

THE EFFECTS OF SHORT-TERM STARVATION AND SUBSEQUENT RE-FEEDING IN
THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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Chase Ullman

Advisor: Dr. Laura Sirot

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ABSTRACT

Starvation is a common phenomenon that negatively affects many organisms' ability to reproduce. Many studies have addressed the negative effects caused by starvation on male fitness specifically in *Tribolium castaneum*. However, few studies have addressed whether the consequences of short-term starvation on male fitness are reversible. In this study, I focus on the effects of short-term starvation and subsequent feeding on male reproductive success in *T. castaneum*. Mating behaviors measured in this study consisted of the average number of mounts per female, number of mated females, latency from mount to female quiescence, female quiescence duration, and insemination success. My results indicate that starvation negatively affected a male's ability to obtain a female mate. Upon re-feeding, male beetles were able to recover their ability to mate with females. Thus, this study has provided evidence suggesting that recovery of mating ability through re-feeding after starvation does occur in *T. castaneum*. The results of this study help further our knowledge of how nutritional stress and re-feeding affect male reproductive success. A mechanism for how males are able to recover from periods of starvation is an interesting future direction.

INTRODUCTION

Effects of Starvation on Reproductive Processes

It is very common for organisms to experience periods of nutritional stress in their natural environments. Nutritional stress is an environmental pressure that causes a response from an organism in order to survive. An organism's survival is dependent on its ability to respond to fluctuating food conditions in nature. Organisms rely on gaining energy through resources in order for them to reproduce, grow, maintain, store, and acquire more resources. Food availability fluctuates and is often unpredictable in the wild (Wang et al. 2006; Rion and Kawecki 2007). An organism will not be able to survive in the wild without access to necessary resources or a mechanism of dealing with fluctuations in resource availability (McCue 2010). Effects of nutritional stress on males include decreased attractiveness, testis size, and total sperm transferred (Hellriegel and Blanckenhorn 2002; Fedina and Lewis 2006). Females are also affected by nutritional stress which results in decreased attractiveness, decreased gonad size, failure to produce eggs, and decrease in offspring produced (Pierce and Ferkin 2005; Perez-Staples et al. 2007; Sbilordo et al. 2011). However, nutritional stress can increase the longevity of organisms (Kolss et al. 2009). Thus, nutritional stress has costs and benefits to an organism's ability to reproduce and sire offspring.

The effects of starvation can have a significant impact on mating processes resulting in a decrease in the male's lifetime fecundity. Nutrition levels during larval development and/or adulthood can have significant impacts on these various mating processes (Engels and Sauer 2007). Mating processes affected by starvation can include latency to sexual maturation, mate choice, courtship, copulation, sperm transfer and storage, and the number of offspring reared. In tephritid fruit flies, post-teneral diet plays an integral role on their sexual development, reproductive performance and their survival (Perez-Staples et al. 2007). Tephritid fruit flies must access sufficient amounts of protein while developing in order to avoid a decrease in these areas of importance. Within the Tephritidae family are Mediterranean fruit flies, in which studies have shown that nutritionally stressed males are less likely to copulate and also experience shorter copulation durations. Females were also found to store less sperm when mating with nutritionally deprived males compared to females whom mated with normally fed males (Yuval et al. 2002). In the closely related Queensland fruit flies, similar results were found as flies whom were deprived of sufficient proteins experienced a significantly lower mating probability as well as experience shorter copulation durations (Perez-Staples et al. 2007). Male fitness is dependent on courtship behavior, which can also be affected negatively by a period of starvation.

Male courtship is vital for obtaining mates in order to reproduce but is often negatively affected by starvation. In *Panorpa cognata*, courtship is negatively affected by nutritional stress as poor conditioned males show a decrease in calling

activity for mates. Moreover, under normal resource conditions, males were able to reach sexual maturity at younger ages, the frequency of mating increased, copulation duration increased and female reproductive outputs increased compared to food-deprived male scorpionflies (Engqvist and Sauer 2003). Similarly, a study on the scorpionfly *Panorpa vulgaris* found that both larval nutrition and adult nutrition had impacts on different mating processes (Engels and Sauer 2007). Food availability during their larval stage ultimately had a factor in the condition and body weight achieved as an adult. Normally fed larvae experienced more desirable phenotypic condition and had larger body weights as adults. Food availability during the larval stage has a major effect on the male's fitness because larger male body weight is correlated to larger salivary gland weight, which produces masses offered to females before copulation. Thus, courtship is an important aspect to a male's reproductive success.

The effects of starvation are not always permanent as studies on *Drosophila melanogaster* indicate that effects of nutritional stress on male reproductive processes are potentially resistable. Starvation resistance results in no phenotypic plasticity during a period of starvation. Meanwhile, reversing the effects of starvation would result in a phenotypic change during the stress period and a return to normal function following the period of stress. *Drosophila melanogaster* has been found to be able to resist starvation (Hoffmann and Harshman 1999). An increase in the lipid content in adults of the *Drosophila* species resulted in an increased starvation resistance. Another factor contributing to this resistance is an increase in

body size, which allows for an increase in energy storage compounds within the organism. However, there are direct trade-offs between starvation resistance and the life history traits of organisms. For instance, a study found that energy reserves in *D. melanogaster* may be used for reproduction or survival under stressful conditions such as starvation (Djawdan et al. 1996). A stronger resistance to starvation can alter developmental time, larval viability, and change patterns of early fecundity (Hoffmann and Harshman 1999). The ability of *D. melanogaster* to resist starvation suggests that the effects of starvation may be reversible in at least some organisms.

Are the Effects of Starvation Reversible?

An organism's ability to reverse the negative effects of starvation is vital for their individual survival and fitness. These negative effects can have a direct impact on an organism's physiological and life history traits. Karan and David proposed that over an evolutionary timescale, populations respond to environmental stresses such as starvation by evolving a capacity for allowing an organism to show phenotypic plasticity during stressful periods in *Drosophila* (Karan and David 2000).

