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Do Female Red Flour Beetles Assess both Current and Future Competition during Oviposition?

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Abstract Female insects must assess multiple factors when laying eggs, including both abiotic and biotic conditions of the laying site. Competition may also play a crucial role in the oviposition decisions of females. Competition at oviposition sites may take two forms: current competition between adults for both food and access to sites for oviposition, and future competition between offspring that will hatch and develop at that site. Here, we test whether female red flour beetles (*Tribolium castaneum*) assess both current and future competition at oviposition sites with a laboratory experiment where we manipulated both the density (current competition) and sex ratio (future competition) of adults at potential oviposition sites. We counted the number of eggs laid in each site to assess oviposition decisions, and then let those eggs develop into adults to determine the fitness consequences of oviposition decisions (measured by the total number of adult offspring produced). Female red flour beetles responded to both density and sex ratio: per capita eggs laid decreased as density increased, but was higher when the sex ratio was male-biased. These oviposition decisions were reflected in the per capita number of adult offspring produced. We provide evidence that female red flour beetles do assess for both current and future competition in their oviposition decisions.

Keywords Fecundity · fitness · oviposition · trade-off · *Tribolium castaneum*

Introduction

Oviposition decisions are integral to the fitness of females that lay eggs (Mousseau and Fox 1998); choosing when and where to lay eggs, as well as how many eggs to lay, can all affect the quantity and quality of offspring (e.g., Scheirs and de Bruyn 2002; Campbell and Runnion 2003). In species that provide no parental care, females can search for specific microhabitats that provide optimal conditions for offspring development (Blouin-Demers et al. 2004; Peet-Paré and Blouin-Demers 2012). In species that reproduce constantly, such as many insects, females can lay eggs in multiple locations and adjust how many eggs they lay in each location based on the quality of that location (e.g., Messina and Renwick 1985; Campbell and Runnion 2003). Females may also choose sites for oviposition that provide the female with higher fitness over the long-term, but may be poor sites for their offspring, thus trading off female fitness with offspring fitness (Scheirs et al. 2000; Mayhew 2001).

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Female oviposition decisions may be akin optimal foraging decisions (Skinner 1985).

While abiotic factors of oviposition sites, such as moisture and temperature, may be readily predicted based on current conditions, biotic factors of oviposition sites may be more difficult to predict. For example, females can determine the current levels of competition based on the number of competitors present in an area (e.g., Brown 1988), but it may be more difficult to assess future competition for their developing offspring. Females could assess future competition between offspring based on the number of eggs or larvae already in a patch (Messina and Renwick 1985; Magro et al. 2017). Females may also be able to estimate future competition based on the number of other ovipositing females at the same site. Do ovipositing females assess both current and future competition, and how do oviposition decisions affect reproductive success of females?

In this study, we use two cues of competition for ovipositing females: the number of adults (both female and male) in a patch and the number of adult females in a patch. The number of adults in a patch is a cue for current competition over food or space, while the number of females in a patch represents a cue for future competition among offspring. We examine two, non-mutually exclusive hypotheses to explain the oviposition decisions of females: (1) females choose the number of eggs to lay as a function of current competition between adults because it decreases the energy available to put towards egg production and (2) females choose the number of eggs to lay as a function of future competition between offspring. More specifically, we test the predictions that (1) females should lay fewer eggs at higher densities by manipulating adult density and (2) females should lay fewer eggs when more females are present, irrespective of overall density, by manipulating sex ratio. We use an experiment with red flour beetles (*Tribolium castaneum*) to test these predictions. We estimate the fitness consequences of oviposition decisions by letting the eggs hatch and develop into adult offspring.

Red flour beetles, and their sister species confused flour beetles (*T. confusum*), have been studied in the laboratory for over 80 years (Chapman 1928; Holdaway 1932; Park 1932). All life stages of flour beetles rely on flour as both a food source and a substrate for burrowing (Good 1936). There is evidence that females choose how many eggs to lay in a food patch and that this choice maximizes fitness by decreasing the mortality rate of offspring or by changing the intensity of future competition between offspring (Campbell and Runnion 2003). The energy available for oviposition is also limited by exploitative competition between adults through negative density dependence (Halliday et al. 2015). Longevity of adult flour beetles varies widely, ranging from a few weeks to over three years under laboratory conditions (Good 1936), although most individuals tend to have shorter lifespans depending on environmental conditions (Halliday and Blouin-Demers 2017). Female flour beetles reproduce continuously throughout their adult life (Good 1936), although reproductive senescence begins within 6 months after eclosion (Halliday and Blouin-Demers 2017). Females lay on average 2–3 eggs per day when suitable substrate is available, with maximum of 13 eggs per day (Good 1936).

