Is negative density dependence in egg laying by female red flour beetles (*Tribolium castaneum*) based on current or future competition?

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April 30th, 2015

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i. ABSTRACT

Food availability can impact individual fitness and affect development and reproduction of different life stages, where a trade-off can be observed between adults and offspring. When the food source available is used as food and oviposition site, females can decide to give priority to one or the other to improve their fitness and limit competition. Negative density dependence can also influence exploitative competition between adults and therefore change female oviposition decision. Female red flour beetles, *Tribolium castaneum*, use flour as a food source and as an oviposition site from which offspring get their food. I tested two hypotheses to explain the oviposition decisions of females: current competition (among adults) and future competition (among offspring) change with negative density dependence and sex-ratio. I examined the impact of density (10, 20 and 30) and sex-ratio (2:1, 1:1 and 1:2) on the number of eggs laid and on success of offspring. Negative density dependence and sex-ratio affected current and future competition. I found that females make an active choice about how many eggs to lay in a patch to limit future competition by adjusting the number of eggs laid so that more energy will be available for offspring. Results are consistent with the above hypotheses and show that negative density dependence has major impact on female oviposition decisions.

ii. RÉSUMÉ

La disponibilité en nourriture peut affecter l'aptitude ainsi que le développement et la reproduction de différentes étapes du cycle de vie, où un compromis peut être observé entre les adultes et leurs descendants. Lorsque la source en nourriture est utilisée pour la consommation et comme site d'oviposition, les femelles peuvent décider de donner priorité à l'un ou l'autre des aspects afin d'améliorer leur aptitude et de limiter la compétition. La dépendance négative de la densité peut aussi influencer la compétition par exploitation entre les adultes et donc changer les décisions d'oviposition des femelles. Les coléoptères rouges à farine femelles, *Tribolium castaneum*, utilisent la farine comme site d'oviposition et pour la consommation où les descendants se procurent leur nourriture. J'ai testé deux hypothèses pour expliquer les décisions des femelles concernant l'oviposition: la compétition actuelle (entre adultes) et la compétition future (entre descendants) varient avec la dépendance négative à la densité et le ratio des sexes. J'ai examiné l'impact de la densité (10, 20 et 30) et du ratio des sexes (2:1, 1:1 et 1:2) sur le nombre d'œufs pondus et sur le succès des descendants. La dépendance négative de la densité et le ratio des sexes affectent la compétition actuelle et future. J'ai trouvé que les femelles font un choix actif sur le nombre d'œufs pondus dans une parcelle de nourriture en ajustant le nombre d'œufs pondus pour que l'énergie disponible aux descendants soit maximisée. Les résultats sont consistants avec les présentes hypothèses et exposent l'impact de la dépendance négative à la densité sur les décisions d'oviposition des femelles.

iii. ACKNOWLEGEMENTS

I would like to thank William Halliday for all the help and time he gave me throughout the year and for answering my questions, Dr. Gabriel Blouin-Demers for the chance he gave me to work in his lab and to accomplish this project, and the University of Ottawa for the opportunity I had to realize a honours project.

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I. INTRODUCTION

Food availability has major impacts on individual fitness, including effects on offspring allocation (Lalonde, 1991), oviposition rate (Berger *et al.*, 2008; Minkenberg *et al.*, 1992), growth rate (Angilletta *et al.*, 2004), maturation rate (Berger et al., 2008) and success of offspring (Fox *et al.*, 1996), as well as on body size of offspring (Both *et al.*, 1999; Hutchings, 1991). It can also impact different life cycle stages of some species in a way that individuals might need to trade-off food for one life stage over food for another. Adults can choose to decrease their own food consumption to increase the survival rate of their offspring (Lankfort *et al.*, 2001; Plaistow and Siva-Jothy, 1999; Stevens *et al.*, 1999). For example, a trade-off can be observed between development and reproduction of adults, where they can either choose to devote energy towards increasing body size instead of their reproductive organs (i.e. capacity for laying eggs) (Enserink *et al.*, 1995; Stevens *et al.*, 1999). Also, if females use the same type of food as their offspring, they can choose to give priority to search for an oviposition site that would be suitable for their eggs or to their own foraging needs (Liu *et al*., 2010).