Phenotypic plasticity induced by starvation and subsequent feeding has been shown in many studies Aphidae family. It has been shown that under periods of short-term nutritional stress, females are able to arrest or resorb their oocytes (Brough and Dixon 1990). Starved aphids that were subsequently fed had increased reproductive rates immediately upon being fed. However, starvation reduced the

aphids overall lifetime fecundity. This is due to the trade off of maintaining the normal rate reproduction by resorption of oocytes during nutritionally stressed periods (Xu et al. 2012). The aphids ability to tolerate periods of starvation via phenotypic plasticity is an important characteristic affecting their overall fitness. Many other insects have the capability of oosorption suggesting that they are able to survive periods of stress and regain functions that benefit their overall reproductive success (Bell and Bohm 1975; Brough and Dixon 1990).

Organisms can also alter internal functions while experiencing starvation periods, which affect their development, behavior, and metabolism. For example, the American cockroach *Periplaneta americana* are able to adjust their midgut cell proliferation activity based on nutritional stresses (Park and Takeda 2008). These organisms can metabolically shut down cell proliferation when experiencing starvation and upon subsequent feeding they can increase cell proliferation. In rats, the reintroduction of food was able to reverse negative effects associated with starvation (Boza et al. 1999). Both metabolic variables and intestinal barrier integrity were able to repair rapidly during a three day refeeding period. Organisms have differing mechanisms activated by subsequent feeding following periods of starvation, which affect different aspects of their biology. The reversibility of the effects of starvation on mating processes is a topic that has not yet been thoroughly investigated. Therefore, the focus of my study was to test whether the negative effects on male fitness caused by starvation could be reversed via subsequent

feeding in the red flour beetle, *Tribolium castaneum*, by comparing several aspects of male reproductive success between males that are fed, starved, and re-fed.

***Tribolium castaneum*: A model system for the study of male reproductive success**

Tribolium castaneum is a widely used model organism to study sexual selection. Intense research of the red flour beetles mating behaviors and sexual selection processes is a result of *T. castaneum*'s importance as a pest and the desire to counter any economic impacts that may result from its increase in fitness. The life history of *T. castaneum* shows phenotypic plasticity in response to changes in abiotic conditions such as temperature, humidity, and food (Ziegler 1976). It is common for red flour beetles to undergo periods of food stress, as they live in a wide variety of food sources that fluctuate in nutritional value and because their populations live in widely separated grain habitat patches. Recent studies have found that under certain conditions, in particular starvation, *T. castaneum* fitness can be negatively affected (Fedina and Lewis 2006; Sbilordo et al. 2011; Lewis et al. 2012). There is a decrease in the amount of sperm stored by females when mating with starved males. Starvation in males also leads to decreased sperm transferred to the female beetles. However, no research has yet been done to explore whether the observed consequences of starvation for male fitness can be reversed in *T. castaneum*.

Sexual Selection

Like in many insect species, sexual selection plays a critical role in the ability for an individual red flour beetle to mate and increase its fitness. Sexual selection arises in response to intersexual mate choice and/or intrasexual competition. Beetles choose mates based on the condition, courtship behaviors, and other aspects of the opposite sex. Intrasexual competition arises when competing for access to the opposite sex. Sexual selection can be divided into three different sub categories: pre-copulatory selection, peri-copulatory selection, and post-copulatory selection.

Pre-Copulatory Sexual Selection

Pre-copulatory sexual selection covers specific traits and behaviors that coincide with mate acquisition. In *T. castaneum*, pre-copulatory sexual selection occurs at two levels, long-range and close-range. In the long-range, chemical signals are thought to be the mechanism for attraction of females rather than visual and acoustic signals (Arnaud and Haubruge 2002). Given that *T. castaneum* inhabit grains and cereals, there is very little light availability leading researchers to suggest that visual signals play little to no role in mate attraction. There has also been no evidence to suggest that acoustic signals play a role in mate attraction as well. In order for *T. castaneum* to attract and locate potential mates, it is commonly thought that males produce chemical signals. In particular, males produce the hormone 4,8-dimethyldecenal (DMD), which attracts both male and female beetles (Arnaud and Haubruge 2002). *Tribolium castaneum* females may use these chemical signals in order to assess potential mates (Lewis et al. 2012). While long-range attraction

plays an integral role in mate acquisition, close-range encounters are equally important.

In close-range encounters in *T. castaneum*, cues such as chemicals, body size, and courtship behaviors may help to attract potential mating partners. In male *T. castaneum* have been found to prefer to mate with females that they have not mated with before rather than mate with the same female multiple times (Arnaud and Haubruge 1999). Moreover, males prefer to mate with mature females over immature females, and are more likely to choose a virgin female mating partner. Thus, male close-range encounters are likely due to the females sexual life.

Mate choice is not limited to only male beetles as it differs for female beetles during close-range encounters. Female *T. castaneum* are thought to exhibit mate choice by deciding if a male is allowed to begin mating or not. Females exhibit different behaviors in order to avoid copulating with a male beetle. They may choose to move away from a male attempting to mount, move backwards and cause the male to slip off, or after the male mounts, dislodge the male by quickly moving away (Pai and Yan 2003). These mechanisms for avoiding copulation are effective in females as only half of all male copulation attempts result in successful intromission (Lewis and Iannini 1995). Thus, pre-copulatory behaviors are useful in exploring sexual selection in *T. castaneum*.

Reproductive Anatomy and Peri-copulatory Sexual Selection

Peri-copulatory sexual selection covers mechanisms and behaviors that are used during copulation by each sex in order to increase fitness. Knowledge of *T. castaneum* reproductive tracts for both males and females and stages of sperm transfer and storage is essential to understanding peri-copulatory sexual selection within the species. The male reproductive system consists of two pairs of male accessory glands, which include short bean-shaped glands and long tubular glands (Fedina and Lewis 2008). These accessory glands produce spermatophores consisting of a membranous sperm containing sac (Bloch Qazi et al. 1996). During copulation, the male spermatophore is deposited into the female's bursa copulatrix, the site of short-term storage, where sperm is released from the spermatophore. The majority of sperm is expelled from the bursa shortly after copulation. The remaining sperm travels to the female's spermatheca, the site of long-term sperm storage, or remains in the anterior bursa of the female. While it takes an hour for sperm quantity in the spermatheca to stabilize, only 4% of the initial sperm transferred from the male beetle end up being stored in the spermatheca (Bloch Qazi et al. 1996). The female is able to use the sperm stored in the spermatheca for fertilization of their eggs for up to 140 days after mating (Bloch Qazi et al. 1996). Since behavioral interactions occur within female reproductive tracts, the basis of their reproductive anatomy helps to explain peri-copulatory sexual selection.