Materials and Methods

We used a culture of red flour beetles that we originally obtained from Carolina Biological Supply Company (Burlington, North Carolina, USA) in 2012. We started with 200 adult beetles and grew the population to over 5000 beetles. We maintained our culture at 30 °C, 70% humidity, and a 12:12 h light:dark cycle. All experiments were conducted in an incubator at 30 °C, the preferred temperature of red flour beetles (Halliday and Blouin-Demers 2014, 2015) and their optimal temperature for oviposition (Halliday and Blouin-Demers 2015; Halliday et al. 2015), and beetles were placed in 2.5 ml of a mixture of 95% all-purpose wheat flour and 5% brewer's yeast. All mention of flour refers to this mixture. We separated beetles by sex during their pupal stage, identifying the sexes based on the presence (female) or absence (male) of ovipositors (Good 1936). Female and male beetles were then left to mature in separate containers with ad libitum flour. One week following eclosion, we marked each beetle on the back using two colours of non-toxic acrylic paint to differentiate between the sexes and allow individuals to be used for several treatments. Marking with paint does not appear to affect longevity because marked individuals in a separate experiment survived and continued reproducing for 6 months (Halliday and Blouin-Demers 2017).

We placed beetles in a petri dish (10 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 (F:M; 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, we used the following ratios: 7:3 at density = 10, 14:6 at density = 20, and 20:10 at density = 30, likewise for the 1:2 sex ratio. Beetles were allowed to lay eggs for two days, at which point we extracted the eggs from the flour with a 250 μm sieve and counted them. We then put the eggs back in the petri dish with 2.5 ml of fresh flour. Adult beetles were separated and put back in their respective male and female containers. Beetles were randomly selected for each treatment and an individual could be used multiple times. Treatments and replicates, however, were randomized to remove any influence of individual beetles and previous experience on experimental outcomes.

Eggs were allowed to hatch and develop into adults to evaluate the fitness effects of competition between adults and of competition between offspring. If the larvae depleted the available flour, we added another 2.5 ml of fresh flour to the petri dish. We did this because larvae would normally be able to disperse from a depleted food patch and seek out a new food source, but this dispersal behaviour was not possible in our experiment. After all pupae became adults, we sifted each petri dish with a 500 μm sieve to obtain and count the number of live adults.

We tested our predictions by analyzing per capita eggs laid (the number of eggs laid per female present in the treatment) using linear models in R (package: stats; function: lm; R Core Team 2014). We built three models, all with per capita eggs laid as the dependent variable. Our first model had density (total number of adults in a treatment) as the only independent variable. Our second model had the number of females in a treatment as the only independent variable. Our third model had density treatment, sex ratio (categorical variable), and their interaction as independent variables; we included sex ratio as a categorical variable to avoid strong multicollinearity between the number of females and the number of adults in a treatment. We then compared these three models using Akaike's information criterion (AIC; package: stats; function: AIC) and selected the best model as the model with the lowest AIC (Burnham and Anderson 2002).

We examined the fitness consequences of oviposition decisions by analyzing the per capita number of adult offspring produced. We compared the same independent variables as in the analysis of per capita eggs laid, but also included variations of these three models that included the per capita eggs laid by females to account for the oviposition decisions of the females in each treatment.

Results

The best model indicated that female oviposition decisions were influenced by sex ratio and density (full model df = 84, $R^2_{adj} = 0.63$, $p < 0.01$; Fig. 1; Tables 1 and 2), and not by the density of all adults alone or the density of females alone (Table 1). The relationship between per capita eggs laid and density was different for each sex ratio ($p < 0.0001$ interaction; Table 2). The treatment with equal sex ratio had the steepest slope (slope = −0.56 ± 0.09, $t_{84} = 6.24$, $p < 0.0001$), the male-biased treatment had the shallowest slope (slope = −0.19 ± 0.13, $t_{84} = 2.89$, $p < 0.01$), and the female-biased treatment had an intermediate slope (slope = −0.22 ± 0.13, $t_{84} = 2.69$, $p < 0.01$). The fitness-density functions for the male-biased and equal sex ratio treatments overlapped, with higher per capita eggs laid in the equal treatment at low density and higher per capita eggs laid in the male-biased treatment at high density. The female-biased treatment had lower per capita eggs laid than the other two treatments at each density.

