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The Impact of the Social Environment on Reproductive Plasticity: The Role of Male Harassment and Larval Competition in *Callosobruchus maculatus*

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Abstract

Effects of the social environment on reproductive behavior are widespread. How the social environment promotes or inhibits behavioral plasticity is less known. I examined the role of male harassment and larval competition on reproductive behaviors in *Callosobruchus maculatus*. For experiment one, I manipulated male harassment level during female oviposition. I measured oviposition substrate preference, clutch size, egg size, and the trade-off between egg and clutch size. For experiment two, I investigated the effect of the social environment during larval development inside the bean. Groups consisted of a control and sex-ratio-based competition treatments. I recorded the mating behavior of all emerged individuals and the clutch sizes of emerged females. My results demonstrated that male harassment, but not larval competition, impacted reproductive behavior. Specifically, male harassment decreased reproductive plasticity. The trade-off between egg and clutch size disappeared, clutch size decreased, and adaptive oviposition preferences were lost. Reproductive plasticity appears to be context-dependent.

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Thesis Title: The Impact of the Social Environment on Reproductive Plasticity: The Role of Male Harassment and Larval Competition in *Callosobruchus maculatus*

LAKE FOREST COLLEGE

Senior Thesis

The Impact of the Social Environment on Reproductive Plasticity:
The Role of Male Harassment and Larval Competition in *Callosobruchus maculatus*

by

Elisabeth Bacon

April 22, 2018

The report of the investigation undertaken as a
Senior Thesis, to carry two courses of credit in
the Department of Biology

Davis Schneiderman
Krebs Provost and Dean of the Faculty

Flavia Barbosa, Chairperson

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ABSTRACT

Effects of the social environment on reproductive behavior are widespread. How the social environment promotes or inhibits behavioral plasticity is less known. I examined the role of male harassment and larval competition on reproductive behaviors in *Callosobruchus maculatus*. For experiment one, I manipulated male harassment level during female oviposition. I measured oviposition substrate preference, clutch size, egg size, and the trade-off between egg and clutch size. For experiment two, I investigated the effect of the social environment during larval development inside the bean. Groups consisted of a control and sex-ratio-based competition treatments. I recorded the mating behavior of all emerged individuals and the clutch sizes of emerged females. My results demonstrated that male harassment, but not larval competition, impacted reproductive behavior. Specifically, male harassment decreased reproductive plasticity. The trade-off between egg and clutch size disappeared, clutch size decreased, and adaptive oviposition preferences were lost. Reproductive plasticity appears to be context-dependent.

Keywords: *Callosobruchus maculatus*, bean beetle, seed beetle, male harassment, larval competition, sperm competition, egg and clutch size trade-off, oviposition, social environment, behavioral plasticity, sex ratio.

Behavioral Plasticity and the Social Environment

DEDICATION

To all my friends and family.

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CHAPTER 1

Behavioral plasticity and the role of the social environment

Behavioral plasticity, or the ability of individuals to modify their actions in response to external stimuli, is a widespread phenomenon (Humfeld et al. 2009; Moretz et al. 2007; Rodd et al. 1997). Stimuli can be either abiotic or biotic (Rudolf and Rodel 2005). Abiotic effects on behavior take on many forms (Guisande et al. 1996; Rudolf and Rodel 2005). For instance, the size of offspring in many species varies with food availability (Guisande et al. 1996). When food availability is low, organisms often invest in larger but fewer offspring (Guisande et al. 1996). Theory predicts that this is because larger offspring are better suited to survive in food-scarce environments (Guisande et al. 1996). Indeed, this has been found to be the case in *Euterpina acutifrons*, a species of marine copepod, where increasing the size of offspring at the expense of offspring number increased offspring survivorship and fitness (Guisande et al. 1996). Another behavioral trait that varies with changes in the abiotic environment is oviposition (Rudolf and Rodel 2005). Tree-hole breeding frogs, for example, lay more eggs in snail shells that have environmental cues (duration of initial water presence) indicating less risk of desiccation and, hence, less offspring mortality (Rudolf and Rodel 2005). In this way, various abiotic signals are detected and used by organisms who adjust their behavior accordingly.

In contrast to abiotic cues, many animals alter their behaviors based on biotic cues (Moretz et al. 2007). These cues can have both indirect and direct effects. Indirect effects on behavior typically occur via modification of morphological features, such as the brain and reproductive anatomy (Brennan et al. 2017; Cornwallis and Birkhead 2007; Maleszka et al. 2009). Gonda et al. (2010) found that tadpoles developed larger

optic tecta under high population densities (Gonda et al. 2010). The optic tectum is the central vision area in the brain and increasing investment for it under higher competition levels was suggested to be a strategy to increase competitive ability for foraging (Gonda et al. 2010). Tadpoles with larger optic tectums likely have increased vision and can outcompete conspecifics when looking for food (Gonda et al. 2010). Therefore, external biotic cues can play a role in behavioral plasticity through phenotype modification.

Direct Effects of Predators on Behavioral Plasticity

Direct effects of the social environment on behavior vary depending on the type of social environment. Broadly speaking, research has focused on two types: those composed of predators or parasites and those composed of competitors and conspecifics (Kiflawi et al. 2003; Resetarits and Wilbur 1991). Many organisms can alter their behavior to minimize exposure to predators (Kiflawi et al. 2003; Resetarits and Wilbur 1991; Rodd et al. 1997). For example, many species, including two species of mosquito and the gray treefrog, preferentially oviposit in areas with fewer predators (Kiflawi et al. 2003; Resetarits and Wilbur 1991). However, males and females do not always respond to predators in the same way (Resetarits and Wilbur 1991). For instance, female gray treefrogs avoid laying eggs around adult sunfish *Enneacanthus chaetodon* and salamander larvae *Ambystoma maculatum* (Resetarits and Wilbur 1991). However, males only avoid calling in areas with *E. chaetodon* but not areas with *A. maculatum* (Resetarits and Wilbur 1991). It is likely that this is because *A. maculatum*

larvae tend to prey on tadpoles but not adult frogs making them a threat to females' eggs but not to calling males (Petranka 1998). Moreover, behavioral responses to predators can vary with predator type (Templeton and Shriner 2004). Guppies, for example, respond differently to aerial versus aquatic predators (Templeton and Shriner 2004). When aquatic predators are present, they tend to school with other fish, inspect the predators, and jump at the surface (Seghers 1974; Seghers and Magurran 1994; Templeton and Shriner 2004). In contrast, when exposed to aerial predators, guppies sink to the bottom, freeze, or hide (Templeton and Shriner 2004). Each of these behaviors is specifically suited to avoid being eaten by a different type of predator (Templeton and Shriner 2004).

Direct Effects of Conspecifics on Behavioral Plasticity

Individuals of many species have also been shown to plastically change their behavior in response to the component of the biotic environment composed of conspecifics (Humfeld et al. 2009; Moretz et al. 2007). Humfeld et al. (2009) found that male spring peepers, *Pseudacris crucifer*, found that they increase the amount of aggressive calls and decrease the amount of advertisement calls when a simulated neighbor went from producing non-aggressive to aggressive calls. Additionally, Moretz et al. (2008) also found a plastic response when zebrafish individuals are exposed to different strains (Moretz et al. 2007). Individuals from the Nadia strain engaged in more aggressive behavior such as biting after being exposed to the more aggressive TM1 individuals (Moretz et al. 2007). TM1 individuals also were more aggressive after

exposure to the Nadia individuals, perhaps as a result of increased escalation by Nadia individuals due to initial aggression of TM1 fish (Moretz et al. 2007). The social environment also altered the TM1 strain's shoaling behavior (Moretz et al. 2007). Typically, individuals of this strain stay closer to the shoal than Nadia fish (Moretz et al. 2007). However, when TM1 fish were mixed with Nadia fish either as adults or as juveniles, they were more likely to leave a future stimulated shoal (Moretz et al. 2007). But conspecific aggression is just one aspect of the conspecific social environment that affects behavioral plasticity.

Oviposition Behavior

Across many species, females respond to conspecific larval density by plastically altering their oviposition preferences (Almohamad et al. 2010; Guedes and Yack 2016; Sato et al. 1999; Yoshioka et al. 2012). It is vital for females of such varied species to select a high quality site in order to ensure their offspring perform well (Almohamad et al. 2010; Guedes and Yack 2016; Yoshioka et al. 2012). Females select sites based on a variety of factors including food availability, predator numbers, and toxicity of host (Gibbs et al. 2005; Janzen 1977; Kiflawi et al. 2003; Resetarits and Wilbur 1991; Yoshioka et al. 2012). In terms of using conspecific larval density as a signal, the oviposition preference-offspring performance theory predicts that, until conspecific larval density causes conspecific competition to increase to a high enough level, females will lay eggs based on which habitats are the most suitable for their offspring (Yoshioka et al. 2012). In fact, in one species of mosquito, there was a tendency for females to

oviposit at sites with low to medium larval densities over sites where no conspecific larvae were present (Yoshioka et al. 2012). This varied based on the clutch size of the female: females with larger clutch sizes preferred sites with around 80 larvae and females with smaller clutch sizes preferred sites with around 10 larvae (Yoshioka et al. 2012). Mosquitos may have been using conspecific larvae as an indicator of high site quality (Yoshioka et al. 2012). However, when larval density levels get high enough that the cost of competition outweighs the cost of a poorer quality site, females of multiple species actively avoid egg laying on hosts or at sites with such high densities (Almohamad et al. 2010; Guedes and Yack 2016; Sato et al. 1999).

Females have several mechanisms to detect the presence of conspecific larvae (Almohamad et al. 2010; Guedes and Yack 2016; Messina et al. 1987), most commonly detection of volatile chemical compounds (Almohamad et al. 2010). Often, volatile compounds serve as indicators of oviposition host quality (Almohamad et al. 2010). Larvae feeding can facilitate the release of such compounds from the host plant (Almohamad et al. 2010) and, in turn, these odorous compounds serve as markers of the presence of conspecific larvae causing females to avoid oviposition at those sites (Almohamad et al. 2010). Additionally, Messina et al. (1987) found that certain insect species can detect conspecific larvae using tactile modalities. In some instances, multiple sensory organs can be involved (Messina et al. 1987). Finally, recent research in the bean beetle *Callosobruchus maculatus* reveals that larvae can be detected inside their bean host by the vibrations they make during feeding (Guedes and Yack 2016). Females then selectively avoid oviposition on beans with high amounts of larvae-

induced vibrations (Guedes and Yack 2016). All of these mechanisms enable females to have plastic oviposition preferences by giving them ways to measure the stimuli of larval density.

Although not well studied, conspecific male harassment has also been shown to affect oviposition behavior (Gibbs et al. 2005; Koch 2005). For example, the presence of harassing males in two species of dragonflies caused a significant decline in the length of time females spent laying their eggs (Koch 2005). Also, in one of those species, *Orthetrum chrysostigma*, females changed ponds significantly more when exposed to harassing males (Koch 2005). This was suggested to be an attempt to run away from said males (Koch 2005). Gibbs et al. (2005) found that the presence of harassing males led to a loss of behavioral plasticity in females in the butterfly *Pararge aegeria*. Females no longer laid their larger eggs on higher quality host plants though they did begin to exhibit an egg size-number trade-off (Gibbs et al. 2005). They decreased egg size while increasing clutch size (Gibbs et al. 2005). Therefore, when reproductive plasticity is able to occur under male harassment, it may be an adaptive response to costs of such harassment.