In the life cycle of red flour beetles (*Tribolium castaneum*), females need to lay their eggs in flour, from which the offspring and the adult beetles will get their food. There is evidence that females choose how many eggs to lay in a food patch (Campbell and Runnion, 2003; Gottlieb *et al*., 2011). Female choice of clutch size tends to maximise their fitness by decreasing the mortality rate of their descendants (Freese and Zwolfer, 1996) or by changing the intensity of future competition between offspring (Guedes *et al*., 2007). Competition for food between adults and larvae can also modify the female's oviposition decision (Cook *et al*., 1994; Vamosi, 2005).

The amount of energy put towards oviposition is also limited by exploitative competition between adults through negative density dependence (Cody, 1966; Halliday *et al.*, 2015). An increase in competition within adult beetles will provide less energy for each individual, and can therefore decrease the number of eggs laid by females. Therefore, negative density dependence also has to be considered in the context of female oviposition decisions.

For my project, I examined two, non-mutually exclusive, hypotheses to explain the oviposition decisions of female red flour beetles (*Tribolium castaneum*) (i.e. number of eggs laid in a food patch by a female): future competition (negative density dependence among offspring) and current competition (negative density dependence among adults). Future competition should be higher when more eggs are laid because more larvae will hatch and consume the flour. If females take future competition into account when making oviposition decisions, then a sex-ratio more female-biased should lead to a decrease in eggs laid per female because females choose to lay fewer eggs when there are more females. The total number of eggs laid in a food patch should therefore not change as the sex ratio changes. Current competition should increase with an increase in the density of adults in a food patch. As density increases, the per capita number of eggs produced should decrease because less resources per individual are available and consequently females have less energy to put into egg production (Both, 1998; Both *et al.*, 2000; Lack, 1947). I also examined the fitness consequences of female oviposition decisions by measuring the offspring size when the food patch was entirely consumed, and the number of offspring that emerged as adults. If females are responding to changes in sex ratio (minimizing future competition), then larval length and the number of adult offspring should not change with sex ratio. Larval length and the number of adult offspring should both decrease as the number of adults increases (Fox *et al.*, 1996; Kawecki, 1995; Parker and Begon, 1986).

II. MATERIALS AND METHODS

Two hundred red flour beetles were obtained from Carolina Biological Supply Company (Burlington, North Carolina, USA). To enlarge the colony, beetles were kept in a large container with *ad lib* flour (composition described below) in an incubator at 30^{o}C, 70% humidity and 12/12 hours dark/light period. For this project, all manipulations were done in an incubator at the same ambient temperature (30^{o}C) and beetles were placed in 2.5 ml of a mixture of 95% all-purpose wheat flour and 5% yeast for all the experiment. All mention of flour refers to the described mixture above.

I sexed the beetles during their pupae stage based on the presence (female) or absence (male) of ovipositors. Sexed beetles were then left to mature in separate containers. One week following eclosion, I marked each beetle on the back using two different colours of non-toxic acrylic paint to differentiate between the sexes and allow individuals to be used for several treatments.

I placed beetles in a petri dish (8.7 cm diameter) with 2.5 ml of flour (pre-sieved through a 250 μm sieve to aid in egg detection) at three densities (10, 20, and 30 beetles) and three sex-ratios (2:1, 1:1, and 1:2) in a fully factorial design with 10 replicates of each treatment combination. Since a 2:1 sex ratio did not always match a whole number, I used the following ratios: 7:3 at density = 10, 14:6 at density = 20, and 20:10 at density = 30.

Beetles were allowed to lay eggs for two days, at which point I extracted the eggs from the flour with a 250 µm sieve. I counted the number of eggs in each petri dish and put them back in the dish with 2.5 ml of fresh flour. Adult beetles were separated and put back in their respective container. Eggs were then allowed to hatch and develop into adults to evaluate the full fitness effects of competition between adults and competition between offspring.

The dishes with the counted eggs were checked every 2 to 3 days. When the larvae depleted the food patch (i.e. when the flour was becoming brown and larvae could be clearly seen), I took a picture of the larvae in the petri dish for each replicate and randomly measured the length of 10 larvae (if possible) using Image J 1.48v. I then added an additional 2.5 ml of flour so that the larvae did not starve and some adult offspring had the opportunity to emerge. I also calculated the number of days between the moment I put the counted eggs with the new flour in the petri dish and the moment larvae needed more food.

After all pupae became adults, I sifted each replicate with a 500 µm sieve to obtain and count the number of live adults. The dead adults were discarded which represent a more accurate metric of fitness for the parent flour beetles.