The best model indicated that mean female fitness (per capita adult offspring produced) was influenced by sex ratio, the per capita number of eggs laid by females, and the interaction between density and the per capita number of eggs laid (full model df = 84, $R^2_{adj} = 0.56$, $p < 0.01$; Figs. 2 and 3; Tables 3 and 4). Females produced more offspring per capita in the male-biased treatment (intercept = 7.15 ± 2.84, $t_{84} = 6.74$, $p < 0.01$; Fig. 2), but the number of offspring produced were not different between the equal sex ratio treatment (intercept = 1.51 ± 6.44) and the female-biased treatment (intercept = −0.18 ± 2.93, $t_{84} = 0.65$, $p = 0.52$). The number of adult offspring produced per female increased as the number of per capita eggs laid increased (slope = 0.51 ± 0.23, $t_{84} = 2.58$, $p = 0.01$), but this relationship decreased as density increased (per capita eggs: density interaction = −0.03 ± 0.01, $t_{84} = 2.38$, $p = 0.02$; Fig. 3). A male-biased sex ratio caused an increase in mean fitness, and mean fitness generally decreased with density, although this effect was modulated by the number of eggs laid per female. The interaction between density and sex ratio was not statistically significant (Fig. 2, Table 3).

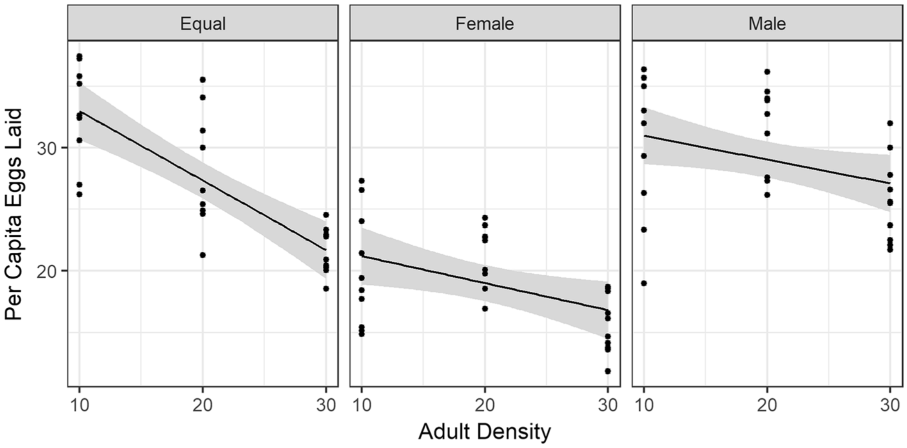

Fig. 1 Partial effects plots for the relationship between per capita eggs laid over two days by female red flour beetles (*Tribolium castaneum*) and adult density treatment in three sex ratio treatments (equal = equal sex ratio; female = female-biased sex ratio; male = male-biased sex ratio). Note that the y axis is displayed as partial residuals. Linear trend lines with 95% confidence intervals are shown for each sex ratio treatment. $N = 30$ for each sex ratio treatment

Discussion

Oviposition decisions are an important aspect of fitness. Females choose where to lay eggs and how many eggs to lay in each patch, which can have large consequences for offspring size and success (Skinner 1985; Campbell and Runnion 2003). While current competition over food among adults is relatively simple to predict, future competition among offspring within an oviposition site may be more difficult to predict. However, insects may use tactics akin to those of optimal foraging to make optimal oviposition decisions (Skinner 1985). Future competition is thought to be an important factor in the oviposition decisions of many insects which modify clutch size depending on the number of other females also laying eggs in the same food patch (e.g., Visser 1996) or based on the size of the food patch (e.g., Bezemer and Mills 2003; Cope and Fox 2003; Campbell and Runnion 2003). In this study, we tested the relative importance of current competition between adults and of 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. If females assessed current competition, but not future competition, in their oviposition decisions, then adult density alone would explain the number of eggs laid by females. If females assessed future competition, but not current competition, in their oviposition decisions, then the number of females alone would explain the number of eggs laid by females. Finally, if females assessed both current and future competition in

Table 1 Model selection for linear models examining the per capita eggs laid by female red flour beetles (*Tribolium castaneum*). *k* is the number of parameters in the model, AIC is the Akaike's information criterion value, and ΔAIC is the difference in AIC between the model and the model with the lowest AIC. $N = 30$ for each sex ratio treatment

