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Butterflies do not alter conspecific avoidance in response to variation in density

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ABSTRACT

High conspecific densities are associated with increased levels of intraspecific competition and a variety of negative effects on performance. However, changes in life history strategy could compensate for some of these effects. For instance, females in crowded conditions often have fewer total offspring, but they may invest more in each one. Such investment could include the production of larger offspring, more time spent engaging in parental care, or more careful decisions about where offspring are placed. For animals that have a relatively immobile juvenile stage, the costs of competition can be particularly high. Females may be able to avoid such costs by investing more in individual reproductive decisions, rearing young or laying eggs in locations away from other females. We tested the hypothesis that conspecific density cues during juvenile and adult life stages lead to changes in life history strategy, including both reproduction and oviposition choices. We predicted that high-density cues during the larval and adult stages of female cabbage white butterflies lead to lower fecundity but higher conspecific avoidance during oviposition, compared to similar low-density cues. We used a 2x2 factorial design to examine the effects of low and high conspecific density during the larval and adult stages of butterflies on avoidance behavior and fecundity. We found that past information about conspecific density did not matter; all butterflies exhibited similar levels of fecundity and a low level of conspecific avoidance during oviposition regardless of their previous experience as larvae and adults. These results suggest that butterflies use a fixed, rather than flexible, conspecific avoidance strategy when making oviposition decisions, and past information about conspecific density has no effect on life history and current reproductive investment. We speculate that this may be partially because current conspecific density *per se* is not a reliable cue for predicting future density and levels of competition, and thus it does not affect the development of life history strategies in this

system.

INTRODUCTION

High conspecific densities can have negative fitness consequences if the presence of conspecifics interferes with an individual’s ability to survive and produce surviving offspring. For instance, crowding is associated with smaller size, lower fecundity, reduced offspring viability, and higher rates of offspring starvation in various invertebrate and vertebrate taxa (Peters & Barbosa, 1977; Tella et al., 2001; C. G. Wiklund & Andersson, 1994). High densities can have especially detrimental effects on species with larval stages that are less mobile or otherwise relatively unable to disperse. Some animals develop in discrete resources such as pools, buds, seeds, or animal bodies and do not continue to feed once the resource is depleted, resulting in competition and negative fitness consequences at high densities (Fox, Czesak, & Savalli, 1999; Harvey, Poelman, & Tanaka, 2013; Hess, Abrahamson, & Brown, 1996; Woolrich-Pina, Smith, & Lemos-Espinal, 2015). The larvae of many holometabolous insects are slow-moving and confined to the immediate area in which they hatch (R. Jones, 1977). High larval densities in these insects can result in negative fitness consequences such as lower body size and pupal mass, longer development time, and decreased survival (Alto, Muturi, & Lampman, 2012; Gibbs, Lace, Jones, & Moore, 2004; Yoshioka et al., 2012). These consequences are often attributed to the effects of intraspecific competition (Gibbs et al., 2004; Kivela & Valimaki, 2008).

Although many species seem to experience such negative effects of offspring competition under high-density conditions (Thompson, Marshall, & Monro, 2015), other species cope with high juvenile densities by adjusting their development and life history strategy (Moran, 1992). For example, salamanders and locusts develop into distinct morphs with different feeding, dispersal, and reproductive habits depending on early exposure to varying densities of conspecifics (Rogers et al., 2003). Larval density cues can also alter resource allocation or the speed of development, leading to changes in life history traits and, ultimately, adult morphology and behavior (Kasumovic & Brooks, 2011; Kasumovic, Hall, Try, & Brooks, 2011). For example, some invertebrates allocate more energetic resources to immune function (Niemela, Vainikka, Lahdenpera, & Kortet, 2012) or organs used to obtain food (Duarte, Re, Flores, & Queiroga, 2014) when exposed to conspecific cues during development. Similarly, crickets and grasshoppers can speed up development in response to high conspecific density, developing into smaller adults that adopt different but potentially adaptive reproductive strategies compared to conspecifics reared at low densities (Applebaum & Heifetz, 1999).