I examined the per capita number of eggs produced (the number of eggs laid per female present in the treatment) and the per capita number of adult offspring produced (the number of adult offspring that survived per female) at the different sex-ratios and densities using multiple regression in R (package: stats; function: lm; R Core Team 2014) with sex ratio, density, and their interaction as independent variables in each analysis. I also examined the total number of eggs laid per treatment using the same method. I examined larval length using a linear mixed effect model in R (package: nlme; function: lme; Pinheiro et al. 2014) with larva length as the dependent variable, sex-ratio, density, and their interaction as fixed effects, and replicate per number of larvae as random effect.

III. RESULTS

Female oviposition decisions were influenced by sex-ratio and density (full model *df* = 87, F = 47.83, R^2 = 0.52, $p < 0.01$). The per capita number of eggs produced decreased with a sex-ratio more female-biased (Table 1) and was influenced by negative density dependence; as density increased, the per capita number of eggs produced decreased (Table 1, Figure 1). The total number of eggs laid in a treatment increased with a sex-ratio more female-biased within the food patch (full model *df* = 87, F = 125.6, R^2 = 0.74, $p < 0.01$, Figure 2) as well as with density (Table 1).

The time elapsed before larvae depleted food was unaffected by the sex-ratio (full model: *df* = 87, F = 19.3, R^2 = 0.31, $p <0.01$, Figure 3), but was shorter as density increased (Table 1). Larvae length was regulated by density and the time elapsed before larvae depleted food (full model: *df* = 740, Figure 4), but was not influenced by the sex-ratio (Table 1). Larvae length affected the time elapsed before larvae depleted food (Table 2). The developmental time decreased as larval length increased. Larval length was also affected by the interaction between density and sex-ratio (Table 2). Larval length decreased as density increased with a female-biased sex-ratio (*df* = 183; Table 2), increased as density increased with a male-biased sex-ratio (*df* = 157; Table 2), and larval length was unaffected by density in the 1:1 sex-ratio (*df* = 198; Table 2).

The total number of offspring that emerged as adults was not influenced by either sex-ratio or density (full model: *df* = 87, F = 2.76, R^2 = 0.06, *p* = 0.07, Figure 5; Table 1). The per capita number of offspring that became adults decreased as sex-ratio became more-female biased and as density increased in the food patch (full model: *df* = 86, F = 33.05, R^2 = 0.54, *p* <0.01, Figure 6; Table 1). Perhaps, when there was a combination of 30 beetles in the patch and more females (2:1 sex-ratio), the number of offspring per female increased (Table 1). The per capita number of adult offspring decreased as density increased with a male-biased sex-ratio (full model: *df* = 28, F = 23.78, R^2 = 0.46, *p* < 0; Table 1), decreased slightly less as density increased with a 1:1 sex-ratio (full model: *df* = 28, F = 27.94, R^2 = 0.49, *p* < 0; Table 1), and decreased even less with density for the female-biased sex-ratio (full model: *df* = 28, F = 16.47, R^2 = 0.37, *p* < 0; Table 1).

IV. DISCUSSION

Oviposition decisions are an important aspect of fitness. Females choose where to lay eggs and how many eggs to lay in each food patch, which can have large consequences on offspring size and success of offspring (Campbell and Runnion, 2003). In this study, I tested the relative importance of competition between adults and future competition between offspring on the number of eggs laid in a patch by red flour beetles, as well as the fitness consequences of these oviposition decisions.

Negative density dependence among adults was regulated by sex-ratio, where an increase of females in a food patch increased the total number of eggs laid in all density treatments, but also decreased the per capita number of eggs laid per female. This suggests that females attempt to limit future competition when there is a female-biased sex-ratio by decreasing their per capita number of eggs, thereby making an active choice about how many eggs to lay in a food patch (Campbell and Runnion, 2003). However, the total number of eggs laid in a food patch does change with a modification of the sex-ratio, which I did not predict. This increase in total eggs, but decrease in per capita eggs, suggests that females are attempting to compensate for increased competition, although they are not compensating enough to stop the increase in the total number of eggs in a patch.

Per capita number of eggs and total number of eggs laid per females were both influenced by the number of adult beetles in the food patch. As density increased in a treatment, the number of eggs laid increased because more females were present and therefore oviposition rate was higher. However, as predicted by my hypothesis, as density increased, per capita eggs per female decreased because less energy was provided per individual for reproduction as well as oviposition rate (Cody, 1966). The presence of more individuals increased current competition for food (i.e. less energy is available per individual) and thus females tend to minimise the impact of competition on their fitness by decreasing the per capita number of eggs they laid (Vamosi and Lesack, 2007). The decision of females to lay fewer eggs when density is higher suggests that more energy will be available for offspring.