Tradeoffs between egg and clutch size, like the one found in Gibbs et al. (2005), mirror the well-established model by Simon and Fretwell (2007), which predicts an optimal balance between the two. Though such tradeoffs have frequently been shown to exist, and some evidence indicates they can be plastic, there is little available research on how the presence of conspecifics may alter the tradeoff (Charnov and Ernest 2006; Fischer et al. 2003; Guisande et al. 1996; Rodd et al. 1997; Walker et al.

2008). However, Rodd et al. (1997) found that guppy individuals inversely altered their offspring number and size based on their origin and the origin of individuals present during rearing. This suggests that guppies did learn and plastically alter their behavior, in part, from their conspecifics (Rodd et al. 1997). Moreover, Creighton (2005) found that burying beetle females decrease brood size but increase offspring body size when in a high-density, but not low-density, population (Creighton 2005). This is likely because larger offspring are better able to compete in the presence of a greater number of conspecific competitors (Creighton 2005). Therefore, there is clearly some existing level of plasticity across differing species in the offspring size/number trade-off.

Mating Behavior

Finally, several studies have found that mating behavior also can respond plastically to the presence of conspecifics (Adkins-Regan and Krakauer 2000; Crowder et al. 2010; Dur et al. 2012; Lehtonen and Lindstrom 2008). In some instances, the presence of conspecifics of both sexes is vital for the normal development of mating behaviors (Adkins-Reagan and Krakauer 2000). For example, in zebra finches, males lose their preference for female over male mating partners when reared with only females and, as a result, make fewer successful copulations (Adkins-Reagan and Krakauer 2000). However, even the social environment during adulthood can lead to changes in mating behavior (Crowder et al. 2010; Dur et al. 2012). For instance, the presence of an incompatible biotype Q in whiteflies leads biotype B females to be less choosy in mating other biotype B males (Crowder et al. 2010). Additionally, alteration of the sex ratio and

encounter rate of the opposite sex within a species also can impact mating behavior (Berglund 1994; Dur et al. 2012). Frequently, when the opposite sex is less common or the encounter rate is lower, the choosy sex reduces their choosiness in order to ensure they still reproduce (Berglund 1994; Dur et al. 2012). Overall, these studies cement the idea that reproductive behavior, like mating behavior, is plastic and can be altered based on biotic signals.

Reproductive Plasticity in *C. maculatus*

In my study, we examined reproductive plasticity in the well-known model organism, the bean beetle *C. maculatus* (Dougherty et al. 2017; Yanagi et al. 2013). Bean beetles have a short and simple lifecycle which makes them ideal for laboratory colonies. Adult females adhere eggs to the surface of seeds from several legume species. This allows for an easy way to obtain a proxy measure of female fitness via egg number (Vamosi 2005). After roughly three days, the larvae in their first instar stage burrow into the bean and eat the cotyledon (Wilson 1994). The larval state is the only time in the lifecycle that *C. maculatus* individuals feed, allowing for direct comparisons between the developmental environment and later fitness (Vamosi 2005). At roughly the 26-day mark, the now fourth instar larvae pupate inside the bean for two days (Wilson 1994). During this whole period of a *C. maculatus* beetle's life, the beetle is restricted to the bean on which its egg was laid (Vamosi 2005). This again allows for a highly controlled environment via control of bean size, temperature, and other factors. After pupation is complete, the adult beetle eats its way out of the bean (Wilson 1994).

Within the first hour, adults will mate and, soon after, the females lay their eggs on beans (Wilson 1994). Most adults die within 7 days (Vamosi 2005). This relatively quick lifespan allows for both a constant flow of available experimental individuals and multigenerational studies.

The mating behavior of *C. maculatus* is particularly well-studied. These beetles engage in multiple mating (Hotzy et al. 2012). Right before copulation begins, males chase females and tap them repeatedly on their backs with their antennae until they successfully insert their genitalia (EB, EG, FB, personal observation). The male genitalia have spikes that tear up the female reproductive tract, which shortens female lifespans (Eady et al. 2007). The longer these spikes, the higher the male fertilization success (Hotzy and Arnqvist 2009), likely due to increased seminal fluid uptake by the female (Hotzy et al. 2012). During the entirety of copulation, males rock back and forth slowly (EB, personal observation). Females then begin to kick their back legs and push them against the part of the male genitalia that remains outside the body (Wilson and Tomkins 2014). This was previously believed to be an indicator of female choosiness by allowing females to shorten copulation duration and also decrease the damage done by the male genital spines (Edvardsson and Canal 2006). More recently, Wilson and Tomkins (2014) found that males appear to have gotten the upper hand in this sexual conflict, as copulation duration was not found to decrease with increased female kicking. Actually, females that kicked longer seemed to induce more damage as they had decreased lifespans (Wilson and Tomkins 2014). Regardless, throughout all aspects

of mating, there are multiple opportunities for both the male and female to respond plastically.

In other life stages, such as oviposition, females have been found to have plastic behaviors (Barde et al. 2012; Cope and Fox 2003; Janzen 1977; Kamble et al. 2016; Kar and Ganguli 2016; Messina 1984; Messina and Renwick 1985a; Mitchell 1975; Tripathi et al. 2013). After mating, when females are depositing their eggs on beans, they select hosts based on a variety of factors such as competition, host quality, and predation risk (Barde et al. 2012; Cope and Fox 2003; Janzen 1977; Kamble et al. 2016; Kar and Ganguli 2016; Messina 1984; Messina and Renwick 1985a; Mitchell 1975; Tripathi et al. 2013). Multiple factors, among them interspecies competition (Kishi and Tsubaki 2014), have been shown to alter this process. This will be further examined in Chapter II.

Bean beetles also have somewhat plastic life history traits in terms of offspring size and number (Kar and Ganguli 2016; Kawecki 1995) Though the life history trade-off between clutch and egg size is well established in other species (Gibbs et al. 2005), it has not been examined in *C. maculatus*. If, as expected, this tradeoff does exist in *C. maculatus*, then it is likely that the social environment will impact it just like it does in other species (Gibbs et al. 2005).

CHAPTER 2

**The effects of male harassment on female reproductive
behavior in the bean beetle**

Introduction

Across multiple species, females and males often have optimal fitness strategies that are incompatible with one another (Berger et al. 2014; Brennan et al. 2010; Dougherty et al. 2017; Harano 2014; Ronn et al. 2007; Zajitschek et al. 2018). Such sexual conflict can be seen in the context of the ideal number of matings (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Zajitschek et al. 2018). Males maximize their fitness by seeking to mate as many times and with as many mates as possible (Gay et al. 2009; Harano 2014). In contrast, females maximize fitness with fewer copulations (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Zajitschek et al. 2018). Typically, between one to a few matings are enough to fertilize all her eggs (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Zajitschek et al. 2018). Copulations beyond this can provide other benefits such as nuptial gifts, insurance of paternal care for offspring, or fitter offspring due to increased genetic diversity (den Hollander and Gwynne 2009; Ronn et al. 2006; Zajitschek et al. 2018). However, the costs are believed to outweigh any such benefits in most cases (Zajitschek et al. 2018). Costs of multiple matings include increased predation risk, physical injury to the female, decreased lifespan and fitness, time lost, and increased risk of pathogen transmission (den Hollander and Gwynne 2009; Harano 2014; Ronn et al. 2006; Zajitschek et al. 2018). As a result of this sexual conflict, both males and females of many species will want to get the upper hand on copulation control.

One way in which males try to gain control over mating is via male harassment (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Zajitschek et al. 2018).

The costs associated with male harassment are often overlooked (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Zajitschek et al. 2018). Male harassment is a form of sexual coercion where males repeatedly attempt to mate with reluctant females (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014). This can have negative impacts analogous to those of multiple matings, such as increased predation risk, physical injuries, loss of time spent doing other activities, lower fitness, decreased longevity or even death (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014). Harassment also has unique costs. For instance, it can interrupt ovipositing females, resulting in smaller clutch sizes or failure to choose optimal egg laying sites (Gay et al. 2009; Gibbs et al. 2005). Negative effects of harassment can also be transgenerational in nature (Zajitschek et al. 2018). This is true for reproductive fitness in *C. maculatus* where daughters of harassed females showed decreased fitness (Zajitschek et al. 2018). Interestingly, granddaughters actually had increased fitness relative to granddaughters from non-harassed and singly mated females indicating a potential tradeoff (Zajitschek et al. 2018). Overall, however, it would appear that male harassment induces a considerable toll on female fitness.

Male harassment has been studied in several bean beetle species (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Ronn et al. 2006; Zajitschek et al. 2018). A relatively recent area of research has focused on the effects of male harassment on female fitness (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Ronn et al. 2006; Zajitschek et al. 2018). The majority of the research demonstrated reduced female lifetime fitness either in the original generation or in the

daughter generation regardless of whether or not the harassing males could mate with the females (den Hollander and Gwynne 2009; Gay et al. 2009; Ronn et al. 2006; Zajitschek et al. 2018). den Hollander and Gwynne (2009) did not find a reduction in female fitness for the original generation due to harassment alone, but they failed to look at following generations. These unusual results could be due to differences in species and in strains as these factors have been shown to mediate the cost of mating (Ronn et al. 2006). Regardless, some evidence suggests that reduction of offspring number due to any form of male presence is caused, in part, by smaller clutch size (Gay et al. 2009; Ronn et al. 2006). Whether this is caused by plasticity or other factors has not been studied.

Similarly, little to no research exists on the effects of male presence on egg size in bean beetles. However, egg size is known to affect important offspring traits (Fox 1994). Smaller eggs develop more slowly and produce less fit offspring (Fox 1994). Yet, there is no difference in offspring survival (Fox 1994). This provides an explanation for why some strains of bean beetle initially have no change in lifetime fitness (den Hollander and Gwynne 2009; Ronn et al. 2006; Zajitschek et al. 2018) and only the following generation shows reduced fitness (Zajitschek et al. 2018). Potentially, these strains decrease egg size but not clutch size under harassment, impacting only the following generations' fitness. Yet, no research has directly examined whether egg size is impacted by male harassment.

Life history theory predicts a tradeoff between clutch size and egg size, and this has been documented in many species (Roff 1993). One prime example conducted by

Gibbs and colleagues (2005) on the butterfly *Pararge aegeria* demonstrated that females traded off an increase in clutch size for a reduction in individual egg size, but only when harassing males were present. This suggests that tradeoffs are social and context dependent.