It is surprising that some organisms seem to lack such adaptive responses to high juvenile densities. It is possible that some of these species do respond in potentially adaptive ways, but that their responses are overlooked when fitness is not assessed in the context of life history theory. For example, high-density conditions often lead to reduced fecundity in insects (Peters & Barbosa, 1977). At first glance, this seems like a cost of living in high-density conditions. However, it may actually be part of an adaptive life history strategy whereby females produce fewer offspring but invest more in each one (Stearns, 1976). The development of this life history strategy may depend upon previous experience with high conspecific density, either during development or immediately before the adult is ready to reproduce. Variation in conspecific density can lead to physiological changes during the juvenile or adult stage, potentially by altering energy acquisition (Joshi & Mueller, 1988) and allocation (Marchand & Boisclair, 1998). These physiological changes manifest as suites of life history traits that together are potentially adaptive under high-density conditions, for example fast growth and dispersal capability (Applebaum & Heifetz, 1999). The previously mentioned trade-off between fecundity and investment in individual offspring may be another example. Investment in individual offspring arguably includes obvious reproductive traits like egg size as well as behavioral traits such as choosiness during egg-laying (Resetarits, 1996). Thus, female butterflies that are less fecund can compensate by producing larger eggs (Garcia-Barros, 2000) or being more careful about where they lay them (Jaumann & Snell-Rood, 2017). In the case of butterflies, egg size was not affected by variation in density in the butterfly *Bicyclus anynana* (Bauerfeind & Fischer, 2005). However, in butterflies and most other animals, little attention has been devoted to the idea that the carefulness of egg-laying or rearing decisions could be a behavioral component of a life history strategy adapted to crowded conditions.

High-density cues can lead to adaptive conspecific avoidance when an animal decides where to rear young or lay eggs. Such conspecific avoidance promotes offspring survival and could compensate for lower fecundity. Females of many species disperse and actively seek out sites with lower conspecific densities (Henaux, Bregnballe, & Lebreton, 2007) or exhibit behavioral avoidance of conspecifics when looking for sites to rear young or lay eggs (Jaumann & Snell-Rood, 2017; Quilodran, Estades, & Vasquez, 2014; Stein & Blaustein, 2015). Some frogs and insects, for example, pay attention to conspecific cues- eggs, larvae, or other adult females- to avoid laying eggs on or in resources that may already have offspring from other females (Prokopy & Roitberg, 2001; Stein & Blaustein, 2015). Such avoidance behavior can directly prevent negative fitness effects of high conspecific density in these females' offspring (Stein & Blaustein, 2015; Sweeney & Quiring, 1998).

A high-density cue during the juvenile *or* adult stage of a female's life could alter investment in reproductive behavior (Muller, Kull, & Muller, 2016; Swanger & Zuk, 2015) as part of a life history strategy in which investment in individual offspring is high. Shifts in energy acquisition and allocation in response to environmental cues can occur throughout an organism's life, affecting its life history strategy. Yet there is limited information about the effects of density cues across life stages on reproductive investment. More specifically, it is unknown if exposure to density cues during the juvenile or adult stage has larger effects on conspecific avoidance during egg-laying. To account for this limitation, we tested the hypothesis that high-density cues during both the juvenile and adult stages lead to changes in reproductive investment. We designed an experiment with low and high conspecific density treatments during the larval and adult stages of *Pieris rapae* (cabbage white) butterflies. High larval densities can massively reduce performance in butterflies (Gibbs et al., 2004; Kivela & Valimaki, 2008), so conspecific avoidance strategies during oviposition in high-density environments should be strongly favored. Indeed, cabbage white females do generally avoid conspecific cues (Sato, Yano, Takabayashi, & Ohsaki, 1999), similar to other butterfly species (Rausher, 1979; Rothschild & Schoonhoven, 1977; Shapiro, 1981; C. Wiklund & Ahrberg, 1978; Williams & Gilbert, 1981). However, it is unclear how information about larval versus adult conspecific density interacts to influence life history traits such as fecundity and oviposition behavior. We test the prediction that females reared in high densities during the larval and adult stages adopt a strategy of producing fewer offspring, but investing more in each one. We expect that these females will be less fecund but more likely to avoid laying eggs near conspecifics than females reared alone. To test this prediction, we created the factorial combinations of life stage and density level by housing female *P. rapae* larvae and adults alone or in groups of five, resulting in four different treatments. We then assayed choosiness during oviposition and measured fecundity in individual female butterflies.