Several different behavioral patterns arise during copulation in *T. castaneum* that can affect sperm transfer, storage, and use. Male beetles display a leg rubbing behavior, which can increase the chances of females allowing sperm transfer during

copulations (Evardsson and Arnqvist; Bloch Qazi 2003). However, not all copulations end in success. It has been found that only 85% of copulations with virgin females and around 80% of copulations with previously mated females result in successful transfer of sperm (Fedina and Lewis 2006).

Several studies have suggested that females also play an active role in determining which male can fertilize their eggs via different mechanisms during copulation (Bloch Qazi 2003; Sbilordo and Martin 2014). In *T. castaneum*, female quiescence is thought to be a mechanism for cryptic female choice. Female quiescence is described as when a female stops moving during a copulation. Sperm transfer is most likely to occur during a period of female quiescence (Bloch Qazi 2003). Further, quiescence durations and number of sperm transferred during copulation are directly correlated. Thus, quiescence may be a mechanism for a female to discriminate against lower quality mates.

Post-Copulatory Sexual Selection

Tribolium castaneum females are highly promiscuous and obtain many mating partners over their lifetime, which in turn makes it an attractive organism to explore the role of male sperm competition and female cryptic choice. The first male to mate with a female has an increased chance of a successful fertilization if the female does not mate with another male relatively quickly (Arnaud et al. 2001). When different males' inseminations are separated by time, there is a reduction in sperm mixing in the bursa due to sperm traveling to the spermatheca (Arnaud et al.

2001). When male inseminations are not separated in time, sperm mixes in the bursa resulting in heterogeneous mixing of sperm in the spermatheca. This is a major finding in the post-copulatory sexual selection of red flour beetles because their results mean that the longer the time between each mating, the less chance the last male has in fertilizing the female's eggs. Males have been observed defending their own sperm against other males by prolonging their copulation time and/or mate guarding after copulation has occurred (Fedina and Lewis 2008).

Cryptic female choice also plays a major role in post-copulatory sexual selection in the form of re-mating. This is accomplished by exerting control over sperm transferred during peri-copulation, and is a mechanism used for cryptic female choice in which the female prefers males in better phenotypic condition (Fedina 2007). Females were found to maximize offspring quality by mating only with higher quality mates in comparison to their previous mates. Since male phenotypic condition can be negatively manipulated by starvation, females who mated with starved males would pursue mates of better condition. Thus, starved males will experience a decrease in their reproductive fitness.

Starvation and Nutrition Effects in *Tribolium castaneum*

Nutritional stress has been shown to affect various phases of reproduction including mating behavior in the red flour beetle. The effects of nutrition on male reproductive performance show that males on low-quality diets have lower mating rates as well as lower insemination success compared to normally fed males (Fedina

and Lewis 2006; Sbilordo et al. 2011; Lewis et al 2012). Additionally, it was found that starved males exhibit decreased leg-rubbing frequencies, as well as shorter copulation times compared to normal males (Fedina and Lewis 2006). This study also focused on female control throughout copulation by using live and dead females. Starved males were mated with both live and dead females and sperm transfer was measured. Live females rejected inseminations and exhibited control based on the male's phenotypic condition.

Female beetles also experience a decrease in reproductive ability when mating with nutritionally stressed males. Female beetles who mated with starved males were less likely to produce eggs (Sbilordo et al. 2011). Moreover, of the females that did produce eggs, far fewer eggs were produced compared to females that mated with normally fed males. This study also observed that eggs of females that mated with starved males contained fewer sperm per egg and eggs of these females were more likely not to contain any sperm. Starvation was shown to result in a reduction of about 47% in the quantity of sperm transferred per spermatophore. Moreover, starved beetles have significantly smaller accessory gland sizes (Fedina and Lewis 2006). With much supporting evidence from previous studies, it can be stated that mating behaviors are negatively affected under periods of nutritional stress in *T. castaneum*.

In this study, I focus on the effects of short-term starvation and subsequent feeding on male reproductive success in *T. castaneum*. Previous research has found that female *T. castaneum* have been able to regain lost fitness abilities due to

subsequent feeding following a short period of starvation (Parthasarathy and Palli 2011). Females were able to induce vitellogenin synthesis and the progression of primary oocytes from the resting stage to the mature stage after being re-fed. This leads to the hypothesis that subsequent feeding following a period of short-term starvation can reverse the negative effects on male fitness associated with starvation. I predict that when starved males are subsequently fed that the negative effects of starvation on the beetles' reproductive success will be reversible. Re-fed male beetles' latency from mount to female quiescence will be similar to fed males and shorter than starved males. Female quiescence duration will be significantly longer in re-fed males than a starved male beetle and similar to that of a normally fed male beetle. Moreover, re-fed male beetles' number of mated females and insemination rate, and average number of mounts will be significantly higher than a starved male beetle and similar to that of a normally fed male beetle.

METHODS

Study Species

Tribolium castaneum beetles used in my experiment were from the Pu11 strain and were obtained from Dr. Yoshi Tomoyasu at Miami University (OH).

Tribolium castaneum is a common pest found all over the globe that spends its life mainly in stored grain and cereal products as well as nuts, dried fruit, spices, chocolate, and yeast. Attributes including short generation time and ease of culture make the red flour beetle an appealing model organism for scientific studies.

Moreover, *T. castaneum* have a high population growth rate, reach maturity fast, develop as larvae fast, and are effective dispersers in stored grains (Mertz 1969). Mating of *T. castaneum* takes place in excavated tunnels within the stored product which they inhabit, and beetles tend to cluster together rather than be randomly distributed among the storage facility (Trematerra and Sciarretta 2004). Adult *T. castaneum* have relatively long life spans with reproductive lifespans of approximately 6-12 months (Nilsson et al. 2002). The beetles' eclose to adulthood after approximately 25 days of development. After eclosion, it takes one to two weeks for adult beetles to sexually mature.