The existence of such a tradeoff between egg and clutch size, and its potential plasticity, has not been well studied in bean beetle species. However, Fox et al. (1997) did find that females of the beetle *Stator limbatus* laid larger eggs but smaller clutches on lower quality seeds. Additionally, though the existence of a plastic tradeoff in bean beetles is uncertain, bean beetle species such as *C. maculatus* and *C. chinensis*, as well as other beetle species such as *N. orbicollis*, have been shown to plastically alter both egg size and clutch size based on environmental conditions ranging from larval competition to host type and availability to population density (Barde et al. 2012; Cope and Fox 2003; Creighton 2005; Fox 1994; Guedes and Yack 2016; Kar and Ganguli 2016; Kawecki 1995; Messina 1984; Messina and Renwick 1985b; Messina and Slade 1999; Yanagi et al. 2013). One study noted that, after being exposed to males with their genitals still intact, female bean beetles laid an equivalent number of eggs to females that had not been exposed to such mates after mating (Zajitschek et al. 2018). However, their offspring were far less fit (Zajitschek et al. 2018). Since clutch size did not change, this difference in offspring fitness could potentially have been due to smaller egg size though it is impossible to rule out the effects of stress (Zajitschek et al. 2018). It is important to note that, in contrast to studies where the females were found to have a decreased clutch size in the presence of males, these females were not given

beans to deposit eggs on until after the males had been removed (den Hollander and Gwynne 2009; Gay et al. 2009; Ronn et al. 2006; Zajitschek et al. 2018).

Regardless of whether females were exposed to males before or during oviposition, there was a reproductive cost to females from male exposure (den Hollander and Gwynne 2009; Gay et al. 2009; Ronn et al. 2006; Zajitschek et al. 2018).

Potentially, the form that cost takes as either a reduction in egg size or clutch size responds plastically to environmental conditions. However, because there is an overall cost, there would likely only be a reduction in one aspect without the subsequent increase in the other. Therefore, if the tradeoff between egg and clutch size is present in bean beetles, the cost of harassment may diminish or even negate it. However, no studies have explored the possibility of a tradeoff between the two in *Callosobruchus* bean beetles and whether male presence alters this tradeoff in any way.

Another understudied area of the effects of male presence on female reproductive behavior concerns oviposition site preferences. Because many insect species like *C. maculatus* cannot travel away from their host as larvae, the quality of their development site is vital to their success (Cope and Fox 2003; Kar and Ganguli 2016; Messina 2004b). The Gibbs et al. (2005) study on a species of butterfly is one of the few studies to examine this. In the absence of males, female butterflies of this species lay their larger eggs on better quality plants (Gibbs et al. 2005). But when males are introduced, the preference disappears (Gibbs et al. 2005). This suggests that another cost of male presence in species where the males harass the females even after the females have mated may be the loss of adaptive oviposition site preferences.

Limited research exists on the impact of male presence on such oviposition preferences in bean beetles. However, the bean beetle species *C. maculatus* do plastically alter their egg laying behavior based on various environmental factors (Cope and Fox 2003; Guedes and Yack 2016; Kar and Ganguli 2016; Kishi and Tsubaki 2014; Messina 1984; Messina 2004; Messina and Renwick 1985b). For instance, they have been shown to prefer to lay eggs on beans with fewer eggs and also with less larval vibrations coming from within (Guedes and Yack 2016; Messina and Renwick 1985b), resulting in decreased resource competition for their offspring. Females also prefer to lay their eggs on larger beans, which provide more nutrients for their offspring, and on undamaged beans over damaged ones (Cope and Fox 2003; Kar and Ganguli 2016). Moreover, females choose young, full grown beans over young, undeveloped beans or old, full grown beans in order to increase both the quality and quantity of their offsprings' nutrients (Messina, 1984). Although they can develop in different varieties of beans, bean beetles are known to prefer their maternal bean likely due to increased survival rates (Messina 2004).

Additionally, a single study by Kish and Tsubaki (2014) did find an impact of male harassment on female oviposition preference. They discovered that, when in the presence of males of another species of bean beetle, both *C. maculatus* and *C. chinensis* females changed their bean type choice (Kishi and Tsubaki 2014). They went from no preference between split and whole beans for *C. chinensis* and a moderate preference for whole beans in *C. maculatus* when in the presence of no males to a strong preference for whole beans in both species (Kishi and Tsubaki 2014). This was

suggested to be a plastic behavioral change due to the fact that females of both species have been known to hide from males between the gaps made by whole beans that are next to each other (Kishi and Tsubaki 2014). They also found an effect of conspecific male harassment on bean type preference but only for *C. chinensis*. (Kishi and Tsubaki 2014). Moreover, no research exists on whether other elements of bean beetle oviposition behavior are impacted by the presence of males and whether female plasticity regarding these elements may actually decrease instead of increase.

This Study

As a result of these gaps in the literature, we conducted an experiment to determine the effects of harassing male presence on *C. maculatus* female reproductive behavior including egg size, egg number, and egg laying site preference. The first prediction is that there will be a tradeoff between egg size and number, unrelated to harassment. Based on how common such a tradeoff is and the fact that both these aspects have been shown to be independently plastic, this is likely to be the case (Creighton 2005; Fox 1994; Gibbs et al. 2005; Kawecki 1995; Messina and Slade 1999; Yanagi et al. 2013).

Our second prediction is that this tradeoff will weaken or disappear in the presence of conspecific, intact males. This is supported by the majority of past research demonstrating that male presence is costly to females and often leads to a reduction in fitness (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Ronn et al. 2006; Zajitschek et al. 2018). Assuming that we also will find a decrease in either clutch

size or egg size when females are in the presence of males, a strong claim can be made that this decrease will not coincide with an increase in the other. If the tradeoff does occur in the presence of males, as well as in their absence, then this fitness cost should not be present as a plastic increase in one variable should offset this reduced fitness due to a costly decrease in the other. More specifically, we predict that clutch size will decrease due to intact male harassment while egg size will not be impacted. Support comes from the fact that our males will be present during the time when females are laying eggs and not before. The majority of studies that had males present during that time found a decrease in clutch size (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Ronn et al. 2006). This could possibly be due to a time cost imparted by males as females now have to spend their time escaping the advancements of males rather than laying eggs.

In addition, we predict that the preference for ovipositing on maternal beans (adzuki) over the nonhost species *Phaseolus vulgaris* (pinto beans) will disappear in the presence of harassing intact males. In other species, presence of males interrupts female preference to deposit eggs on higher quality hosts that improve offspring survival, and it is likely that the same could occur in *C. maculatus* (Gibbs et al. 2006; Kishi and Tsubaki 2014). Even though one study did not find any change in preference between split and whole beans for *C. maculatus* females exposed to conspecifics (Kishi and Tsubaki 2014), it is possible that this bean trait simply does not have a relevant enough fitness cost. In contrast, *Phaseolus vulgaris* beans contain a lectin that is toxic to *C. maculatus* so their innate non-preference for it is vital to their survival (Janzen 1977).

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As a result, it is likely that selective pressure will select for females who can respond adaptively to changes in their environment and that this plastic response may be inhibited by costly social contexts.

Methods

General Population

Our laboratory population of *Callosobruchus maculatus* originated from several hundred individuals of a Brazilian strain that were obtained from Carolina Biological Supply. Lab populations were maintained in 3 separate 4-liter plastic containers with mesh openings for ventilation. Containers were kept approximately half full with adzuki beans. From those general population containers, a small subset of beetles was put in smaller containers with new adzuki beans once a week to oviposit. The date was recorded on the side of the containers. Approximately 3 weeks from the day these containers were set up, beans were isolated in well plates and the date was recorded on the side. This allowed us to obtain virgin individuals of the same age for experiments. The well plates were put in an incubator at 27 degrees Celsius and checked every day for emergences of adults. Any wells that had multiple emergences on a single day were not used. Beetles that emerged in wells without any other conspecifics were given an ID, sexed, and massed on a Mettler Toledo xp26 scale. The beans from all wells with emergences were placed in a waste container and put in the freezer prior to being discarded.

Mating and Treatments

After a minimum of 24 hours since emergence, males were haphazardly assigned to one of two roles: copulation and harassment. Females were mated with a single male from the copulation group. They were placed in a small petri dish under a

dissecting scope. Females were always placed first and were given 5 minutes to acclimate before males were placed with them. Matings were observed to ensure that intromission occurred. Latency to mate and copulation duration were recorded. Females and males were weighed before and after mating to check if the spermatophore was transferred. Within that same day, females were added to an oviposition cup where they had 10 adzuki beans and 10 pinto beans of roughly the same shape and size as determined visually. They were then haphazardly assigned to one of three treatments: control, low harassment, or high harassment. The control treatment consisted of a solo female in a bean cup, low harassment had one virgin male and the high harassment condition had three virgin males in the oviposition cup with the female. Other than the presence/absence and number of males, oviposition cups were identical. All cups were labeled with the female's ID and the time females were added and placed in an incubator for 48 hours.

After 48 hours, each beetle was transferred to a fresh oviposition cup for an additional 48 hours. At this point, any dead males were replaced with new virgin males. Like before, each cup contained 10 adzuki and 10 pinto beans. 48-hour time periods were chosen as we had previously observed that it takes about that amount of time for females to lay more than one egg per bean when a total of 20 beans are available to her. Since *C. maculatus* females have been shown to initially equally distribute eggs across all bean types until the majority of beans have one egg before they become start to express bean preferences, it was important to give them enough time to get past that phase (Mitchell 1975). This second batch of cups were labeled 96 hours and with

the female's ID. Any containers with dead females were not used in the data set. Then, all cups were placed back in the incubator. After this second 48 hour time period, all females were removed and preserved in ethanol.

Counting and Measuring Eggs

All the bean cups from both the 48 and 96 hour time periods were kept after the removal of beetles for counting and measuring eggs, except for those where females died during testing. For each cup, the beans were placed under a dissecting scope (Leica MZ60) and the number of eggs on each bean type was counted and recorded. Beans were manipulated using featherweight forceps to avoid damaging eggs. Then, the beans were placed back in the cup and put in a temperature controlled room at 27 C to develop.

Once the eggs were counted, up to 3 eggs per bean were measured using a Leica MZ16A stereoscope with a Leica DFC429 digital camera to take initial photos of individual eggs. Each egg photo was given a unique ID. Later, the photos were analyzed using the ImageJ software measuring tool. To estimate egg size, both length and width of eggs were recorded. The volume of the eggs was calculated using the protocol from Yanagi et al. (2013). The quality of the photo based on the clear demarcation of the egg outline and on the angle of the egg was quantified from one to five with five being highest quality. Only egg photos quantified as a five were used.

Statistics

One-way ANOVAs were run to test for effects of the harassment treatment on mating behavior. Additionally, general linear mixed models were conducted to check for the effects of harassment treatment on clutch size, egg size, and oviposition site preference. This was followed by appropriate post hoc tests. Finally, linear regressions were run to check for the existence of a trade-off between clutch and egg size within each treatment group.

Results

Although we collected oviposition data at two time periods (48 and 96 hours), our 96-hour data set was considerably smaller due to female mortality. In addition, including the two time periods in the statistical models resulted in too many variables and interactions that could not be interpreted. For these reasons, we decided not to include the 96-hour oviposition data set in the analysis presented here.

I- Effects of harassment treatment on mating behavior

To check whether mating behavior differed among the different harassment treatments, we ran two One-Way ANOVAs with treatment as the independent variable and latency to mate and copulation duration as dependent variables. As expected, we found no effect of treatment on either latency to mate ($F_{2,76} = 0.0663$, $P = 0.936$; Figure 2.1a) or copulation duration ($F_{2,75} = 0.221$, $P = 0.802$; Figure 2.1b).