The number of days before larvae depleted the food patch was only modulated by density. As the number of adult beetles increased in a food patch, it took less time for larvae to deplete food. These results suggest that there is more competition for food during larval stage when density increases because more eggs are laid in the food patch, which is consistent with the above hypothesis. Larval length affected the developmental time of larvae but was regulated by density, indicating that negative density dependence among offspring affects larval length. When the patch is more female-biased, larval length decreases with the increase of the number of adult beetles in the treatment. It seems that under a more competitive scenario, larva seems to have a shorter length because less food is available per individual. Thus, this increased competition scenario forces larvae to accelerate their transformation process by growing faster, shown by the decrease of developmental time with the increase in density. Moreover, the actual increase in larval length was 0.3 mm for an increase of 10 adult beetles for the male-biased sex ratio, and a decrease of 0.4 mm for an increase of 10 adult beetles for a female-biased sex ratio. It confirms that larvae adjust their length with the amount of energy available per individual in the patch to make sure they will survive through pupation and become adults.

I found no relationship between the number of offspring and sex-ratio, as I hypothesized. The number of offspring was the same as sex-ratio and density changed, which may result from a choice by females to minimize future competition. However, the number of offspring per female was negatively influenced by a female-biased sex-ratio and by an increase in density. As there were more females in the food patch, the per capita offspring decreased because females lay eggs so that the energy available for offspring will support them. Also, per capita egg was affected by the interaction of density and sex-ratio; as density and the number of females increased in the food patch, the number of offspring for each female decreased but stayed higher than in a patch with more males or in a 1:1 sex-ratio. It showed that females lose in fitness as density increased, but did not seem to change the overall increase in the total number of offspring. It suggests that females tended to foresee larval mortality by laying more eggs when density is higher in the patch to increase their fitness but also to prevent larvae from starvation by laying less eggs that they would have laid if the sex-ratio was more male-biased or equal (Minkenberg *et al.*, 1992; Parker and Courtney, 1984). Total survivorship of larvae into adults and the adjustment of number of eggs laid to increase total fitness are results of female oviposition decisions.

V. CONCLUSION

Current and future competition among the different life stages of red flour beetles seem to be controlled by female oviposition decisions which is supported the majority of my hypotheses. However, more work should be done to clarify how a population of beetles more female-biased change the total number of eggs laid by females and how the number of offspring is influenced when the density of adults present during oviposition change in the patch. I investigated how changes in density and in sex-ratio modify female oviposition decision, but more studies should also be done to investigate more about how changes in food availability or in food quality modify female choice and how competition with other species impact fitness.

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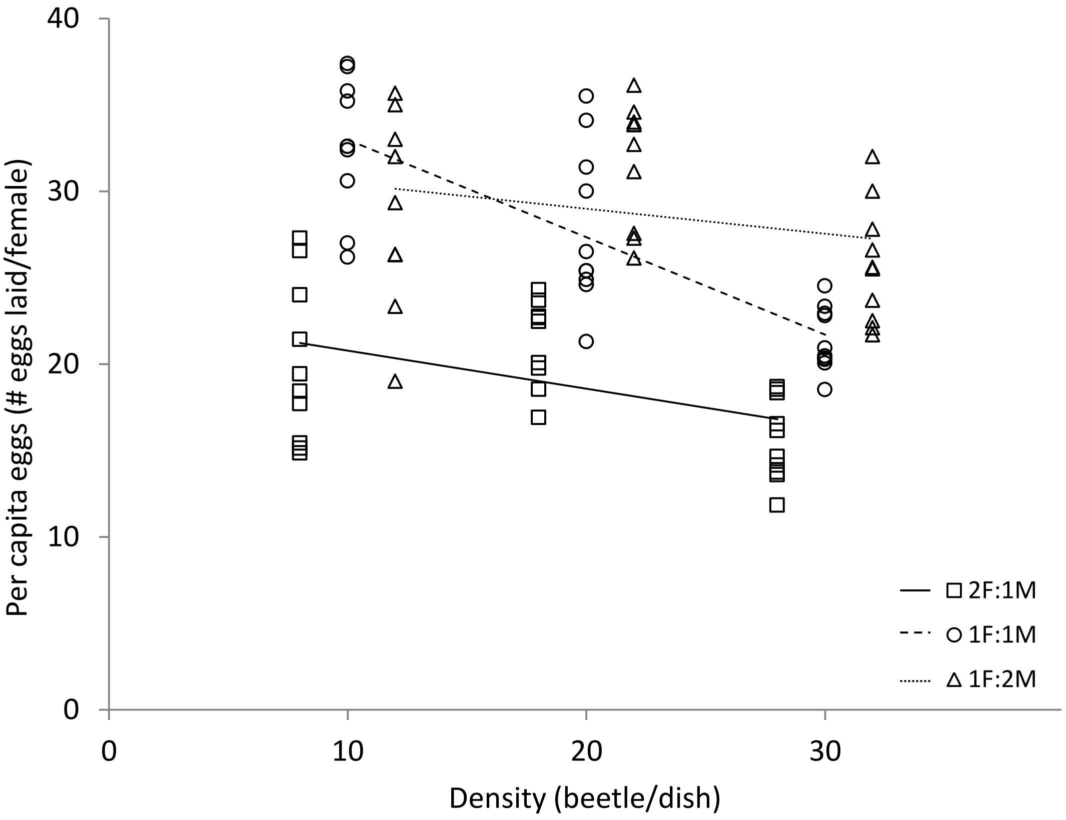
VII. APPENDIX