Rearing Conditions

Stocks and beetles were maintained continuously at 30° C in a dark incubator throughout the experiments. All beetles were kept in 16 oz. mason jars and mesh

was used to prevent beetles from escaping. Fed beetles were supplied with organic whole-wheat flour. The beetles were subcultured every other week using #25 sieves in order to separate adults from larvae and pupae, and to prevent cannibalism between adult beetles and pupae. Larvae were placed in their own jar while the pupae were collected and sexed. Shape of the genital papillae were used to determine the sex of each pupa. The papillae of females are much larger those of males and appear to look like two finger-like structures anterior to the pointed urogomphi (Fig. 1). After the pupae were sexed, they were placed into separate containers according to their sex. After eclosion, female beetles were fed with organic wheat flour and were allowed to sexually mature for at least two weeks before experiments began. Male beetles were allowed to sexually mature for at least two weeks after eclosion and then were placed into one of three treatment groups differing in feeding condition.

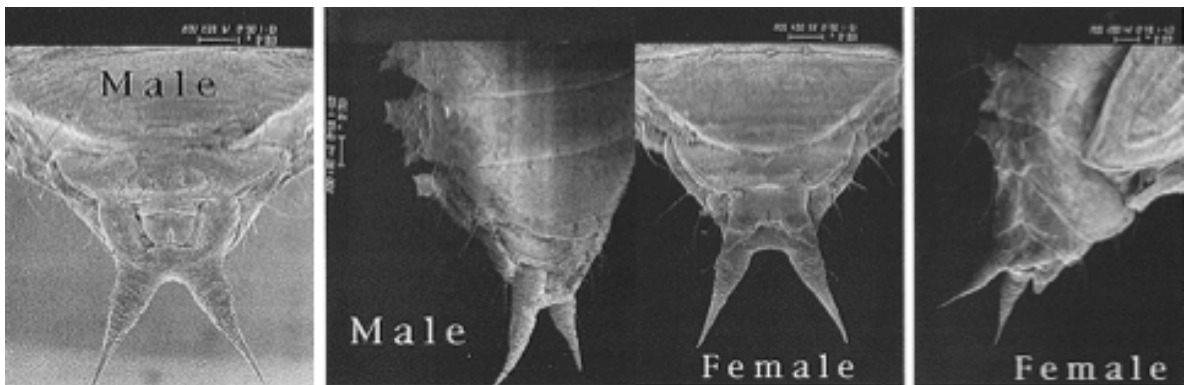


Figure 1. Male and female genital papillae in *Tribolium castaneum* pupae (Photographs from Figure in Beeman et al. 2009).

Treatments

Experimental groups consisted of 25 males for each condition. Each group of males underwent their treatment for 10 days total and each male was placed individually in a 25 x 100 mm test tube. Males in the “Fed” treatment were supplied with standard food for 10 days. Males in the “Starved” treatment were fed for 5 days and then kept without food for the following five days. Males in the “Re-fed” treatment were given standard food for five days after five days of starvation.

Mating Behavior

On the day of the experiment, for each trial, five virgin female beetles were placed into a 3.5-cm diameter plastic petri dish mating arena in a dark room. Individual male beetles were placed into the mating arena and had the opportunity to acclimate for five minutes. Each individual male beetle was placed under a cup in order to keep the beetles from interacting. The cup was then removed and males were allowed to interact for 15 minutes with the females. Each mount attempt was recorded. Latency from male mount to female quiescence was measured from the time the male mounted the female until the female stopped leg movements for a period greater than five seconds. The duration of female quiescence was also recorded. Immediately following a copulation, the mated female was removed and replaced by another virgin female to maintain female density during trials. Each mated female was placed individually in a 1.5 mm eppendorf tube until dissection.

The number of females that the male beetle copulated with during the 15-minute observational period was also recorded.

Dissections and Insemination Success

Dissections of the female occurred 22 to 28 hours after a copulation occurred. Before dissection, each mated female was placed on ice to reduce its mobility. After, female beetles were then placed in phosphate-buffered saline (PBS) on a microscope slide. The females abdomen was pressed lightly using forceps in order make her ovipositor extrude. The ovipositor was then grasped with another pair of forceps and pulled slowly from the female's body. After pulling the female reproductive tract out, both the bursa and spermatheca were torn apart to release potential sperm in a separate drop of PBS. A 22x22-mm coverslip was placed over the sperm and sperm presence was observed under 400x magnification using phase shift. Insemination success was determined by whether sperm was present in the female reproductive tract.

Data Analysis

The effect of male treatment on average mounts per female, the number of mated females, latency from male mount to female quiescence, duration of female quiescence, and insemination rate were analyzed for normality using a one-sample Kolmogorov-Smirnov test. The data for all measures were not normally distributed. Therefore, all data were analyzed using a Kruskal-Wallis one-way ANOVA.

RESULTS

I found an effect of starvation but no effect of re-feeding of males on the average mounts per female. There was a marginally significant difference in average mounts per female between male treatments ($\chi^2= 5.812$; d.f.= 2; $p= 0.055$; Figure 2). Starved males had a significantly higher average amount of mounts per female beetle compared to re-fed male treatments ($U= 189.5$; $Z= -2.390$; $p= 0.017$ one-tailed). However, there were no significant differences in average mounts comparing fed and starved treatments ($U= 360.0$; $Z= 0.924$; $p= 0.178$ one-tailed) and fed and re-fed treatments ($U= 238.0$; $Z= -1.448$; $p=0.074$ one-tailed). Additionally, an effect was found of starvation and re-feeding of males on the number of mated females. There was a significant difference in a number of mated females between male treatments ($\chi^2= 6.742$; d.f.= 2; $p= 0.034$; Figure 3). Re-fed treatments mated with a significantly higher number of females than starved treatments ($U= 444.0$; $Z= 2.589$; $p= 0.010$ one-tailed) and fed treatment mated with a significantly higher number of females than starved treatments ($U= 229.0$; $Z= -1.643$; $p= 0.050$ one-tailed). However, there was no significant difference in mating rate between fed and re-fed treatments ($U= 354.0$; $Z= 0.816$; $p= 0.207$ one-tailed).