II- Effects of experimental variables on female egg size and clutch size

We used a natural log transformation to normalize female mass, clutch size and egg size. We then ran linear mixed models to explore the effect of female size on egg and clutch sizes. In general, one can expect larger females to produce larger eggs and clutches, and in that case we should correct egg and clutch size for female mass in all subsequent analyses. Our goal here was to check whether this is true of our data set and make the appropriate corrections before proceeding with the analyses. For these models, we included female ID, female mass, bean type and harassment condition as dependent variables. We included female ID as a random effect nested within bean

type. At this stage, no interactions between variables were included. We found that female mass had a significant effect on egg size ($F_{1,119} = 8.03$, $P = 0.005$ and $F_{1,186} = 64.3$, $P < 0.0001$ respectively), while bean type and harassment treatment had no effect ($F_{1,119} = 3.06$, $P = 0.08$ and $F_{1,116} = 0.14$, $P = 0.87$ respectively). When examining clutch size, we found that bean type and harassment treatment both had a significant effect ($F_{1,116} = 5.42$, $P = 0.02$ and ($F_{1,116} = 5.28$, $P = 0.006$ respectively). Surprisingly, female mass did not have an effect on clutch size ($F_{1,116} = 0.63$, $P = 0.43$). Based on these results, we compared egg size to female size by calculating the residuals of the regressions between female mass and egg size. We proceeded with data analysis using the residuals of egg size in subsequent models.

Egg size

We ran a linear mixed model testing the effects of harassment treatment, bean type and the interaction of these terms on the residuals of egg size. We included female ID as a random effect nested within bean type. We found no effect of harassment treatment, bean type or the interaction between them on egg size (Figure 2.2, Tables 2.1 and 2.2).

Clutch size

We ran a linear mixed model testing the effects of harassment treatment, bean type and the interaction of these terms on clutch size. We included female ID as a random effect nested within bean type. As shown in Figure 2.3 and Tables 2.3 and 2.4, we found a significant effect of harassment treatment, bean type and the interaction

between them ($F_{2,116} = 3.96, P = 0.02$; $F_{1,116} = 16.28, P < 0.0001$ and $F_{2,116} = 4.38, P = 0.01$ respectively). Tukey HSD post hoc tests showed that females in the no harassment treatment had significantly larger clutch sizes, and clutch sizes were larger on adzuki beans (Figure 2.3).

III – Egg size/clutch size trade-off

We ran linear regressions for each of the harassment treatments to test for a tradeoff between egg and clutch size. We initially ran separate analyses for eggs on pinto and adzuki beans, but, as there were no significant differences between them, we pooled the data together. Only in the no harassment treatment did egg size significantly predict clutch size indicating the existence of a tradeoff: as egg size increased, clutch size decreased ($R^2 = 0.52, P < 0.01$, Figure 2.4a). There was no significant relationship between egg size and clutch size in either the low harassment or high harassment treatments ($R^2 = 0.06, P = 0.257$ and $R^2 = 0.03, P = 0.463$ respectively; Figures 2.4b-c).

To test whether this relationship between clutch size and egg size varied significantly between treatment conditions, we performed a linear regression where egg size was the independent variable; and clutch size, harassment condition, and the interaction between the two were the predictor variables. As expected, egg size varied significantly with clutch size ($F_1 = 4.76, P = 0.03$; Table 5). Additionally, we found a significant effect of the interaction between clutch size and harassment condition on egg size ($F_2 = 6.35, P < 0.01$, Table 5) indicating that the relationship between clutch size and egg size varies based on harassment level.

IV- Effect of harassment on bean preference

As reported under section II, we ran a general linear mixed model to test if harassment treatment had an effect on female oviposition site preference. Our independent variable was clutch size, and the predictor variables were female ID, harassment condition, bean type, and the interaction between bean type and harassment condition. Female ID was a random effect nested within bean type. We found that clutch size significantly varied by harassment treatment and bean type ($F_{2,116} = 3.96, P = 0.02$ and $F_{1,116} = 16.28, P < 0.0001$ respectively, Figure 2.3, Table 2.4). Importantly, we found a significant interaction between bean type and harassment treatment ($F_{2,116} = 4.38, P = 0.01$, Figure 2.3, Table 2.4), demonstrating that female preference for bean type depended on the harassment treatment. A post hoc Tukey HSD test revealed a preference for adzuki beans over pinto in the no harassment treatment, but this preference was not present in the low or high harassment treatments (Figure 2.3, Table 2.3).

V - Larval mortality

There was a total of 1400 hatchlings with only one emerging from a pinto bean. Therefore, out of 1746 eggs laid on pinto beans, only one became an adult. There were 2216 eggs on adzuki and 1399 of them made it to adulthood. The percentage of eggs laid by each female that made it to adulthood and emerged from a bean was calculated. To determine if larval mortality varied with treatment, we ran a One-Way ANOVA. The dependent variable was emergence rate and the independent variable was the harassment treatment. There was a significant difference in emergence rate

between treatments ($F_{2,57} = 3.84$, $P = 0.02$; Figure 2.5, Tables 2.6 and 2.7). A Tukey HSD post hoc test revealed that there was a significantly higher emergence rate, or significantly less larval mortality, in the no harassment condition than in the high harassment condition. There was no significant difference between the low harassment treatment and any other treatment (Figure 2.5).

Discussion

In accordance with predictions, there is a significant trade-off between egg size and clutch size and this trade-off dissipated when oviposition occurred in the presence of conspecific males. Moreover, as expected clutch size (but not egg size) was significantly affected by male harassment. In the presence of high male harassment, *C. maculatus* females deposited fewer eggs overall. Additionally, females' significant preference for adzuki beans over pinto beans as hosts disappeared in the presence of harassing males. These findings together strongly support the idea that female *C. maculatus* reproductive and oviposition behavior is plastic and is impacted by male harassment.

When females were allowed to oviposit in isolation, they laid significantly more eggs on adzuki beans than on pinto beans. As adzuki beans are the favored bean over the toxic pinto beans (Janzen 1977), this strongly suggests that females favor the superior host bean species. The fact that only one out of 1400 offspring emerged from a pinto bean further supports this claim. This is not surprising and is in agreement with past research that found clutch size to be a plastic trait in *C. maculatus* females that responds to host quality (Guedes and Yack 2016; Messina and Renwick 1985b; Messina and Slade 1999). Moreover, the finding that females laid larger clutches on higher quality beans replicates past studies in which the same phenomenon was observed (Barde et al. 2012; Cope and Fox 2003; Guedes and Yack 2016; Kar and Ganguli 2016; Messina 1984; Messina and Renwick 1985b). Though, as far as we are aware, no past studies have examined differences in preference between host species toxic to *C.*

maculatus and nontoxic hosts, our finding that females preferentially deposit eggs on their native bean adzuki over the non-native pinto in the absence of harassment is in accordance with existing literature (Messina 2004; Messina and Slade 1997). Such literature has found that *C. maculatus* significantly choose to oviposit on the bean they developed on, in our case adzuki, over any other bean (Messina 2004; Messina and Slade 1997). Females selectivity based on host quality also occurs in other species such as the butterfly *Pararge aegeria* further supporting our finding (Gibbs et al. 2005).

In contrast, females did not alter egg size based on bean type. Since egg size has been previously demonstrated to be a plastic trait (Yanagi et al. 2013), it is possible that females chose not to alter it as it is not advantageous for them to do so based on bean host. Since they were already preferably selecting higher quality beans, changes in egg size may not have an impact on female fitness.

The trade-off between egg size and clutch size under no harassment was observed across both bean types. The larger the average size of the females' eggs, the fewer the amount of eggs laid. As far as we are aware, this is the first time that the existence of this trade-off in *C. maculatus* has been demonstrated. However, the existence of an egg size and clutch size trade-off is widespread among animal species and a major prediction of life history theory (Roff 1993), making this finding consistent with past studies (Carriere and Roff 1995; Creighton 2005; Gibbs et al. 2005).

Moreover, the fact that this trade-off disappears under male harassment conditions adds to the growing body of literature on the negative effects that such harassment has on females (den Hollander and Gwynne 2009; Gay et al. 2009; Harano

2014; Ronn et al. 2006; Zajitschek et al. 2018). Theory predicts that females will have different strategies, investing more in either larger egg size or large clutch size depending on environmental context (Carriere and Roff 1995; Gibbs et al. 2005). This is supported by studies on other species that found this to be the case (Carriere and Roff 1995; Creighton 2005; Gibbs et al. 2005). Such plasticity enables females to optimize their reproductive strategy for the given environment (Carriere and Roff 1995; Creighton 2005; Gibbs et al. 2005). For instance, in the burying beetle *Nicrophorus orbicollis*, females produce smaller broods with larger body sizes when population density is higher to ensure higher quality offspring at the expense of raw number of offspring (Creighton 2005). Larger body size enhances competitive ability, something that is more important in higher population densities (Creighton 2005). If future studies do find similar plastic alterations in this tradeoff in *C. maculatus* under various environmental conditions, male harassment would pose a threat to the survival of female offspring as it would prevent females from optimizing the tradeoff for a given context. It is possible that this loss of plasticity led to the observed significant increase in offspring mortality under high harassment. However, further research would need to determine if this mortality is simply a result of reduced female body condition or, as suggested, a result of plasticity loss.

As previously stated, females laid fewer eggs under harassment, resulting in fewer potential offspring. Once again, this provides further support for the idea that male harassment is costly (den Hollander and Gwynne 2009; Gay et al. 2009; Harano 2014; Zajitschek et al. 2018). Furthermore, it expands upon past studies that found that

male presence during ovipositing led to decreased *lifetime* egg production by demonstrating that it additionally leads to decreased *current* egg production (Gay et al. 2009; Ronn et al. 2006). Potentially, the reduction in lifetime clutch size is caused by this initial decrease in egg production though it is possible that egg reduction occurs throughout the remainder of females' lives.

The mechanism by which clutch size is reduced in the presence of harassing males, however, has yet to be examined. Though it is possible that females plastically responded to male harassment by decreasing clutch size, it seems unlikely as a decrease in one trait without a subsequent increase in the other would be detrimental to the females' immediate fitness (Fox 1994). Such an increase in egg size was not found suggesting that clutch size was reduced as a direct result of the cost of harassment and not as a result of a plastic choice. However, there was also not a decrease in egg size suggesting that females may have maintained some control of oviposition and maximized size as much as they could within the constraints of harassment. It is possible that they were attempting to select for larger eggs at the expense of smaller clutch size but the maximum egg size was reduced due to harassment. This would make sense as Yanagi et al. (2013) found that *C. maculatus* females produce larger eggs in stressful environments. It is important to note, however, that egg size may simply not be affected by harassment. Additionally, it is possible that females were simply minimizing current reproductive output in order to maximize future reproduction under less costly conditions. However, that is unlikely as increased chance of remating due to male presence leads to a reduction in lifespan, suggesting that it would be to the

detriment of females to wait to lay more eggs as their lifespans may be reduced (Eady et al. 2007). If anything, we should expect females under harassment to maximize current reproduction as a form of terminal investment.