Model	*k*	AIC	ΔAIC
Per Capita Eggs = Adult Density + Sex Ratio + Interaction	7	514.77	0.00
Per Capita Eggs = Females Density	3	547.58	32.81
Per Capita Eggs = Sex Ratio	4	550.30	35.53
Per Capita Eggs = Adult Density	3	584.79	70.02

Table 2 Final model output for linear models examining the per capita eggs laid by female red flour beetles (*Tribolium castaneum*). N = 30 for each sex ratio treatment, df = degrees of freedom, and Sum Sq is the sum of squares for each parameter

Parameter	Sum Sq	*df*	*F*	*p*
Intercept	9393.3	1	390.62	< 0.0001
Adult Density	636.2	1	38.87	< 0.0001
Sex Ratio	505.9	2	15.46	< 0.0001
Adult Density: Sex Ratio	170.6	2	5.21	< 0.0001
Residuals	1374.8	84		

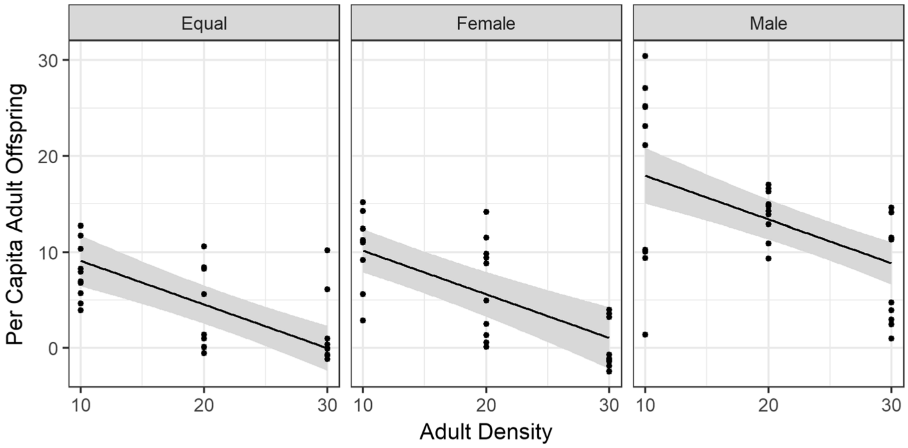

Fig. 2 Partial effects plots for the relationship between per capita number of adult offspring produced by female red flour beetles (*Tribolium castaneum*) and adult density treatment in three sex ratio treatments (equal = equal sex ratio; female = female-biased sex ratio; male = male-biased sex ratio). Note that the y axis is displayed as partial residuals. Linear trend lines with 95% confidence intervals are shown for each sex ratio treatment. *N* = 30 for each sex ratio treatment

their oviposition decisions, then both adult density and sex ratio would explain the number of eggs laid by females.

We found support for both of our hypotheses: both adult density (current competition) and sex ratio (future competition) caused decreases in the number of eggs laid by females. Treatments with a male-biased sex ratio also had higher mean fitness (more adult offspring produced) and an increase in density caused a decrease in mean fitness. Negative density dependence among adults was modulated by sex ratio: an increase in females in a food patch decreased the per capita number of eggs laid. This suggests that females attempt to limit future competition between offspring when there is a female-biased sex ratio by decreasing their per capita number of eggs laid, thereby making an adaptive choice about how many eggs to lay in a food patch (Skinner 1985). This adaptive choice indeed led to higher mean fitness (more per capita adult offspring produced) for individuals in the lowest density treatment than predicted based on density alone for the male-biased treatment, whereas individuals in the equal sex ratio and female-biased treatments had similar fitness-density functions. Other insects also attempt to limit future competition in their oviposition decisions (e.g., Visser 1996; Bezemer and Mills 2003).

