quality diet (two-sample t-test of feeding duration: low condition = 2053.12±232.39 seconds; high condition = 1244.81±185.72; t_{29} = 1.69, P = 0.01; Fig. 1.4). Because of this, in addition to feeding duration I measured two behaviours unlikely to be influenced by female preference in addition to feeding duration. These additional behaviours were thought to be part of male effort to increase feeding duration and ultimately the duration of
mating after copulation, and thus they were expected to be correlated. I examined these using linear regressions.

When females dismount during feeding, males almost always sing, producing short bursts of trills until the female remounts. Singing rate is a measure of how many times a male stridulates over the total time a female is dismounted between her post-copulatory bouts of gland feeding, in order to standardize the measure of male singing between matings of differing durations. Tegminal stridulation is a costly behaviour in several orthopteran species (Simmons et al., 1992; Hoback and Wagner, 1997), and the singing rate can be thought to be a form of male effort similar to mate guarding that maintains coupling during nuptial feeding (Fulton, 1915).

The last variable measured was the proportion of remounts that were solicited by the male. Female remounts during post-copulatory feeding are solicited either by the male, when he follows the female and repeatedly stridulates, or without male solicitation, when a female returns to feed on a stationary male who has not stridulated or moved little or not at all. Thus I also used the proportion of male solicited remounts of the total number of remounts as a measurement for male effort expanded in maintaining the coupling during nuptial feeding. Because proportions tend to have non-normal distributions, the data was arcsine-transformed for parametric analysis. Observations were made from 8 focal males for each treatment, for a total of 16 focal males. In addition, 16 non-focal males and 32 non-focal females were used in matings with the focal males and also to interact with focal males to set up either male-biased or female-biased OSR treatments.

All mating trials were conducted in a dark room under red light. The focal male and the female were observed in a 9cm x 9cm x 11cm (h/w/l) plastic container with a piece of fibreglass mesh
placed inside for substrate. Trials were recorded using Sony Handycam model DCR-HC26. Each trial was recorded until the end of mating, or terminated if the pair had not begun courting by 10 minutes or had not started mating after one hour from the start of observations. Mating ended when the female reached down and consumed the spermatophore, which usually coincided with the male no longer pursuing or singing to her. Both the focal male and the female were weighed immediately before and after the mating trial.

### 1.2.4 Statistical analysis

There was no significant covariation between the three measures of male reproductive effort, feeding duration, proportion of male-solicited remounts, and the singing rate (see supp. Fig. 1 and supp. Table 1). Thus individual repeated measures two-factor ANOVA tests were conducted to test for effects of OSR on the male reproductive efforts when each male was mated with a high condition female and a low condition female. OSR and female condition were used as the two factors and female condition on male identity as repeated measures. Female condition and OSR were my factors and female conditions against male identity were repeated measures.

If, as I hypothesized, males experiencing a female biased OSR show post-copulatory mate choice, all three response variables should be higher in matings with high condition females compared to low condition females. In addition, the feeding duration in matings with high condition females should be greater for all three response variables when males are exposed to a female-biased OSR compared to when they are exposed to a male-biased OSR.
To check for the probability of type two error, I conducted power analyses using G*Power version 3.1.9.2. And in addition to the power analysis, the effect sizes of my study were compared, using meta-analytical techniques, with effect sizes of previous studies looking at similar effects. The effect sizes were calculated from the studies according to the methods in McCartney and Rosenthal (2000) and Fritz, Morris, and Richler (2011), which were then standardized to $r$ values using Comprehensive Meta Analysis software. This gave me another way to analyse whether the significance (or lack thereof) of my tests were due to low power, or perhaps due to there not being a detectable effect comparable to previous studies. A total of eight studies with 18 tests between them were used to compare the effect of female quality and OSR on feeding duration or other related variables. For singing rate, effect sizes found in two studies with three tests between them were compared with those of my study, although conclusions that could be drawn from this comparison were limited due to the small number of studies compared. Effect size comparisons for ratio of remounts could not be conducted as there were no studies looking at similar effects. The studies used for this analysis is listed in the results.
Figure 1.1. Oviposition pattern and eggs of *Oecanthus nigricornis*. A) Oviposition scars in a raspberry stem; B) longitudinal section of A; C) egg; D) projection of egg cap; E) egg cap (reproduced from Fulton, 1915).
Figure 1.2. Characteristic mating position of the *Oecanthus* tree crickets. In this genus, the female mounts the male and feeds from his metanotal gland for extended periods of time as shown above, before and after copulation (reproduced from Fulton, 1915).
Figure 1.3. Schematics of the first eight matings in the experiment. ♂ = male, ♀ = female. The number behind the symbol represents the identity of the individual (e.g. the two non-focal males used in mating 1 and mating 2 are the same individuals). Each focal male cricket was in one of the two OSR treatments: Female-biased OSR or male-biased OSR, and were given 48 hours between matings. N = 8 per treatment.
Figure 1.4. Duration of nuptial feeding for females of high or low condition. X±SE, n = 16 for each female condition.
1.3 Results