In order to determine if this reduction in clutch size is directly caused only by the cost of harassment or also indirectly via female control of oviposition, future research could examine if the number of nonviable eggs dumped by females varies by harassment condition. Because *C. maculatus* females have been shown to egg dump in less than ideal oviposition conditions, allowing them to lay more eggs later on, the plasticity of egg laying during harassment can be checked by measuring if egg dumping levels increase under it or not (Wang and Horng 2004). If egg dumping does increase, it would suggest that females are still controlling oviposition, but, if not, it would lend more evidence for the idea that clutch size reduction is simply a direct cost of male harassment.

Regardless of whether harassment impacts clutch size directly or has a more generalized negative impact on the effectiveness of the trade-off itself, there was a clear cost of harassment on female fitness in *C. maculatus*. Future research will be needed to determine the exact mechanism of this cost. For instance, perhaps this is simply a time cost as females have to spend a greater portion of their time avoiding males instead of laying eggs. On the other hand, this cost could be a result of reduced female health due to increased stress or even injuries incurred during mating attempts by males.

In conjunction with the costs of male harassment discussed so far, we also found that female preference for the higher quality adzuki beans disappeared in the presence of males. Though this effect was previously unknown in any species of bean beetle, it closely mirrors the effects of male harassment on female *P. aegeria* butterflies where the preference for higher quality host plants disappeared (Gibbs et al. 2005). This decrease in female choosiness for more suitable oviposition sites resulted in major fitness consequences. Just how immense these consequences were can be shown by the fact that only one out of over a thousand eggs laid on pinto beans successfully emerged as an adult. Similar to the findings for reductions in egg and clutch size, the mechanism of this cost could also be a result of time loss or a result of female health reduction.

In the future, it would be interesting to know if all plastic oviposition behavior changes equally under male harassment or if some behaviors are more resilient than others. Additional research could also examine the even more intriguing effects of other environmental conditions to determine what else has an impact on the plasticity level of all variables examined in this study. It would also be of interest to determine just how long lasting the effects of male harassment on female reproductive plasticity are once females are alone again.

These results indicate that male harassment has additional costs in the form of plasticity loss that were not previously known. This paper highlights the limitations of plastic reproductive behaviors in *C. maculatus* females. It also demonstrates that the

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social environment has wide reaching interactions with behavior and suggests that, in general, when examining any form of behavior, external context matters.

Figures and tables

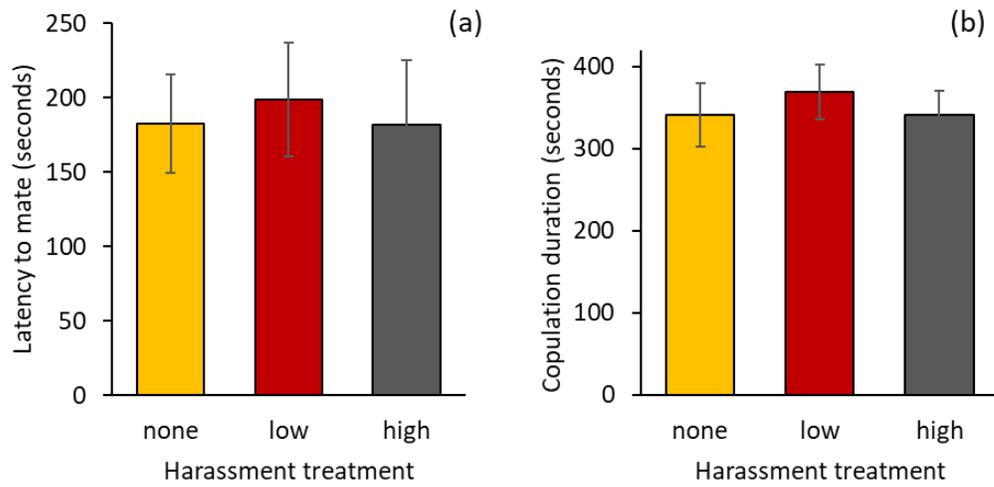


Figure 2.1 –Latency to mate (a) and copulation duration (b) did not differ among harassment treatments. Means and standard errors for each treatment are shown.

Figures and tables

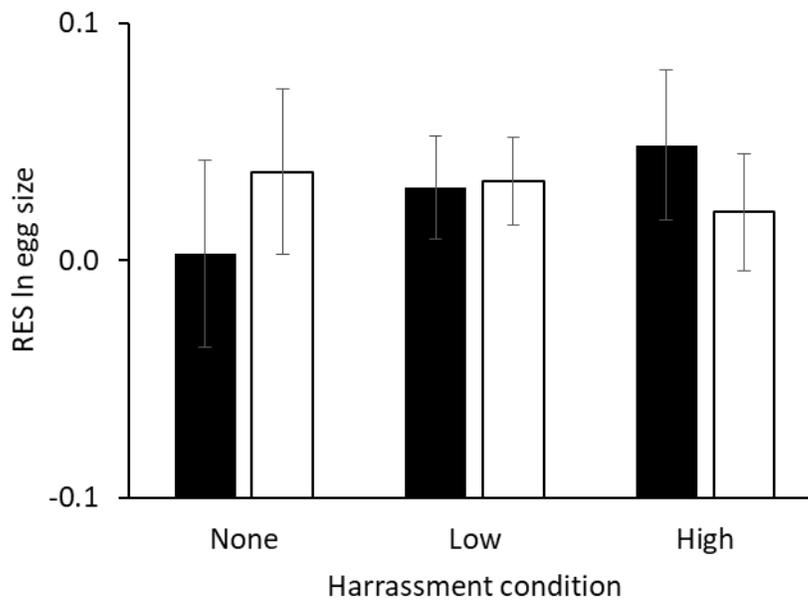


Figure 2.2 – Harassment treatment, bean type (adzuki in black; pinto in white) and the harassment*bean type interaction term did not have an effect on egg size. Means and standard errors for each treatment group are shown. See Table 1 for descriptive statistics and Table 2 for the full model effects.

Figures and tables

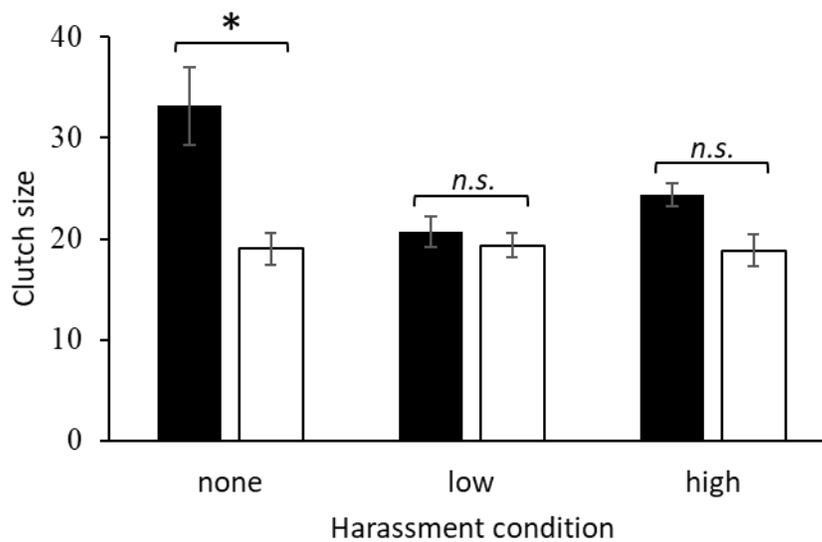


Figure 2.3 – Harassment treatment and bean type (adzuki in black; pinto in white) had an effect on clutch size, as well as the harassment treatment*bean type interaction. This indicated that the preference for adzuki beans as oviposition substrate varied among harassment treatments: there was a significant preference for adzuki (black) over pinto (white) in the no harassment treatment, but no preference in the low and high harassment treatments. Means and standard errors are shown, and asterisks indicate statistical significance at $p < 0.05$.

Figures and tables

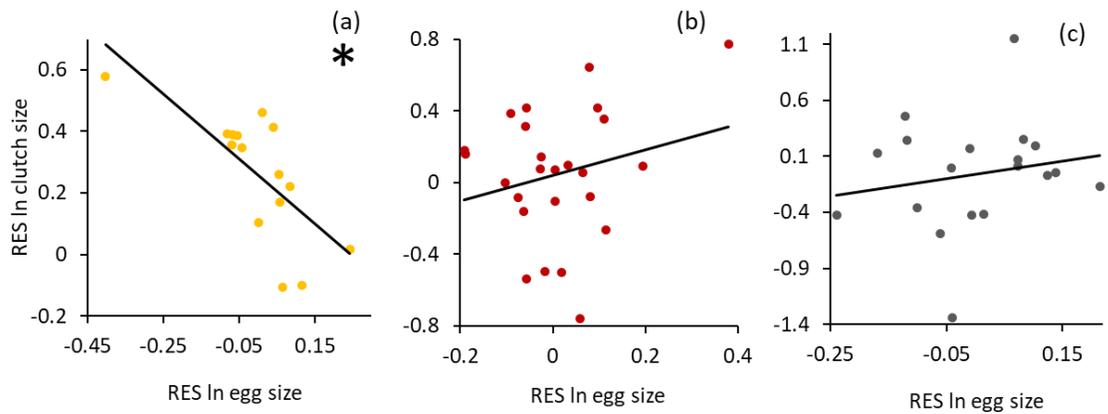


Figure 2.4 – The relationship between egg size and number varied among harassment treatment: there was a significant negative relationship in the no harassment treatment (a, yellow) and no relationship in the low (b, red) or high (c, gray) treatments. Data are shown for the 48-hour period and both types of beans pooled together. The asterisk denotes a statistically significant linear regression at $p < 0.05$.

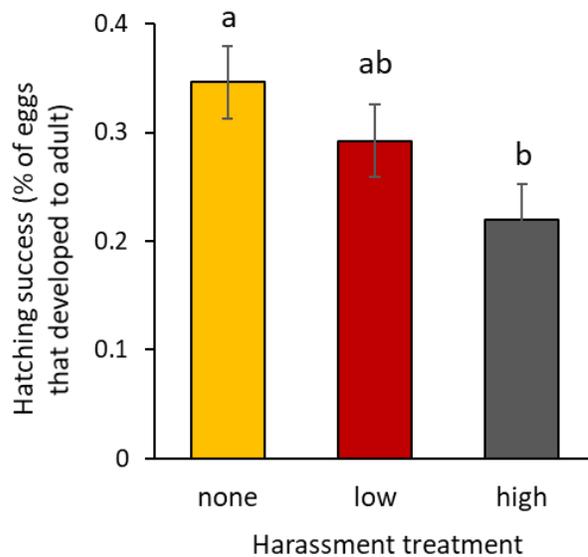
Figures and tables

Figure 2.5 – Offspring mortality varied among harassment treatments: the adult emergence success rate was significantly higher in the no harassment treatment than in the high harassment treatment. Posthoc comparisons using Tukey’s HSD are shown: treatments not indicated by the same letters are significantly different at $p < 0.05$. Means and standard errors for each treatment are shown, and difference in letters indicate statistical significance at $p < 0.05$.