VII.i. Table 1. Summary of multiple regression models with the estimate, the standard error and the t-value for each variable of the models. Significant p-values are identified (*).

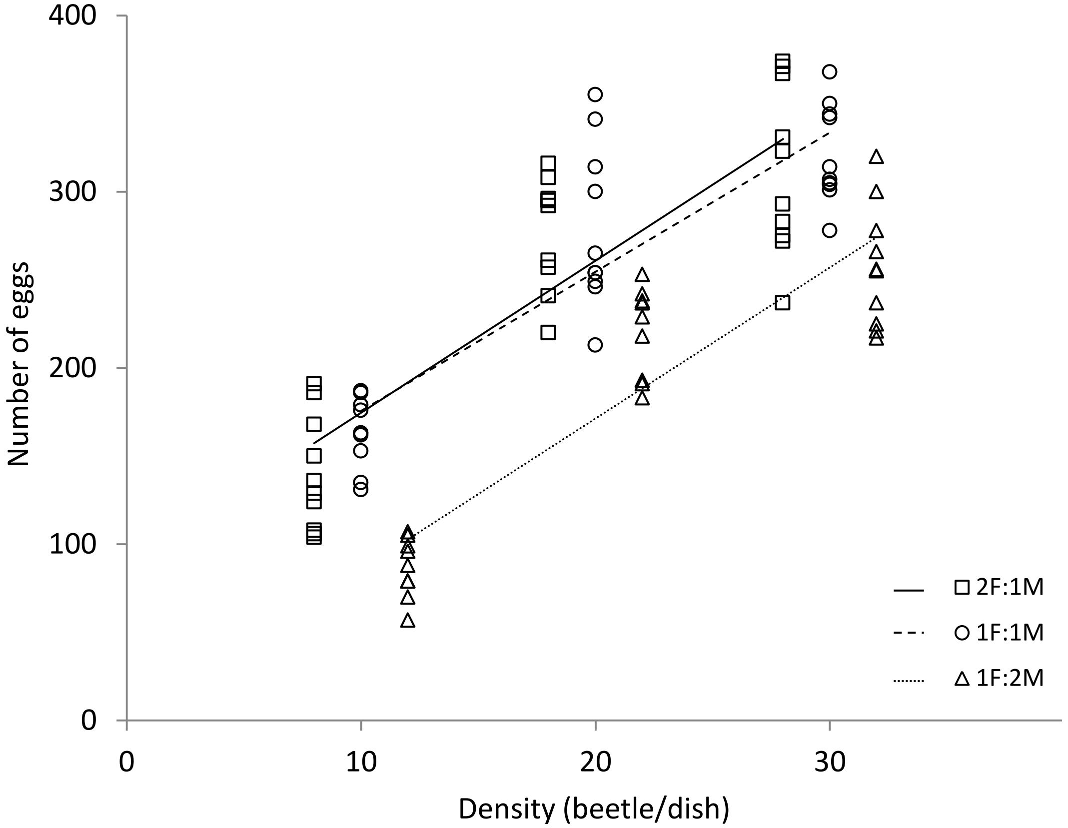
Model	Estimate	Standard error (SE)	t	p
Per capita number of eggs				
sex-ratio	-28.48	3.46	$t_{1,87} = 8.24$	< 0.01*
density	-0.31	0.06	$t_{1,87} = -5.26$	< 0.01*
Total number of eggs laid				
sex-ratio	162.16	32.69	$t_{1,87} = 4.96$	< 0.01*
density	8.36	0.56	$t_{1,87} = 15.05$	< 0.01*
Time elapsed before depletion				
sex-ratio	1.28	1.69	$t_{1,87} = 0.76$	0.45
density	-0.18	0.03	$t_{1,87} = -6.17$	< 0.01*
Total number of offspring				
sex-ratio	-68.73	34.72	$t_{1,87} = -1.98$	0.05
density	-0.74	0.59	$t_{1,87} = -1.27$	0.21
Per capita number of offspring				
sex-ratio	-55.10	11.60	$t_{1,86} = -4.75$	< 0.01*
density	-1.20	0.28	$t_{1,86} = -4.3$	< 0.01*
interaction sex-ratio and density	1.33[1]	0.54	$t_{1,86} = 2.48$	< 0.01*
subset 2:1 ratio	-0.81[1]	0.17	$t_{1,28} = -4.88$	-
subset 1:1 ratio	-0.42[1]	0.08	$t_{1,28} = -5.29$	-
subset 1:2 ratio	-0.36[1]	0.09	$t_{1,28} = -4.06$	-