The predictions for the post-copulation singing rates were not supported, as neither female condition (F$_{1,19} = 0.65$, P = 0.4288) nor OSR alone (F$_{1,19} = 0.067$, P = 0.80) had significant effects on singing rate. The only significant result, a significant interaction between OSR and female condition on singing rate (F$_{1,19} = 4.94$, P = 0.04) was opposite to the prediction; singing rate significantly increased but only in matings with high condition females when males had experienced a male-biased OSR (t$_{12} = -2.74$, P = 0.02, Fig. 1.5). The tests of the effects of OSR had a somewhat high power (1-β) of 0.70, the effect of female condition had a low power of 0.10, and the test of their interaction had a somewhat high power of 0.74.

Also in contrast to predictions, neither OSR nor a female condition had significant effects on the differences in feeding duration (OSR: F$_{1,19} = 0.03$, P = 0.86; female condition: F$_{1,19} = 0.04$, P = 0.85; Fig. 1.6) or on the differences in the proportion of male-solicited remounts (OSR: F$_{1,19} = 0.26$, P = 0.62; female condition: F$_{1,19} = 0.12$, P = 0.74; Fig. 1.7), nor were there significant interactions between these treatments (feeding duration: F$_{1,19} = 0.01$, P = 0.91, Fig. 1.6; remounts: F$_{1,19} = 0.07$, P = 0.80, Fig. 1.7). The power of these tests were high for OSR (1-β = 0.81) and female condition (1-β = 0.98) but low for the interactions in feeding duration (1-β = 0.05) while the powers were low (OSR: 1-β = 0.19, interaction: 1-β = 0.07) or medium (female condition: 1-β = 0.52) for male-solicited remounts.

Almost all of the effect sizes in previous studies looking at changes in feeding duration or other comparable variables due to female quality or OSR were substantially greater than the effect sizes in my study (Table 1). The only similar effect sizes as mine were from the study by Simmons and Kvarnemo (1997), which tested the individual effects of increased encounter with
females and increased female size on nuptial gift size of male katydids and found them to be non-significant.

The effect of the interaction between female quality and OSR on singing rate had a comparable effect size (0.454) to previous studies, while the effect size of female quality on singing rate was comparable with a non-significant effect of encounter rate with females on male katydid’s chirp duration in the Allen and Bailey (1994) study (Table 2). The effect size of OSR was much lower than any of the previous studies.
Figure 1.5. Singing rate for males mated to high and low quality female (with 48 hours between matings). F-biased OSR = matings with males that experienced female-biased OSR, M-biased OSR = matings with males that experienced male-biased OSR. * denotes a significant difference. X±SE, n=8 per treatment

Figure 1.6. Feeding duration for males mated to low quality and high quality female (with 48 hours between matings). F-biased OSR = matings with males that experienced female-biased OSR, M-biased OSR = matings with males that experienced male-biased OSR. X±SE, n=8 per treatment
Figure 1.7. Proportion of male-solicited remounts for males mated to high quality and low quality female (with 48 hours between matings). F-biased OSR = matings with males that experienced female-biased OSR, M-biased OSR = matings with males that experienced male-biased OSR. X±SE, n = 8 per treatment
Table 1. The effect sizes (standardized to r) of studies that looked at the effect of female quality and conspecific encounters on feeding duration or similar metrics. Within each study, only the tests that had variables comparable to this study are included here. The effect sizes of the two tests in Simmons and Kvarnemo (1997) are bolded to show their comparable size to the effect sizes of this study.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Species</th>
<th>Test type</th>
<th>Independent variable</th>
<th>Dependent variable</th>
<th>p</th>
<th>r</th>
<th>n</th>
<th>Tests per study</th>
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<td>Repeated-measures ANOVA</td>
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<td>Salivary mass</td>
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<td>Species</td>
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<td>Simmons &amp; Kvarnemo 1997</td>
<td><em>Kwanaphila nartee</em> (katydid)</td>
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<td>Perez-Staples, Cordova-Garcia, Aluja 2014</td>
<td><em>Anastrepha ludens</em> (tephritid fly)</td>
<td>Linear regression</td>
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Table 2. The effect sizes (standardized to r) of studies that looked at the effect of mate quality and conspecific encounters on signing rate or other similar metrics. Within each study, only the tests that had variables comparable to this study are included here.