Figures and tables

Table 2.1 – Mean, standard error (SE) and sample sizes (n) of egg size in the different harassment treatment groups and bean types. Egg size values were natural log-transformed and are shown as the residuals of the regression of egg size on female body mass.

| Harassment treatment | Bean type | Mean | SE | n |
|-----------------------------|------------------|-------------|-----------|----------|
| None | Adzuki | -0.027 | 0.04 | 16 |
| | Pinto | 0.007 | 0.03 | |
| Low | Adzuki | 0.001 | 0.02 | 25 |
| | Pinto | 0.004 | 0.02 | |
| High | Adzuki | 0.019 | 0.03 | 19 |
| | Pinto | -0.010 | 0.03 | |

Figures and tables

Table 2.2 – Linear mixed model testing for differences in egg size according to bean type and harassment treatment.

| Source of variation | df | <i>F</i> | <i>P</i> |
|----------------------------------|-----------|-----------------|-----------------|
| Harassment treatment | 2,118 | 0.13 | 0.87 |
| Bean type | 1,118 | 0.02 | 0.89 |
| Harassment treatment * bean type | 2,118 | 0.58 | 0.56 |

Figures and tables

Table 2.3 – Mean, standard error (SE) and sample size (n) of clutch size in the different harassment treatment groups and bean types. Clutch size values were natural log-transformed.

| Harassment treatment | Bean type | Mean | SE | n |
|-----------------------------|------------------|-------------|-----------|----------|
| None | Adzuki | 3.33 | 0.02 | 16 |
| | Pinto | 3.12 | 0.02 | |
| Low | Adzuki | 2.89 | 0.02 | 25 |
| | Pinto | 2.85 | 0.03 | |
| High | Adzuki | 3.03 | 0.04 | 19 |
| | Pinto | 2.93 | 0.03 | |

Figures and tables

Table 2.4 – Linear mixed model testing for differences in preference for bean type according to harassment treatment. We tested for the effect of bean type and harassment treatment in clutch size. The bean type*harassment treatment interaction tests for differences in preference for bean type according to harassment treatment. Significant values are in bold and marked with asterisks ($P < 0.05$).

| Source of variation | df | F | P |
|----------------------------------|-----------|----------|-------------------|
| Harassment treatment | 2,116 | 3.96 | 0.02* |
| Bean type | 1,116 | 16.28 | <.0001* |
| Harassment treatment * bean type | 2,116 | 4.38 | 0.01* |

Figures and tables

Table 2.5 – Linear regression testing for differences in egg size according to clutch size and harassment treatment. The clutch size*harassment treatment interaction term tests for differences in the egg size/clutch size relationship according to harassment treatment. Significant values are in bold and marked with asterisks ($P < 0.05$).

| Source of variation | df | SS | F | P |
|----------------------------------|-----------|-----------|----------|------------------|
| RES clutch size | 1 | 0.06 | 4.76 | 0.03* |
| Harassment treatment | 2 | 0.04 | 1.73 | 0.19 |
| Harassment treatment * bean type | 2 | 0.17 | 6.35 | >0.01* |

Figures and tables

Table 2.6 – Mean, standard error (SE) and sample size (n) of adult emergence success in the different harassment treatment groups. Adult emergence success is shown as the percentage of eggs laid by a female that developed to adulthood.

| Harassment treatment | Mean (%) | SE | n |
|-----------------------------|-----------------|-----------|----------|
| None | 34.61 | 0.03 | 15 |
| Low | 29.23 | 0.03 | 25 |
| High | 21.93 | 0.03 | 20 |

Figures and tables

Table 2.7 – One-Way ANOVA testing for differences in adult emergence success according to harassment treatment. Adult emergence success was defined as the percentage of eggs laid by female from which adults emerged. The significant value is in bold and marked with an asterisk ($P < 0.05$).

| Source of variation | df | SS | MS | F | P |
|----------------------------|-----------|-----------|-----------|----------|--------------|
| Harassment treatment | 2 | 0.14 | 0.07 | 3.8 | 0.02* |
| Error | 57 | 1.06 | 0.02 | | |
| Total | 59 | 1.21 | | | |

CHAPTER 3

The impact of larval competition on bean beetle mating behavior

Introduction

The quality of the oviposition site is imperative to the success of many species where larvae are unable to move between hosts (Cope and Fox 2003; Kar and Ganguli 2016; Messina 2004). To increase the probability that their offspring will survive, females of these species consider several factors when choosing oviposition sites, including the level of competition, the quality and quantity of resources, and the risk of predation or parasitism (Barde et al. 2012; Cope and Fox 2003; Janzen 1977; Kamble et al. 2016; Kar and Ganguli 2016; Messina 1984; Messina and Renwick 1985b; Mitchell 1975; Tripathi et al. 2013). Due to the importance of oviposition site selection, plastic responses to such factors is key.

One such species where oviposition choice is critical to the success of the offspring is the bean beetle *Callosobruchus maculatus* (Cope and Fox 2003; Tripathi et al. 2013). With host variety being equal, arguably the most vital factor for them is the amount of larval competition or, in other words, the number of other larvae present within a single host bean. Bean beetles actively try to reduce such competition in a few ways. When ample beans are available, females will equally distribute their eggs among beans in order to reduce competition among offspring (Mitchell 1975). They also avoid laying eggs on beans where eggs from other females have already been deposited as another way to decrease competition (Messina and Renwick 1985b). *C. maculatus* females have even been shown to detect vibrations from larvae within beans and avoid beans that have high signal levels (Guedes and Yack 2016).

Being able to choose hosts with lower levels of competition is imperative as larval competition results in an array of negative effects (Credland et al. 1986). Among many species of beetle, larval competition has been shown to lead to increased mortality of offspring and smaller adult body size (Colegrave 1993; Credland et al. 1986; Creighton 2005; Shade and Vamosi 2012; Vamosi 2005). Specifically, the decrease in body size, likely as a result of decreased water uptake from the bean due to competition, often leads to a reduction in adult female fecundity (Colegrave 1993; Credland et al. 1986; Creighton 2005; Schade and Vamosi 2012; Vamosi 2005). Moreover, in certain strains of bean beetle, females that emerge from beans with high larval competition have lower lifetime egg production than females from beans with low levels of competition (Colegrave 1993; Credland et al. 1986; Vamosi 2005). Therefore, it would appear that larval competition leads to a reduction in female fitness.

That being said, there are conflicting findings on whether lifetime egg reduction is a direct result of larval competition or an indirect result of a decrease in female mass (Colegrave 1993; Vamosi 2005). In contrast to Vamosi (2005), Colegrave (1993), found no impact of competition alone on female fecundity. However, his study used a comparatively low level of larval competition for his treatment group with a maximum of only one competitor (Colegrave 1993). Vamosi (2005) used up to two competitors in his maximum larval competition treatment. This difference in level of competition could explain the conflicting findings. Possibly, only at a high enough level of larval competition can the impact of competition on lifetime egg production be detected.

Additionally, an alternative explanation is that this effect of competition varies based on bean beetle species and on host species (Vamosi 2005). There are likely other factors that mediate this relationship that have yet to be examined.

A largely understudied area of larval competition in bean beetles is its possible impact on mating behavior and sperm competition (Gay et al. 2009). According to sperm competition theory, males in populations with fewer potential mates, more male competition, or both should invest more in testes size, sperm quality, or sperm number (McNamara et al. 2016). What little research exists on larval competition's effect on mating behavior in *C. maculatus* suggests that larval density has no impact on sperm competition, namely testes size and sperm length (Gay et al. 2009). However, this study failed to account for differences in sex ratio within each bean that could be mediating the relationship between sperm competition and larval competition (Gay et al. 2009). This matters as the sociosexual environment is known to alter mating behavior in *C. maculatus* (van Lieshout et al. 2014; Wilson et al. 2014; Wilson and Tomkins 2014). More specifically, within female-biased evolutionary lines, female beetles become more resistant to longer copulations, kicking males sooner when they have previously been exposed to a male-biased social environment as opposed to a female-biased social environment (van Lieshout et al. 2014). Kicking has been suggested to be a marker of female resistance indicating that earlier kicking during mating means choosier females (van Lieshout et al. 2014). Likewise, even during copulation, the presence of additional males caused females to kick earlier (Wilson and Tomkins 2014). Likely, females kicked earlier when more males than females were present either before or during copulation

because they could afford to reject more often since more mates were available (van Lieshout et al. 2014; Wilson and Tomkins 2014). However, copulation duration and copulation latency, traits more commonly associated with sperm competition, have not been shown to be impacted by the sex ratio of the sociosexual population present either before or during copulation (van Lieshout et al. 2014; Wilson and Tomkins 2014). But sperm competition is only one aspect of mating behavior and it is possible that other aspects are impacted. Studies have yet to examine whether sex ratio during larval development and not just during adulthood could affect *C. maculatus* mating behavior.

After identifying these gaps in knowledge, we wanted to determine if initial, and not just lifetime, egg production is affected by high levels of larval competition (two or more competitors). We predict that the same trend that exists for *C. maculatus* females on adzuki beans, where increased levels of larval competition lead to decreased lifetime egg production, will hold true for initial egg production (Vamosi 2005).

Additionally, we aimed to determine if bean beetles can respond plastically to larval competition by altering their egg laying behavior when raised with only female or only male competitors. We predict that females originating from beans with only other females will produce different sized clutches than females originating from beans with only other males. This is because other aspects of egg production during larval competition are already known to be plastic (Yanagi et al. 2013). For instance, in both *C. chinensis* and *C. maculatus*, when controlling for body size, females that emerged from beans with competitors plastically alter their egg laying behavior by laying larger eggs than females raised on beans with no competitors (Yanagi et al. 2013). This is likely

because larvae from larger beans have shorter development times and can better compete (Yanagi et al. 2013). Moreover, we suspect that it's the female, not the male, competitors that cause this behavior. The more females, the larger the number of eggs, and, therefore, the higher the amount of conspecific competition. Increasing male competitors, however, does not directly effect egg amount. Therefore, we predict that, if a trade-off is indeed found in the previous chapter, then clutch size will decrease to compensate for larger more competitive eggs under female-biased larval competition.

Finally, we wanted to examine if larval competition does in fact alter mating behavior and if this relationship is mediated by developmental sex ratio within beans. We predict that females exposed to only other female larvae during development will be less choosy as indicated by a longer latency to kick. Because females are already known to be able to plastically alter their kicking behavior in response to the adult social environment, we suspect that this might also apply to variations in their developmental social environment (van Lieshout et al. 2014; Wilson and Tomkins 2014).

We also predict that copulation duration will be longest with males that developed in beans that only produced other males. This male-biased developmental environment is likely to lead to an increase in male-male competition via sperm competition. Though sperm competition has not previously been found to be impacted by larval competition as a whole, it is possible that the developmental sex ratio will mediate this relationship (Gay et al. 2009). Therefore, males raised in male only developmental environments could potentially have a different behavioral response to males raised in a mixed sex or female-biased developmental environment or in

isolation. Males that perceive more competition may want to mate longer with females as longer copulation duration has been shown to be correlate positively with larger ejaculate size (Edvardsson and Canal 2006). Additionally, though past studies found no change in copulation duration as a result of differences in the sex ratio of the adult environment (van Lieshout et al. 2014; Wilson and Tomkins 2014), it is possible that that aspect of mating behavior is determined beforehand by the developmental environment.