I found no effect of starvation or re-feeding of males on female quiescence or insemination rate. There was no significant difference for the average latency from mount to female quiescence ($\chi^2= 2.333$; d.f.= 2; $p= 0.311$; Figure 4). There was no significant difference for the average duration of female quiescence between male treatments ($\chi^2= 1.339$; d.f.= 2; $p= 0.512$; Figure 5). Furthermore, there was no

significant difference in insemination rate across the different male treatments ($\chi^2=1.085$; d.f.= 2; $p= 0.581$; Figure 6).



Figure 2. Number of mounts until successful copulation of fed, starved, and re-fed male *T. castaneum* beetles. Treatments with different letters are significantly different from each other. The heavy line indicates the median, the box indicates the interquartile range and the whiskers indicate all other values. ° denotes outliers; * denotes extreme outliers

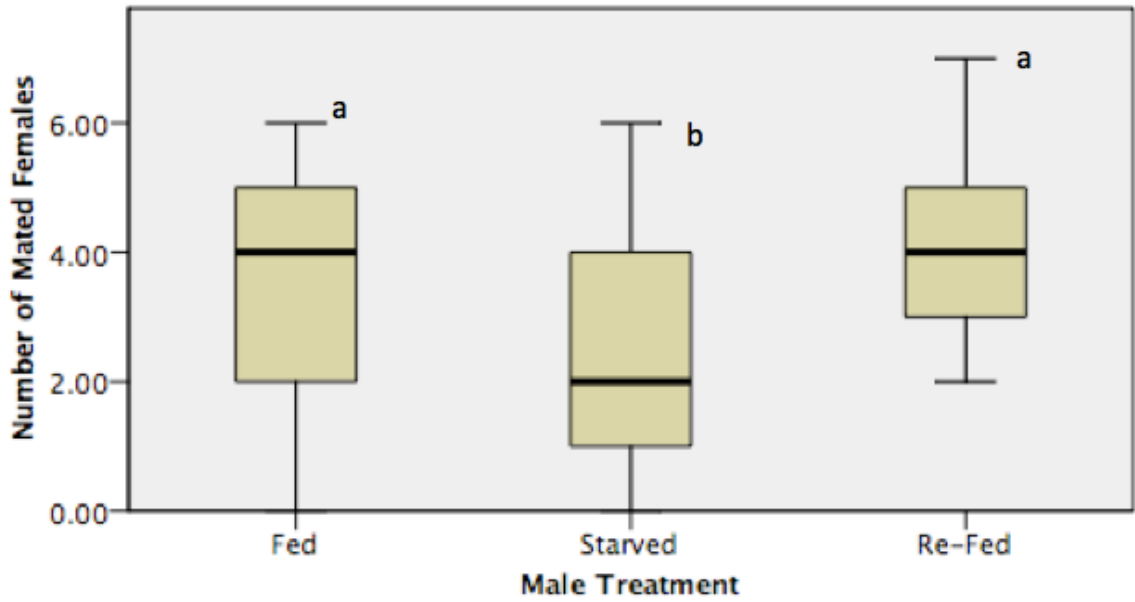


Figure 3. Number of mated females of fed, starved, and re-fed male *T. castaneum* beetles. Treatments with different letters are significantly different from each other.

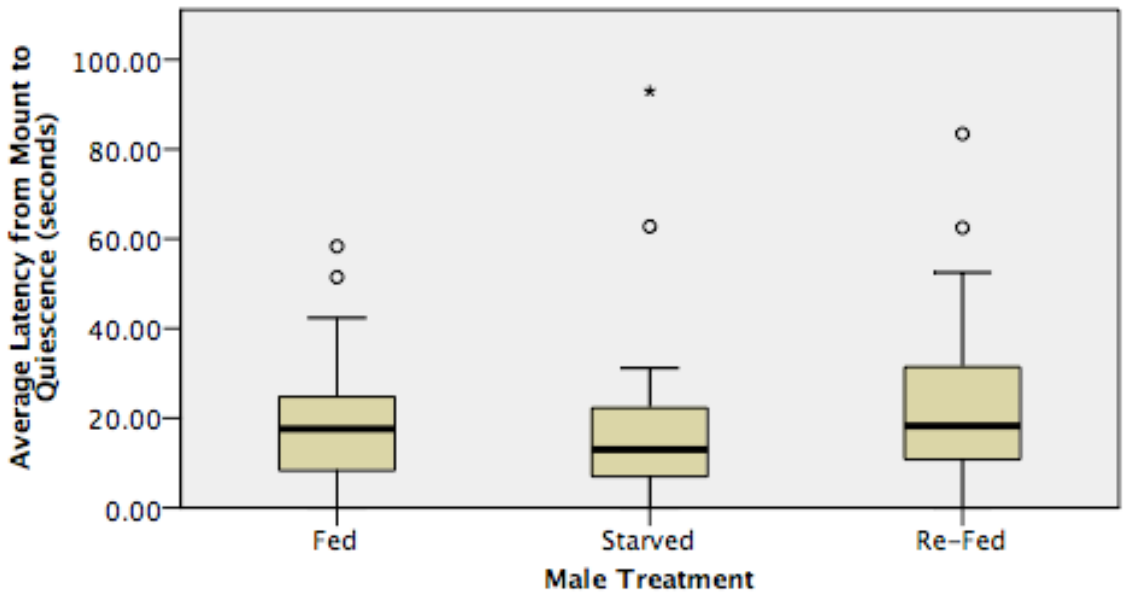


Figure 4. Latency from mount to female quiescence in females mated with fed, starved, and re-fed male *Tribolium castaneum* beetles. ° denotes outliers; * denotes extreme outliers



Figure 5. Duration of female quiescence in females mated with fed, starved, and re-fed male *T. castaneum* beetles. ° denotes outliers; * denotes extreme outliers