[1] Units are in offspring per female.

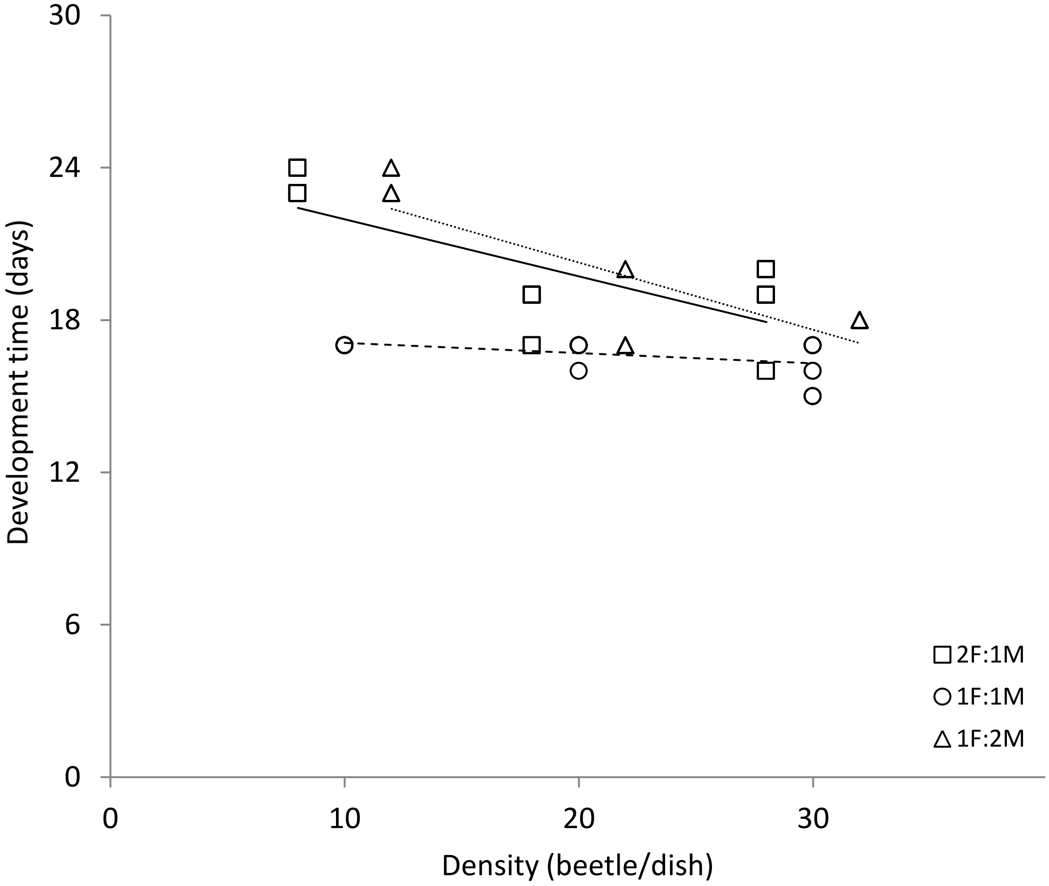
VII.ii. Figure 1. Per capita eggs (number of eggs laid per female) for a 2:1, 1:1 and 1:2 (female:male) sex-ratio of adult red flour beetles (*Tribolium castaneum*) for a two day period at three density treatments (10, 20, and 30). Linear trend lines are shown for each different sex-ratio.