Methods

General and Experimental Populations

Populations were kept and beans were isolated as described in the previous chapter. A subset of the general population of adult beetles was placed in other smaller containers with fresh adzuki beans. These smaller containers had approximately 200 beans and 100 beetles of both sexes. These beetles were removed after 48 hours, a period during which they oviposited. The beans were then examined under a LEICA MZ60 dissecting scope and all the beans with fewer than three eggs were removed. Among the remaining beans, half of the beans were haphazardly chosen to belong to the solo treatment and half to the competition treatment. The half chosen to be in the solo treatment had all but one egg scraped off using a razor blade and the ones for the competition treatment were left intact. All the beans were massed. Originally, the beans were isolated in 1.5ml microcentrifuge tubes but no emergences occurred. Therefore, this experimental set up was repeated two more times. For the two subsequent repetitions, the beans were placed in well plates where each plate and well had an ID.

Emergences and Mating

For both non-experimental and experimental emergences, all beetles were weighed and given unique IDs. Emergence order was also recorded. All experimental beetles that could be determined to have been the first to emerge from a bean were used for mating experiments. We also used some individuals that were second or third

to emerge when the first emerged from that bean failed to mate. These experimental individuals were paired with non-experimental individuals for mating trials. Trials were conducted as described in the previous chapter. Once again, copulation duration, latency to kick by female, and latency to mate were recorded.

Clutch Size

All experimental individuals were preserved by either being placed in ethanol (for batch 2) or put in the freezer inside labeled tubes (for batch 3). Before being preserved, all experimental females that mated were allowed to oviposit by being placed individually in bean cups with 10 adzuki beans for 48 hours. Egg number for each bean cup was counted under the dissection scope and recorded.

Statistics

The effects of larval competition on copulation behavior and clutch size were determined by running multiple One-Way ANOVAs. We analyzed the data by dividing the experimental individuals in treatments according to presence or absence of competitors in their development bean, as well as the sex of the competitors. Therefore, treatments were: no competition, female competitors, male competitors and mixed competitors. We analyzed the data separately for experimental males and females as we make different predictions about how the sexes would respond to competition.

Results

One-way ANOVAS were run to check for differences in mating behavior between treatment conditions. Beetles that emerged from beans that had other eggs but failed to produce bean mates were excluded from analysis. Time constraints for processing the experimental individuals resulted in insufficient samples for the competition treatments. Nevertheless, we proceeded to analyze and present the data we obtained.

We ran a linear mixed model to test for the effect of female size on clutch size. Female mass, competition treatment (without regard for competitor sex) and the interaction between female mass and treatment were the independent variables. We found no effect of treatment and no interaction, but a positive and significant effect of female mass on clutch size ($F_{3,45} = 3.11$, $P = 0.03$). Therefore, in subsequent analyses, we corrected clutch size by female mass by using the residuals of a linear regression between these two variables.

I - The effects of larval competition on female mating and reproductive behavior

For females, the effect of competition treatments including treatments with only female bean mates, only male bean mates, or no bean mates, on female mass ($F_{2,48} = 0.6776$, $P = 0.5126$; Figure 1a and Table 1), latency to mate ($F_{2,49} = 0.5613$, $P = 0.5741$; Figure 1b and Table 1), female latency to kick males during copulation ($F_{2,48} = 0.8551$, $P = 0.4316$; Figure 1c and Table 1), and clutch size controlled for female mass ($F_{2,47} = 0.24$, $P = 0.78$; Figure 1d and Table 1) were measured. Competition did not have a significant effect in any of these traits. We did not test for differences in female copulation

duration as this variable appears to be primarily under male control (Wilson and Tomkins 2014). Therefore, our results demonstrated that female mating behavior was not affected by larval competition.

II - The effects of larval competition on male mating behavior

Within the male group, the competition treatments included only female bean mates, only male bean mates, mixed sex bean mates, or no bean mates. The independent variable was treatment group and the dependent variables were male mass ($F_{3,44} = 0.9007$, $P = 0.4485$; Figure 2a), latency to mate ($F_{3,46} = 2.2398$; $P = 0.0963$; Figure 2b), and copulation duration ($F_{3,46} = 0.0996$, $P = 0.9598$; Figure 2c). Larval competition had no significant effects on any of these variables.

III - The effect of larval competition on clutch size

A One-Way t-test was run to test for an effect of general larval competition on clutch size. Here we compared the effect of presence versus absence of competition without regarding the sex of the competitors, as competitor sex is not predicted to affect clutch size. We did not find a significant effect ($t_{47} = 0.45$, $P = 0.51$, Figure 3).

Discussion

None of our hypotheses were supported by our data. Larval competition had no effect on immediate female clutch size. Moreover, the sex ratio during development had no effects on reproductive behaviors in females or males. Latency to kick for females, latency to mate for males and females, and copulation duration for males were all unaffected.

There are multiple reasons for our failure to detect significant effects. First, it is possible that the effects simply do not exist. This could be true for one, a combination, or all of the behaviors examined. It is possible that the need to alter these behaviors plastically based on larval competition is simply not there. Even if the developmental environment does indeed have an impact on other future behaviors, it may not be beneficial for male or female beetles to adjust their reproductive behaviors based on it. This is because the developmental environment within one bean is rarely representative of the social environment during mating in either sex ratio or number.

Our failure to find an effect of general competition on immediate clutch size seems to be in disagreement with past studies that found lifetime clutch size to be reduced under such circumstances (Colegrave 1993; Credland et al. 1986; Creighton 2005; Schade and Vamosi 2012; Vamosi 2005). However, it is possible that larval competition only impacts lifetime fitness (Vamosi 2005) and not immediate fitness by simply reducing the total number of eggs available to be laid or by reducing female lifespan subsequently limiting time available to oviposit but having no effect on oviposition rate.

Additionally, we failed to find an effect of sex ratio during larval competition on clutch size. Females from beans with only other females did not have significantly smaller clutch sizes than any other larval competition or control group. This result is not supported by Yanagi et al. (2013) who found that egg size increases under larval competition and with our previous finding that a trade-off between egg and clutch size exists so therefore we should expect to see a decrease in clutch size. Since females are expected to increase egg size at the cost of clutch size when there are higher levels of competition, we expected that a higher amount of females during development would serve as an indirect measure of increased future larval competition. Our failure to find this could be because only lifetime (Vamosi 2005), and not immediate, clutch size is affected.

Moreover, our failure to detect an effect of male-biased larval competition on females' latency to kick also does not coincide with past research (van Lieshout et al. 2014; Wilson and Tomkins 2014). For instance, past studies have found that females kick sooner, indicating non-preference, when more males are present immediately before or during copulation (van Lieshout et al. 2014; Wilson and Tomkins 2014). It is possible, however, that the effects of the social environment immediately before or during copulation on behavior overrides the effects of the social environment during development. This would make sense as those environments closest in time to mating are the most relevant to females when they are deciding how choosy to be.

Finally, our prediction that male copulation duration would be higher for males from male-biased developmental bean environments as opposed to isolated, mixed sex,

or female-biased environments was not upheld. Once more, this contrast with past research that found that longer copulation means more ejaculate transfer, which would be adaptive under such increased sperm competition (Edvardsson and Canal 2006). However, another study called that finding into question as they did not find any increase in ejaculate transfer when rival males were present during copulation (Wilson et al. 2014). If this second study is correct and male ejaculate size does not increase with longer copulations, then even if males did perceive an increase in sperm competition in male-biased beans, altering copulation duration would not be an effective way of increasing their competitiveness. But, as Wilson et al. (2014) only presented rival males after copulation had begun, future research would need to check to ensure that this is not simply the result of being exposed to the social-sexual environment for too short a length of time. Other research seems to be in line with our finding as well. For instance, some studies have found that the sex ratio of the adult sociosexual environment has no impact on copulation duration (van Lieshout et al. 2014; Wilson and Tomkins 2014). Again, if ejaculate amount does not increase with longer copulation duration, this makes sense as we would not expect to see behaviors that favor unnecessarily increased mating length.

Possibly the most obvious suggestion for why our hypotheses were not supported and for the various inconsistencies with past research is that our sample sizes were simply too small to detect any existing effects. Because we analyzed the data by dividing up the treatment group into subgroups based on sex ratios, we severely reduced each group's sample size. Although we could have pooled all the competition

individuals together, this could have caused us to miss patterns in the data as we have opposite predictions for mating behaviors based on the developmental sex bias. As is, we did not have enough statistical power. We recommend that future research replicates our study but with much larger sample sizes.

It is also important to note that we were unable to determine egg viability. Therefore, some eggs could have produced larvae that simply did not make it to adulthood. Yet, those larvae could still have had an impact on others. Since we had no way of checking for or sexing larvae, it is possible that our competition sub-groups are inaccurate. For instance, a male put into the male only group could actually have been exposed to female larvae as well that simply did not make it to adulthood. Future research should figure out a way to control for this.

Finally, we could have failed to support our predictions because the amount of larval competition in our treatment was not high enough. Once again, since we had no way of knowing which eggs made it to the larval stage, beans that only yielded two adults but had at least three eggs might have only had two larvae as well. If that was the case, perhaps this was not a strong enough manipulation of larval competition to produce any effects.

In the future, studies could be conducted to determine if developmental sex ratio is relevant to changes in any other behavior in *C. maculatus* such as egg size. It would also be interesting to specifically examine how developmental environments affect antennation behavior during matings. In a different vein, researchers could

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explore how, if at all, female and male genitalia are impacted by the sociosexual developmental environment and larval competition in general.