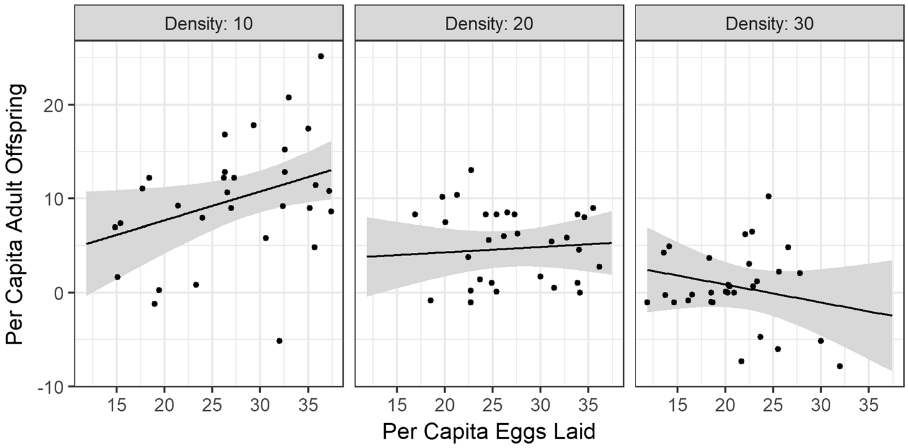

Fig. 3 Partial effects plots for the relationship between per capita number of adult offspring produced by female red flour beetles (*Tribolium castaneum*) and per capita eggs laid in three adult density treatments (10, 20, and 30). Note that the y axis is displayed as partial residuals. Linear trend lines with 95% confidence intervals are shown for each density treatment. N = 30 for each sex ratio treatment

Table 3 Model selection for linear models examining the per capita adult offspring produced by female red flour beetles (*Tribolium castaneum*). *k* is the number of parameters in the model, AIC is the Akaike's information criterion value, and ΔAIC is the difference in AIC between the model and the model with the lowest AIC. *N* = 30 for each sex ratio treatment

Model	*k*	AIC	ΔAIC
Per Capita Adults = Adult Density + Sex Ratio + Per Capita Eggs + Adult Density: Per Capita Eggs	7	548.49	0.00
Per Capita Adults = Adult Density + Sex Ratio + Per Capita Eggs + Adult Density: Per Capita Eggs + Sex Ratio: Per Capita Eggs	9	548.64	0.15*
Per Capita Adults = Adult Density + Sex Ratio	5	551.55	3.06
Per Capita Adults = Adult Density + Sex Ratio + Per Capita Eggs	6	552.35	3.86
Per Capita Adults = Adult Density + Sex Ratio + Interaction	7	552.57	4.08
Per Capita Adults = Adult Density + Sex Ratio + Interaction + Per Capita Eggs	8	552.61	4.11
Per Capita Adults = Female Density	3	570.83	22.34
Per Capita Adults = Adult Density	3	592.23	43.74
Per Capita Adults = Per Capita Eggs	3	596.53	48.04

The per capita number of eggs laid was an imperfect predictor of fitness on its own, but when density and sex ratio were also taken into account, the per capita number of eggs laid became an excellent predictor of fitness. The significant interaction between the per capita eggs laid and density on the mean fitness suggests that eggs from lower density treatments were of higher quality than eggs laid in high density treatments, or at least that the eggs from lower density treatments had a higher chance of success. This idea could be tested in the future by taking eggs laid under different density treatments, allowing them to develop alone in the absence of competition with other offspring, and documenting the production of offspring. This result could explain why females choose to lay fewer eggs in a patch when more females are present: eggs are less likely to develop into adult offspring under such conditions.

Table 4 Final model outputs for linear models examining the per capita adult offspring produced by female red flour beetles (*Tribolium castaneum*). N = 30 for each sex ratio treatment, *df* = degrees of freedom, and Sum Sq is the sum of squares for each parameter

Parameter	Sum Sq	*df*	*F*	*p*
Intercept	0.00	1	0.00	1.00
Per Capita Eggs	158.03	1	6.64	0.01
Sex Ratio	1112.57	2	23.37	< 0.0001
Adult Density	9.16	1	0.38	0.54
Per Capita Eggs: Adult Density	134.53	1	5.65	0.02
Residuals	1999.56	84		

It is currently unknown whether females based their estimate of future competition on the number of competing females or on the number of eggs in the patch. Other insects assess the number of eggs in a patch (e.g., Messina and Renwick 1985); however, this may be difficult for flour beetles in flour patches where the eggs are covered in flour and thus difficult to detect. More work is needed to tease these potential effects apart, such as presenting females with flour patches that already contain eggs but no other females, and flour patches without eggs but with other females. Indeed females prefer flour without eggs to flour with eggs as oviposition sites (Ghent 1963), so it is likely that females do assess the number of eggs already present in a flour patch.

In conclusion, females must assess multiple factors when making oviposition decisions. Females must first select sites with appropriate abiotic conditions for egg development, and then must assess at least two levels of competition: current competition with other adults and future competition between offspring. Our results suggest that red flour beetles do assess both forms of competition when making decisions about the number of eggs to lay in a flour patch, which follows the predictions of optimal oviposition theory (Scheirs and de Bruyn 2002).

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