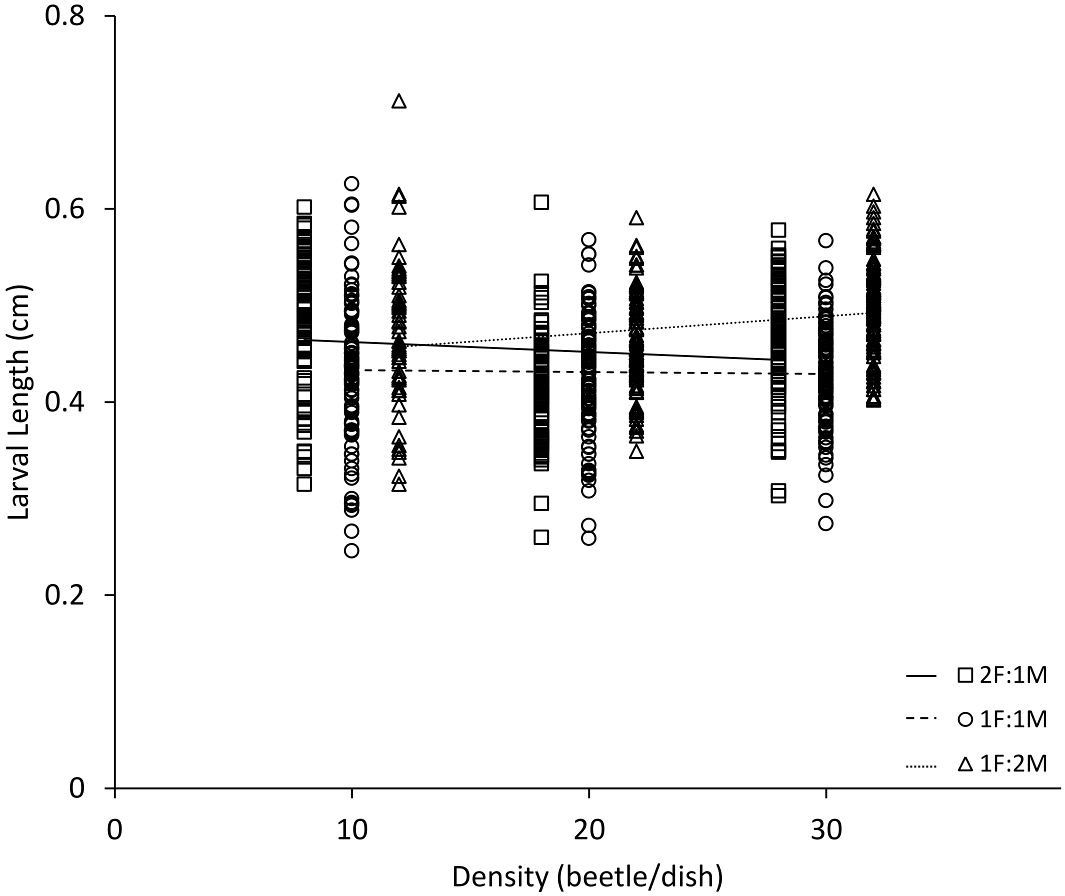
VII.iii. Figure 2. Number of eggs laid by females red flour beetles (*Tribolium castaneum*) for a 2:1, 1:1 and 1:2 (female:male) sex-ratio of adults for a two day period at three density treatments (10, 20, and 30). Linear trend lines are shown for each different sex-ratio.



VII.iv. Figure 3. Developmental time of larvae (number of days between the moment the counted eggs with the new flour was placed in the petri dish and the moment larvae depleted food) for a 2:1, 1:1 and 1:2 (female:male) sex-ratio of adult red flour beetles (*Tribolium castaneum*) for a two day period at three density treatments (10, 20, and 30). Linear trend lines are shown for each sex-ratio.



VII.v. Figure 4. Larval length of 10 random larvae (*Tribolium castaneum*) for a 2:1, 1:1 and 1:2 (female:male) sex-ratio of adults for a two day period at three density treatments (10, 20, and 30). Linear trend lines are shown for each different sex-ratio. Symbols are offset in order to aid in clarity.