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1.4 Discussion

*Oecanthus nigricornis* females feed on glandular substances produced by the males which are both beneficial to the females by increasing their lifespan (Brown, 1997b) and are costly to the males as the glandular secretions become depleted after multiple matings (Brown and Kuns, 2000). The more a female feeds from the gland, the more sperm is transferred to that female (Brown, 1997b) similar to how larger gifts (spermatophylax meals that males attach to the spermatophore in this case) lead to greater number of sperm transferred in other gift giving orthopterans (Sakaluk 1984; Wedell and Arak, 1989; Simmons, 1995). Male *O. nigricornis* on high-protein diets have been shown to transfer higher quality gifts to high condition females (Brown, 2011) and thus they may strategically allocate their gifts to matings with higher quality mates due to the cost of producing the gifts. Mating efforts by males such as singing and soliciting female remounts after copulation are also thought to increase feeding duration (Fulton, 1915) which ultimately increases sperm transfer by delaying the female’s removal of the spermatophore that is external to her genital chamber. Because these behaviours are also costly to male insects (Simmons et al., 1992; Hoback and Wagner, 1997; Hockham and Vahed, 1997; Bateman and MacFadyen, 1999; Jordan et al., 2014) males may also vary their allocation of these mating efforts based on female condition in conjunction with gift size.

Due to these female benefits and male costs, I predicted that male *Oecanthus nigricornis* would invest more reproductive effort in post-copulatory mating interactions with high condition females compared to low condition females, thus exerting post-copulatory mate choice, when they experienced a female-biased OSR prior to mating. In contrast, males should discriminate less against mates when the chance of finding another mate decreases as males become less limited in the amount of resources he can invest into a mating (Bonduriansky, 2001). And if the
number of receptive females is low enough (for example, in a male-biased OSR) such that males are not resource limited, no discrimination should be beneficial to the male to maximize his reproductive fitness (Edward and Chapman, 2011). Thus, I expected longer feeding duration, a greater proportion of male-solicited remounts, and greater singing rate when male crickets were mated with high condition females only in female-biased social environments.

However, the results are not consistent with the predictions. Two of the three measurements of male reproductive effort allocation, the feeding duration and the proportion of male-solicited remounts, showed no effect of female condition or the OSR treatments. The third variable, singing rate, did increase in matings with high condition females when there was a male-biased OSR, but this pattern was not predicted.

The first thing to note is that my tests varied with respect to statistical power. Power – the probability of accurately rejecting the false null hypothesis – is thought to be strong at 0.8 (but see Colquhoun (2014) for a critique of this convention). While some of my tests have power of about 0.8 others have quite low power, and thus some real effects may not have been detected by the tests. Low power means that conclusions made from some of the non-significant results of this study must be drawn with caution, and they may be the primary explanation for many of the unexpected findings of this study.

However, effect sizes comparisons for feeding duration and singing rate showed that only the interaction between female quality and OSR on singing rate, the one significant result of my study, was of a comparable size as previous studies. The few effects that were close in size to my other results were not only rare (2/18 tests compared for feeding duration, 1/3 for singing rate), but also found to be not significant in their respective studies. While it should be noted that these
comparisons of effect size are not definitive evidence due to their basic nature and the low number of studies compared, they do suggest that the non-significant results of my study may not simply be due to low power but are representations of real phenomena. If true, this would mean that *O. nigricornis* do not show post-copulatory mate choice in the ways that I predicted.

As discussed, male resources become depleted following multiple matings in species where males provide costly investments (Verrell, 1989; Brown and Kuns, 2000; Nandy et al., 2012). And yet, neither feeding duration nor proportion of male-solicited remounts was found to vary with OSR. One reason males may not have shown preferential allocation of reproductive effort despite the costs is that males in my experiments had sufficient time to replenish the contents of their gift glands. They were mated only twice during their lifetime with 48 hours between matings, when a male *O. nigricornis* can mate at least twice a day in nature (Bussière et al., 2005). Thus, even males that were on low-quality diet may not have been resource-limited and did not to preferentially allocate nuptial gift to higher condition females.

The one significant result of my study is that males sang more toward high condition females during post-nuptial feeding when they were exposed to more males. One explanation for this is that increased contact with other males which was a cue for increased sexual competition and consequently potential sperm competition with rival males. The long duration of post-nuptial feeding in *O. nigricornis* (typically lasting around 20 minutes on average but sometimes over an hour) is argued to function in transferring more ejaculate (Brown, 1997b; Brown and Kuns 2000). Thus, a male increases the duration of post-copulatory nuptial feeding by singing more after having recently encountered more rivals. Females mate multiply in this species, up to around four times in the field according to a conservative estimate using sperm analysis (K. Ercit, personal communication, September 16, 2015), and so an increase in nuptial feeding can increase
the proportion of offspring sired by the donor male compared to his rivals as shown in other gryllids and some Diptera (Sakaluk, 1986; Parker et al., 1990) and importantly increase the refractory period of females during which the female will lay eggs without mating with a rival male through chemicals in the ejaculate (Gwynne and Simmons, 1991; Sakaluk, 2000).