Figures and Tables

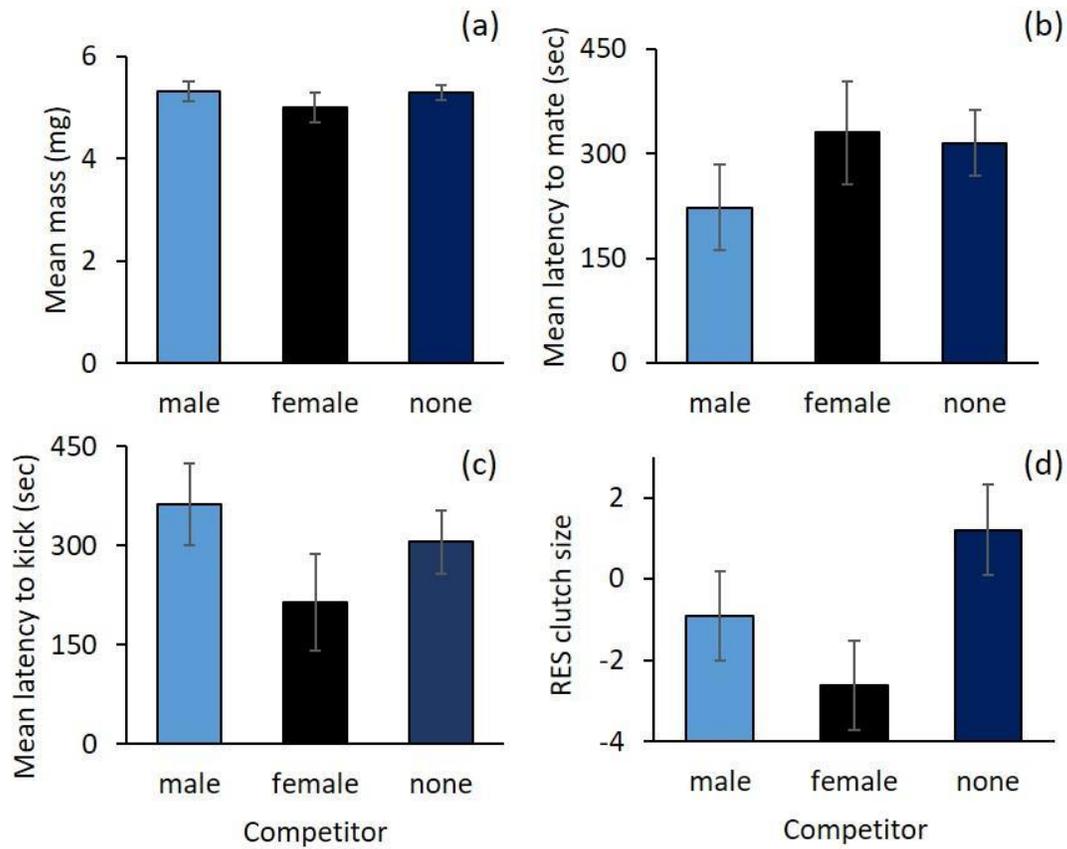


Figure 3.1 – In females, larval competition treatments did not have an effect on (a) mass, (b) latency to mate, (c) latency to kick or (d) clutch size. Competition treatments consisted of competition with males (light blue), females (black), and no competition (dark blue). Means and standard errors are shown.

Figures and Tables

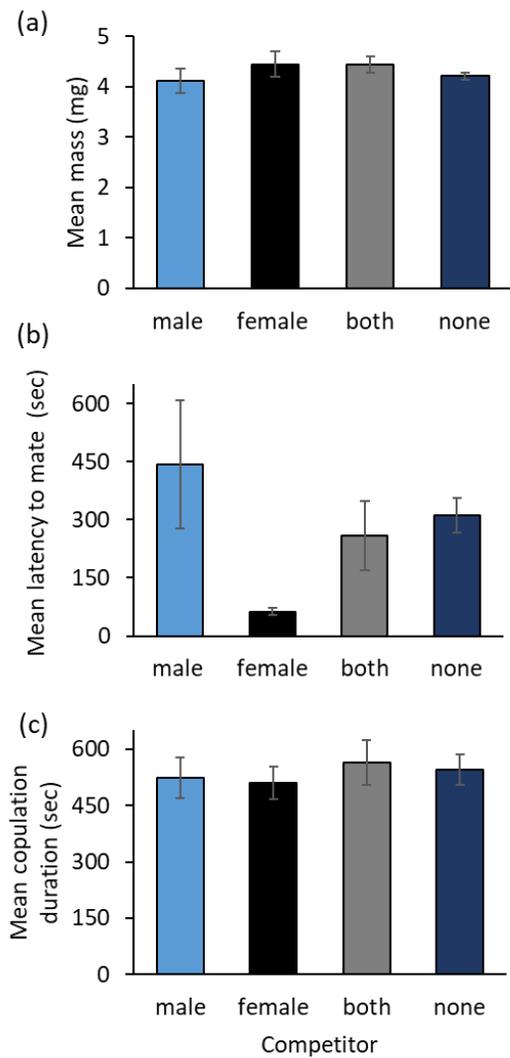


Figure 3.2 – In males, larval competition treatments did not have an effect on (a) mass, (b) latency to mate and (c) copulation duration. Competition treatments consisted of competition with males (light blue), females (black), both (gray) and no competition (dark blue). Means and standard errors are shown.

Figures and Tables

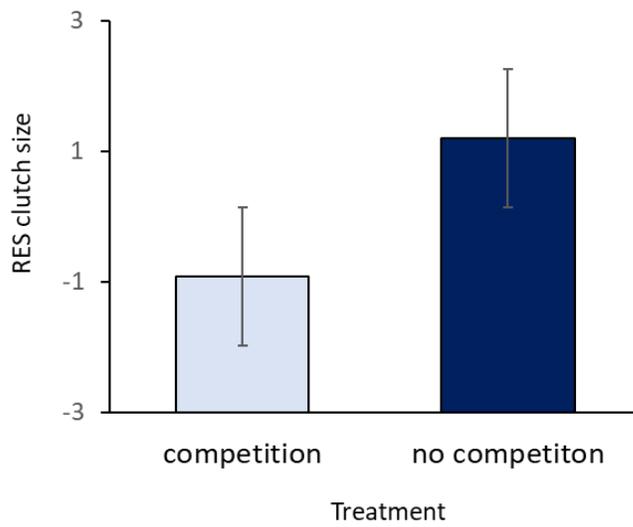


Figure 3.3 – Female clutch size did not vary significantly in the presence (light blue) or absence (dark blue) of larval competition. Means and standard error bars are shown.

Figures and Tables

Table 3.1 – One-Way ANOVAs testing for differences in females for (1) mass, (2) latency to mate, (3) latency to kick and (4) clutch size according to larval competition treatment.

| 1- Source of variation in female mass | | | | | |
|---|-----------|-----------|-----------|----------|----------|
| | df | SS | MS | F | P |
| Competition treatment | 2 | 0.89 | 0.45 | 0.68 | 0.51 |
| Error | 48 | 31.71 | 0.66 | | |
| Total | 50 | 32.61 | | | |
| 2- Source of variation in female latency to mate | | | | | |
| | df | SS | MS | F | P |
| Competition treatment | 2 | 71227.6 | 35613.8 | 0.56 | 0.57 |
| Error | 49 | 3108969.1 | 63448.3 | | |
| Total | 51 | 3180196.7 | | | |
| 3- Source of variation in latency to kick | | | | | |
| | df | SS | MS | F | P |
| Competition treatment | 2 | 47983.1 | 23991.5 | 0.85 | 0.43 |
| Error | 48 | 1346664.8 | 28055.5 | | |
| Total | 50 | 1394647.9 | | | |
| 4- Source of variation in RES clutch size | | | | | |
| | df | SS | MS | F | P |
| Competition treatment | 2 | 125.53 | 62.765 | 0.25 | 0.78 |
| Error | 47 | 11712.28 | 254.61 | | |
| Total | 49 | 11837.81 | | | |

Figures and Tables

Table 3.2 – One-Way ANOVAS testing for differences in males for (1) mass, (2) latency to mate and (3) copulation duration according to larval competition treatment.

| 1- Source of variation in male mass | df | SS | MS | F | P |
|---|-----------|-----------|-----------|----------|----------|
| Competition treatment | 3 | 0.63 | 0.21 | 0.91 | 0.45 |
| Error | 44 | 10.21 | 0.23 | | |
| Total | 47 | 10.84 | | | |
| 2- Source of variation in male latency to mate | df | SS | MS | F | P |
| Competition treatment | 3 | 515342.1 | 171781 | 2.24 | 0.09 |
| Error | 46 | 3527982.3 | 76695 | | |
| Total | 49 | 4043324.5 | | | |
| 3- Source of variation in copulation duration | df | SS | MS | F | P |
| Competition treatment | 3 | 11682.9 | 3894.3 | 0.09 | 0.96 |
| Error | 46 | 1797680.6 | 39080.0 | | |
| Total | 49 | 1809363.5 | | | |

CHAPTER 4

Conclusion and future directions

Results suggest that the specificity of the social environment matters in regards to its influence on reproductive behavior. In this study, we found that only male harassment had an impact on reproductive behavior, namely the disappearance of the trade-off between egg and clutch size, reduction in clutch size, and loss of advantageous oviposition site preference. In sharp contrast, larval competition had no influence on any examined reproductive behaviors. These included female clutch size, female latency to kick during mating, male's latency to mate with females, and male copulation duration.

This was in contrast to our predictions that stated that there should be a change in the level of behavioral plasticity in response to both male harassment and larval competition. One explanation is that there may be a time element to behavioral plasticity where only the social environment present within a certain time frame of a behavioral display is relevant. Since male harassment occurs right around and during the time where reproductive behaviors are occurring, it is possible that this has a stronger influence on such behaviors than does the more temporally distant developmental environment. This would make evolutionary sense as the sex ratio and level of competition within a bean is not necessarily representative of the social environment during reproduction and would likely not exert a selective pressure. In contrast, the level of male harassment is a representation of a specific aspect of the social environment that occurs during reproduction serving as a force of selection whereby individuals that are successfully able to adjust their behavior have more offspring (this study; Janzen 1977; Kawecki 1995; Yanagi et al. 2013). For instance,

females that choose to oviposit on non-toxic beans over toxic beans produce more offspring that make it to adulthood (this study; Janzen 1977).

However, it is also possible that we did not have an accurate enough representation of larval competition to induce any changes in behavioral plasticity. Since we were unable to account for competition from larvae that failed to make it to adulthood, many individuals may have been grouped in the wrong competition treatment. In this instance, any existing plasticity in clutch size and mating behavior may have gone undetected. Furthermore, even if most larvae did make it to adulthood, many beans had only two emergences. Based on past studies, this is likely not a strong enough measure of competition to select for reproductive behavioral plasticity (Colegrave 1993). This accounts for the fact that, unlike various studies (Credland et al. 1986; Vamosi 2005; Yanagi et al. 2013), we found no effect of larval competition on reproductive behavior.

Finally, our study, in conjunction with other studies, indicates that the social environment can inhibit, in addition to promote, reproductive plasticity. Past research has found that the social environment often promotes plasticity (Almohamad et al. 2010; Berglund 1994; Creighton 2005; Crowder et al. 2005; Dur et al. 2012; Guedes and Yack 2016; Humfeld et al. 2009; Kar and Ganguli 2016; Kiflawi et al. 2003; Kishi and Tsubaki 2014; Koch 2005; Moretz et al. 2008; Resetarits and Wilbur 1991; Rodd et al. 1997; Sato et al. 1999; Templeton and Shriner 2004; van Leishout et al. 2014; Wilson and Tomkins 2014; Yanagi et al. 2013; Yoshioka et al. 2012). For instance, one study found that, when controlling for body mass, female bean beetles lay larger eggs at

higher larval competition levels (Yanagi et al. 2013). Additionally, female bean beetles have been found to kick sooner during mating indicating their resistance when more males are present during or before copulation (van Leishout et al. 2014; Wilson and Tomkins 2014). In turn, a few studies have found that the social environment can limit plasticity (Gay et al. 2009; Gibbs et al. 2005). This is further supported by our study where the initial trade-off between egg and clutch size was not maintained under male harassment as a result of a decrease in clutch size. Also, our study found a loss of adaptive oviposition site choice as a result of male harassment.

That being said, the exact mechanisms of reproductive behavioral plasticity gain or loss in many species, including *C. maculatus*, are relatively unknown and should be examined further. Additionally, it would be of interest to determine the ways in which other forms of behavioral plasticity other than reproductive plasticity are impacted by the social environment.

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