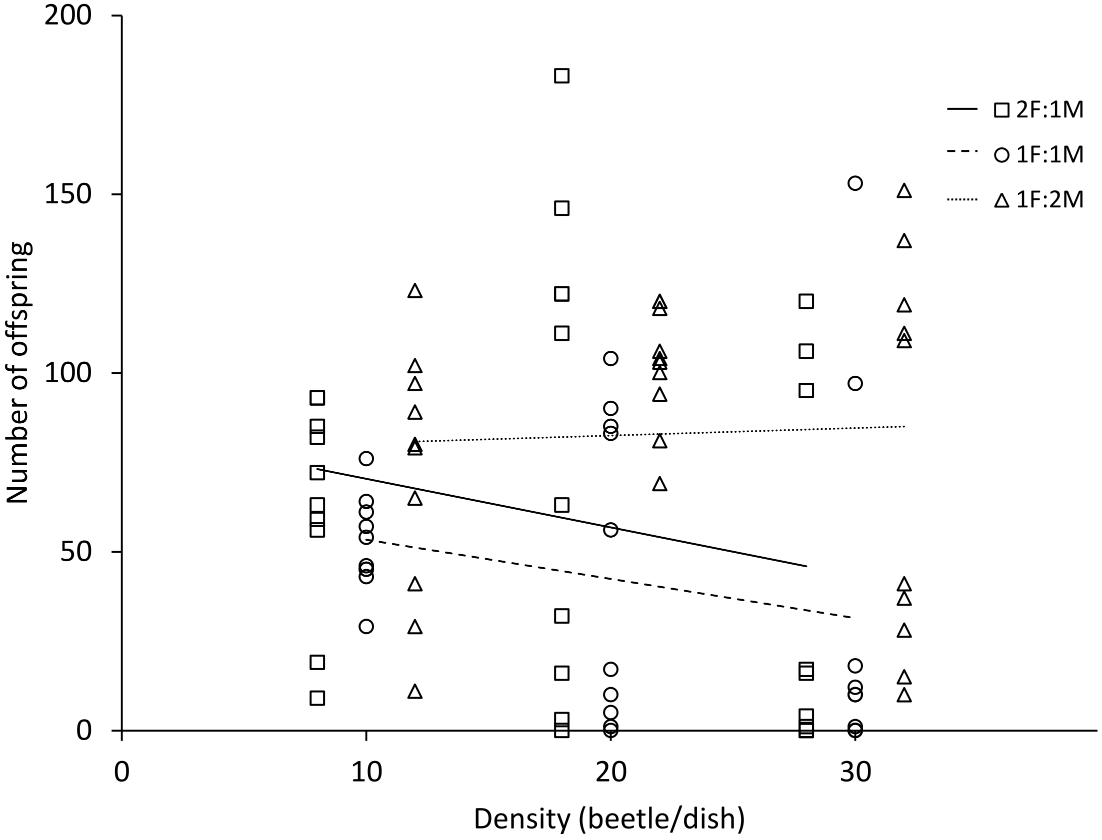


VII.vi. Table 2. Summary of linear mixed effect model with the estimate, the standard deviation and the t-value for each variable of the larval length model. Significant p-values are identified (*).

Model	Estimate	Standard deviation (SD)	t	p
Larval length				
density	0.005	0.001	$t_{1,87} = 4.95$	< 0.01*
time elapse before depletion	0.004	0.001	$t_{1,87} = 3.8$	< 0.01*
sex-ratio	0.09	0.05	$t_{1,87} = 1.82$	0.07
interaction sex-ratio and density	-0.0087[1]	0.002	$t_{1,86} = -4.29$	< 0.01*
subset 2:1 ratio	-0.0031[1]	0.0006	$t_{1,86} = 5.05$	< 0.01*
subset 1:2 ratio	-0.0044[1]	0.0007	$t_{1,86} = -6.09$	< 0.01*
subset 1:1 ratio	-0.0001[1]	0.0005	$t_{1,86} = 0.048$	0.96

[1] Units are in centimeter.

VII.vii. Figure 5. Number of adult offspring red flour beetles (*Tribolium castaneum*) for a 2:1, 1:1 and 1:2 (female:male) sex-ratio of adults for a two day period at three density treatments (10, 20, and 30). Linear trend lines are shown for each different sex-ratio.



VII.viii. Figure 6. Per capita number of adult offspring (number of offspring per female red flour beetles; *Tribolium castaneum*) for a 2:1, 1:1 and 1:2 (female:male) sex-ratio of adults for a two day period at three density treatments (10, 20, and 30). Linear trend lines are shown for each different sex-ratio.