I note that OSR affected male singing rate only in matings with high condition females (not with low condition females), and examining the pattern of change in high condition females only shows that it is similar to that found in Bussière et al. (2005). In other words, in my study a male allocated more reproductive effort to high condition females only after he had interacted with multiple males prior to mating, while Bussière et al. (2005) showed the same effect when all females were fed high-protein diets. Thus, it remains possible that a decrease in singing rate with high condition females in female-biased OSR represents a decrease in male investment towards all females, not just high condition females. My tests may not have detected this difference for low condition females due to low power of female condition in my analysis. If this is the case, my results would not be showing post-copulatory mate choice but rather males changing their reproductive effort regardless of female condition as shown by Bussière et al. (2005). I had hypothesized that a male would show choice when resources become limited due to female-biased OSR, as strategically allocating more effort to higher condition females would increase his fitness (Edward and Chapman, 2011). However, a study in katydids by Sevgili and Reinhold (2007) showed that males may not display mate choice based on female quality, even if they provide substantial nuptial gift investment and are under female biased OSR conditions.

Another possible explanation for the observed pattern of greater post-copulatory singing rate with high condition females in male-biased OSR is decreased reproductive effort towards high condition females. Bateman and Ferguson (2004) found that male tettigonids are more likely to
accept smaller females as mates. Furthermore, Simmons and Kvarnemo (1997) showed that with a female-biased OSR, male tettigonids gave smaller spermatophylax gifts to larger, more fecund females. The authors posited that because females actively seek mates when there is female-female competition in such female-biased conditions (see Gwynne and Simmons, 1990), this active mate seeking behavior by females can increase the number of matings by high condition females and consequently the degree of sperm competition experienced by her mates. Intense sperm competition diminishes paternity returns for heavy investment, thus leading to lower investment in high condition females. The fact that they reported similarly low effect of female quality and OSR individually on the amount of nuptial gift given by the male as was found in this study also lends more weight to the fact that similar mechanisms of sperm competition might be at work in O. nigricornis as well. A further study of the degree of sperm competition in O. nigricornis under different conditions may prove useful in testing this hypothesis.

Furthermore, the low effect size of female quality and OSR also requires attention as these are not consistent with the findings of Brown (2011) and Bussière et al. (2005). Brown found that feeding duration increased when high condition males are mated with low condition females, with effect size of female condition substantially higher than that of the present study. Furthermore, high condition males also had more amino acids in their glandular secretions when mating with high condition females. Bussière et al. (2005) found that the duration of nuptial feeding is decreased when a male encounters multiple sexually receptive females prior to mating. Thus, both these studies found evidence of post-copulatory male choice through differential allocation of nuptial gift. In contrast my results showed that variation in female quality or OSR had no effect on either feeding duration or singing rate in matings with low condition females. This inconsistency may be due to the fact that while Bussière et al. (2005) allowed each male to
interact with 12 conspecifics, I did not create such extreme OSR conditions and thus the focal males may not have experienced a strong enough sex-bias prior to mating. For the effect of female quality, the results may be different due to the fact that males in this study always encountered conspecifics prior to mating (either two males or two females) unlike in Brown (2011) where males did not encounter any conspecifics other than the mating females.

In conclusion, contrary to my hypothesis, male’s preferential investment towards high condition females through greater allocation of nuptial gift and mating efforts did not occur when OSR was female biased. Instead, male singing rate towards high condition females compared to low condition females increased under male-biased OSR. Although some studies showed that males may discriminate against higher quality females under female-biased OSR (Simmons and Kvarnemo, 1997; Bateman and Ferguson, 2004), my study provide some evidence that males might allocate more reproductive effort towards high condition females under male-biased OSR but not under female-biased OSR. However, the overall low power of some of the tests may mean many other real effects were not detected, although comparisons of effect sizes offer some support of the results. Further study would be required to verify this pattern of male reproductive effort in *Oecanthus nigricornis*, and whether similar phenomena occur in other species.
1.5 Supplementary figures and tables

Supplementary Figure 1. Scatterplot matrix of the three variables used to measure male mating effort. n=8 per variable.
**Supplementary Table 1. Regression analyses of the three interaction variables.** Remounts = arcsine-transformed proportion of male-solicited remounts.

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References