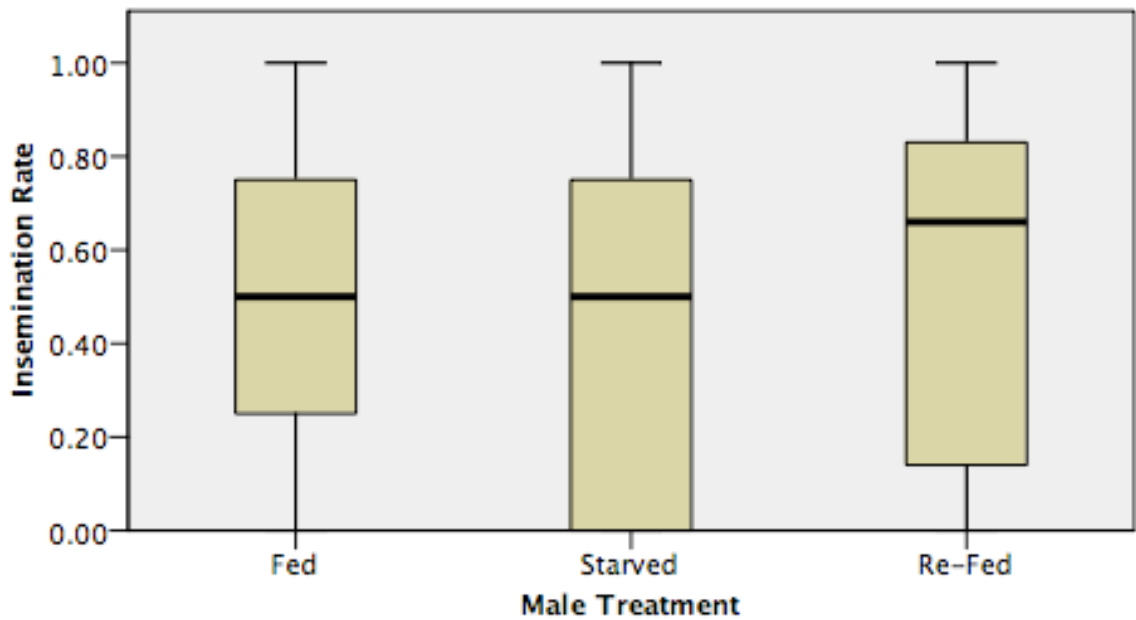


Figure 6. Insemination success of fed, starved, and re-fed male *T. castaneum* beetles. Insemination success was determined by whether sperm was present or not in the female reproductive tract following a copulation.

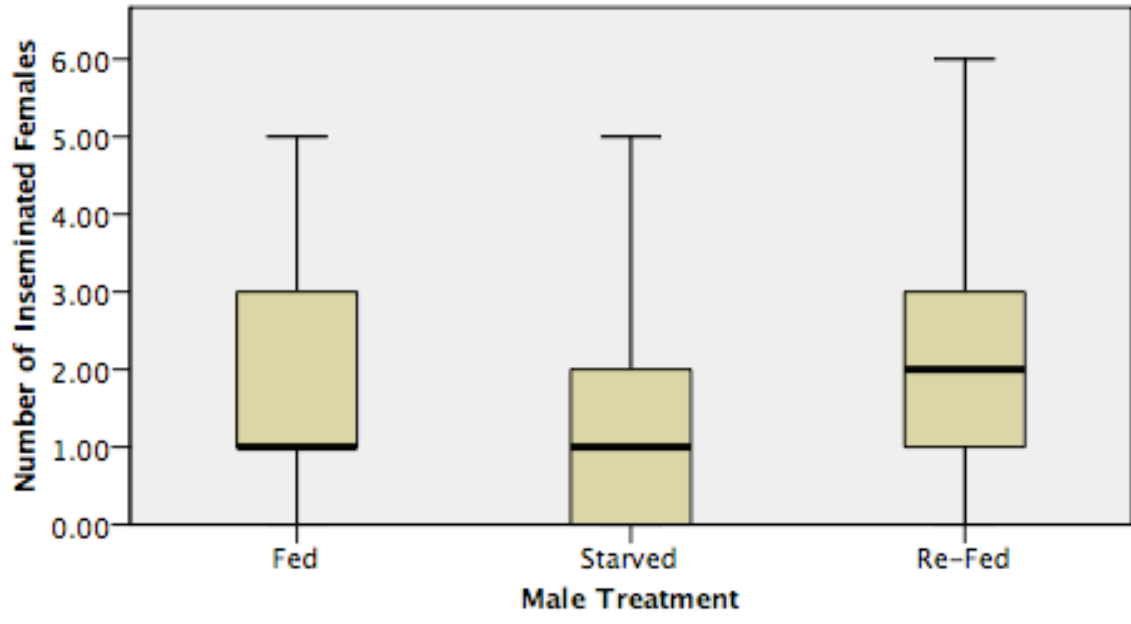


Figure 7. Number of inseminated females that were mated with fed, starved, and re-fed male *T. castaneum* beetles. There were no significant differences between male treatments.

DISCUSSION

I predicted that starvation would reduce the mating and insemination success of male *T. castaneum*. The data collected in this study showed that fed males mated with more females than starved males. However, all other mating behavior and insemination measures failed to show significant differences between fed and starved male. Trends in the data collected suggested that starved males had higher average mounts per female. There was no trend in the data collected for latency from mount to female quiescence or female quiescence duration. Moreover, there was no trend in the data for insemination rate. I also predicted that subsequent feeding following a period of short-term starvation would reverse the negative effects on male fitness associated with starvation. The data collected supported this hypothesis, showing significant differences between starved and re-fed males when measuring the number of females mated. Re-fed males mated with significantly more females than starved males, which suggest that re-fed males were able to regain change in phenotypic condition during starvation.

Pre-Copulatory Reproductive Success

Of the mating behaviors measured in this study, the pre-copulatory behaviors measured were the average mounts per female and the number of mated females. I found no difference between fed males and starved males in the average mounts per female; the data show an insignificant trend that fed males mounted fewer females and performed similarly to re-fed males. This finding is consistent with previous research showing no difference in the frequency of mountings

between fed and starved males (Sbilordo et al. 2011). I also found that starved males had a significantly higher average of mounts compared to re-fed beetles. The trend for the average mounts per female does not support my prediction that fed and re-fed males would mount more females than starved males. Previous research has shown that female choice can occur in which they dislodge a mounted male (Pai and Yan 2003). It is possible that starved males on average mounted more females due to the fact that starved males more consistently fell off of female beetles compared to the other treatments. Thus, they may have needed a higher amount of mounts until they could successfully mate with a female. The number of mated females may be related to the average mounts per female. Therefore, another possible explanation for these results is that fed and re-fed males spent more time mating with females than starved males did, which took away from their total number of mounts.

The number of mated females also showed differences between the male treatment groups. It was found that both fed and re-fed male beetles mated with a greater number of females than starved males, which supported my prediction. Male starvation has been shown to drastically affect reproductive success in *T. castaneum* (Sbilordo et al. 2011). The results of my experiment are supported by previous literature, which has found that starved males mate with fewer females than fed males (Lewis et al. 2012). My data also showed that re-fed males were able to perform similar to the fed beetle treatments. Based on these results, it appears that the re-fed beetles were able to overcome the effects of short-term starvation in terms of obtaining mates.

There are multiple mechanisms for how male beetles were able to overcome periods of nutritional stress and acquire females for mating. One possibility could be that during periods of starvation, males are unable to expend energy during courtship. Thus, they are unable to hold on to females during mounts or keep up with moving females. The reintroduction to a food source could give them the ample amount of energy needed to expend during pre-copulation. Another potential mechanism could include the male's ability to stimulate females during courtship via leg-rubbing. Starved males leg-rubbing frequency is much lower than fed males (Fedina and Lewis 2006). However, the general consensus is that females do not use male leg-rubbing behaviors as a signal of male quality meaning that this behavior does not affect male fitness (Fedina and Lewis 2008). Chemical signaling is another mechanism that could affect the way females assess a male beetle's condition. In *T. castaneum*, males produce chemical signals such as 4,8-dimethyldecanal (DMD), which attracts females (Arnaud and Haubruge 2002). It has been found that starved *T. castaneum* males produce less amounts of DMD than fed males (Hussain 1994). A study using *Tenebrio molitor* found that the chemical signals produced by starved males were less attractive to females (Rantala et al. 2003). This suggests that starved *T. castaneum* males may be less attractive to females due to an inferior ability to produce chemical signals.

According to the results collected in my experiment, it is possible that re-fed males were more attractive to females compared to starved males due to the fact that they were able to obtain more mates. It should be noted that there was no male-male competition in my study since males were alone with the female beetles.

Recovery in attractiveness can be attributed to re-fed beetles' ability to increase pheromone production upon being re-introduced to a food source. A similar pattern occurs in meadow voles. In many rodents, sexual behavior depends on odor attractiveness, proceptivity, and receptivity which are lost during periods of nutritional stress (Pierce and Ferkin 2005). It was found that upon re-feeding, food-deprived female meadow voles all three components of sexual behavior were able to return to normal levels. Interestingly, odor attractiveness took the longest of the components to be affected by starvation. This suggests that odor attractiveness is most important to female meadow voles in obtaining mates. The same could be true in male *T. castaneum* as restoration of DMD production upon re-feeding is a potential mechanism in which male beetles can reverse the effects of starvation.

Another mechanism for reversing the effects of starvation could be altering internal functions in order to improve deteriorated fitness. Cockroaches and rats are able to alter internal functions when undergoing periods of stress (Boza et al. 1999; Park and Takeda 2008). Alteration of these internal functions via control of cell proliferation is signaled during periods of starvation and re-feeding which affects their development, behavior, and metabolism in both organisms. It is possible that male *T. castaneum* are able to increase cell proliferation during re-feeding allowing for increase in metabolic activity. With increased metabolic activity, more energy can be devoted to keeping up with females and obtaining successful matings. Due to starved males producing less DMD than fed males, the site of DMD production in starved males may be damaged. Increased cell proliferation caused by reintroduction to a food source may repair any damages to

glands involved in DMD production. Unfortunately, the sites of DMD production in males are not fully known (Fedina and Lewis 2008). Further inspection of glands responsible for DMD production and its relationship with starvation and re-feeding should be addressed in order to see if this is a viable mechanism for recovering from starvation.

Peri-Copulatory and Post-Copulatory Behaviors

Male treatment did not affect any quiescent behavior displayed by female beetles. While starved males average latency from mount to female quiescence was slightly lower than fed and re-fed males, there was no significant difference between the treatments. I found the same pattern for the average duration of female quiescence. I predicted that fed and re-fed males would have similar results and would differ significantly from starved males. A previous study suggests that female quiescent behavior could be a mechanism for cryptic female choice (Bloch Qazi 2003). Sperm transfer is more likely to occur during periods of female quiescence, and longer quiescence durations are associated with higher numbers of sperm transferred to the female. It should be noted that the quantity of sperm transferred was not measured in my experiment. Female quiescent behavior had never before been looked at as a mating behavior when comparing males of different nutritional qualities. The findings of my study show that male nutrition had no effect on female quiescent behaviors, and therefore were not helpful in determining whether the effects of starvation are reversible in male *T. castaneum*.

Post-copulatory behaviors measured in my experiment were insemination rate and the total number of inseminated females. Both of these behaviors were quantified as sperm being present or not in the female reproductive tract. These behaviors are considered post-copulatory behaviors since it occurs following a mating between a male and female beetle. Insemination rate was not different among the treatment groups and there was no trend in the data. Likewise, the total number of inseminated females was not different among the treatment groups. However, there was a trend in the data that suggested fed and re-fed performed similarly and were inseminating more females than starved males. My results failed to coincide with previous studies that have shown fed males more often transferring spermatophore compared to starved males (Fedina and Lewis 2006). In the previous study, females were dissected immediately following copulations and insemination was recorded. However, in my study dissections to observe whether sperm was present occurred 24 hours after mating. A previous study has found that male spermatophores can be expelled before sperm is released (Fedina 2007). Removal of spermatophore before sperm transfer is complete is common in insects (Eberhard 1996). It is likely that waiting to dissect the female after mating could have resulted in a number of inseminations being compromised via spermatophore expulsion. It is still unknown whether spermatophore expulsion is a mechanism for cryptic female choice in *T. castaneum*.

It is important to note that the presence of sperm or no sperm in the female reproductive tract is not the most efficient way to quantify insemination measures when comparing males of differing condition. Due to limitations of my study, I was

unable to quantify the total sperm transferred during each mating or the size of male accessory glands. A previous study has shown that starved males have significantly higher sperm viability than fed males (Fedina and Lewis 2006). However, fed males transferred significantly more sperm, which resulted in significantly more sperm reaching the spermatheca than starved males. Moreover, fed males had larger accessory gland sizes, which have been found to produce proteins that incapacitate stored sperm from previous matings (Fedina and Lewis 2006). Larger accessory gland sizes also produce larger spermatophores which contain higher quantities of sperm (Fedina and Lewis 2006). These same results were duplicated in a follow up study, which concluded that sperm quantity is more important than sperm quality when successfully inseminating females and accessory gland size plays a crucial role in sperm competition in *T. castaneum* (Sbilordo et al. 2011). It is possible that starvation had the same effect on the male beetle's total sperm transferred and size of the male accessory gland as shown in previous literature. Thus, these behaviors would have been helpful in determining the effects of starvation and re-feeding in male *T. castaneum* and should be assessed in future experiments.

Variation between strains in stress resistance could explain why my results on quiescence and sperm transfer differ from the results of previous studies. The majority of previous research has suggested that fed males are reproductively superior to starved males (Fedina and Lewis 2006; Sbilordo et al. 2011; Lewis et al. 2012). However, another experiment has contradicting results regarding the effects of male nutritional status. In this study, the researchers found that diet had no

significant impact on male reproductive performance (Ming and Cheng 2012). This contradiction is most likely a result of a difference in strains used by the two different research groups. In fact, different strains of the same species such as *Drosophila* have been known to vary in their ability to resist certain stresses (Hoffman and Harshman 2001; Rion and Kawecki 2007). Moreover, differences in population stress resistance have been found in house flies, cockroaches, and ant lions (Hicks et al. 2004; Mira and Raubenheimer 2002; Arnett and Gotelli 2003). Variations in strains may be an explanation for why no significant results were found by this study when comparing fed and starved males. It is very possible that the Pu11 strain used in this experiment has developed the ability to better resist the effects of starvation than the strains used in other experiments. Therefore, male peri-copulatory and post-copulatory behaviors may have not been affected by starvation in this strain.

Beyond Insemination

While sperm transfer mechanisms are important for a male's overall fitness, cryptic female choice also plays a key role. Female cryptic choice occurs after mating with the male. In some species, including *T. castaneum*, females have the ability to regulate and control the amount of sperm that is stored in the spermatheca (Bloch Qazi et al. 1998). Furthermore, females also have the ability to expel the spermatophore shortly after matings take place (Fedina 2007). Re-mating is also a common mechanism of post-mating female choice (Eberhard 1996). Female *T. castaneum* are more likely to accept a male's spermatophore if they had previously

mated with a low quality male (Fedina and Lewis 2007). A male's condition is important to females as they prefer higher quality males (Fedina and Lewis 2006). Male phenotypic quality is based on male body condition, which can be negatively affected during periods of starvation. Due to the fact that females discriminate against male phenotypic condition, a male must be able to either resist starvation or overcome any negative effects associated with starvation.

Compensation

Compensation is a likely mechanism for how male *T. castaneum* can regain phenotypic condition upon re-feeding following short-term starvation. Compensatory growth is a type of rapid growth to compensate for a period of food stress (Metcalf and Monaghan 2001). During periods of starvation, growth rates in organisms typically decline immediately and any available resources are allocated towards maintenance (Dmitriew 2011). With the reintroduction of food, growth rates have been found to quickly recover (Broekhuizen et al. 1994). In rats, re-feeding allowed for regained function growth variables, metabolic variables, and intestinal barrier integrity (Boza et al. 1999). Many fish species have shown the capacity for compensatory growth upon re-feeding following periods of starvation (Ali et al. 2003). Furthermore, a study on the damselfly found that compensatory growth after food deprivation and re-feeding eliminated the effects of starvation on mass and storage molecules (Stoks et al. 2006). Unfortunately, compensatory effects on reproduction have not yet been thoroughly studied. However, compensatory growth and its effect on reproduction were investigated in the grain

aphid. It was found that subsequent feeding allowed aphids to allocate their energetic commitment towards reproduction following periods of starvation (Xu et al. 2012). After the reintroduction of food, reproductive rates immediately improved. While it is common for organisms to show compensation and allocation following periods of nutritional stress, much is unknown addressing compensatory growths relationship with reproduction.

Conclusion and Future Directions

Re-feeding can lead to regaining lost fitness abilities associated with short periods of starvation. While the majority of behaviors measured in this experiment showed resistance to starvation, the male's ability to obtain mates was found to suffer during periods of nutritional stress as predicted. Thus, any evidence supporting the hypothesis that negative effects of starvation are reversible could only be shown through a male's ability to obtain mates. Upon the reintroduction of food, male beetles were able to recover their ability to achieve female mates providing evidence for recovery. This study has provided evidence suggesting that recovery of mating ability through re-feeding after starvation does occur in *T. castaneum*. Since *T. castaneum* are a major pest of stored grains, this study may provide further knowledge that can be applied to pest management of these beetles. Due to the fact that the effects of starvation are reversible in these beetles, the use of insecticides may be the most effective way to control their populations from infestation of stored products.

For future studies, it may be beneficial to conduct this experiment using a strain of beetles that have previously shown to be affected by differences in nutritional condition. Having beetles that are known to be less resistant to starvation effects would allow for re-feeding effects to more easily be detected. Looking at mating behaviors such as copulation duration, male leg rubbing, and the number of sperm transferred and stored by the female may also be useful measures in testing whether re-feeding leads to recovery in *T. castaneum*. Another potential avenue for research could be determining if DMD production can be recovered via subsequent re-feeding after starvation. As shown in this study, re-fed males were able to recover their ability to obtain female partners but it is not yet determined how they recover. Due to the fact that starvation reduces DMD production in males (Hussain 1994), it may be interesting to find if this chemical production is recovered in re-fed males.

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