METHODS

Butterfly Collection and Husbandry

Around 50 female cabbage white butterflies were collected from gardens at the University of Minnesota St. Paul campus from May to October 2014. The butterflies used in our study were 48 lab-reared offspring from these wild mothers. To collect eggs, we housed wild mothers in 61x61x61 cm clear vinyl and mesh "Bug-Dorm" cages in a greenhouse, ensuring that there were no more than ten females to a cage. Each cage was supplied with a damp washcloth, cup of water covered with tulle, and non-host plant watered daily to provide humidity. A small sponge soaked with 10% honey solution provided carbohydrates. An organic cabbage leaf was placed in each cage to promote oviposition. The washcloth, cup of water, and sponge were refreshed daily. Cabbage leaves were also replaced daily. If eggs were observed on a leaf after 24 hours in a cage, it was stored in an incubator set at a 14-hour photoperiod, 23°C, and 70% relative humidity until the eggs hatched. To control for larval nutritional environment, we transferred larvae from the cabbage to 5-oz plastic cups containing artificial agar-based diet modified from established methods (Troetschler, Malone, Bucago, & Johnston, 1985). Because first-instar larvae are delicate, we waited until they were second instars to transfer them to the diet (7-10 days post laying). The diet contained wheat germ (5% by weight), casein (3%), sucrose (2.4%), Wesson salt mix (0.9%), torula yeast (1.2%), cholesterol (0.36%), linseed oil (0.47%), vitamin mix (1%), sorbic acid (0.15%), ascorbic acid (0.3%), cellulose (1%), and cabbage powder (1.5%). Small amounts of methyl paraben and streptomycin were added to prevent the growth of fungi or bacteria in the diet. Most of the ingredients were purchased from Frontier Agricultural Sciences-Entomology Division. The streptomycin was purchased from Sigma-Aldrich.

Butterflies were allowed to pupate and emerge as adults in the diet cups. Emerged females were individually marked with a Sharpie pen on their hindwings and transferred to mating cages in the greenhouse for three days because mating generally stimulates egg development (Papaj, 2000). After mating, the butterflies were moved to experimental cages where they were subjected to a behavioral assay measuring egg-laying behavior in response to conspecific cues. Butterflies in mating and experimental cages were cared for in a manner identical to wild females although the plants inside the cages differed. Larval density was manipulated in the diet cups, and adult density was manipulated in the mating cages (see below). Starting in late August, overhead lights were used to give a 15-hour photoperiod to preserve normal butterfly behavior.

Density Manipulations

Females were provided with conspecific density cues during both the larval and adult stages. During the larval stage, caterpillars were reared in either a low-density treatment of one caterpillar per 5-oz diet cup or a high-density treatment of five caterpillars per cup. Previous work in butterflies shows negative effects of density at five larvae per plant (Gibbs et al., 2004). A density of five caterpillars per cup is likely comparable in terms of nutrition, but encourages social interaction more because the larvae are confined to a smaller space. Five is also the maximum number of pupae per cup that minimizes disease and allows for enough space for individuals to eclose without physical deformities (personal observation). Cups were checked daily; most cups never ran out of food, but if caterpillars consumed most of the food in their cup, they were transferred together to a new diet cup.

Adult females were exposed to either a low-density treatment of one female and one male per mating cage or a high-density treatment of five total females and five total males per mating cage. We assessed the oviposition behavior of individual females in each of the four factorial combinations of these larval and adult treatments. All females were tested alone to avoid confounds involved with testing multiple females together, such as social cues unable to be controlled for. Only one out of five individuals from each larval and adult high-density treatment was included in analysis to avoid pseudo-replication.

Host Plant Preparation

Cabbage host plants (*Brassica oleraceae* var. Earliana) were grown under natural light in greenhouse facilities at the University of Minnesota St. Paul campus. All plants experienced identical growing conditions to minimize variation. Plants were grown from seed in Sungro Sunshine Professional Growing Mix soil, Mix #8/LC8 (SKU: 5292601), with 70-80% sphagnum. Five grams of Osmocote Classic 14-14-14 N:P:K slow release fertilizer was applied twice to each cabbage plant, approximately 4 weeks apart. Batches of cabbages were grown in the early and late summer for a continuous supply of fresh, mature leaves.

We conducted behavioral assays from June to December 2014, with supplemental greenhouse lighting to maintain a 15-hour photoperiod during the later months. These assays were designed to identify effects of prior experience with low and high conspecific density cues on the oviposition behavior of female butterflies with regard to the presence of conspecific cues. For each run of the behavioral assay, we placed two cabbage leaves in each of at least ten experimental cages. To spread potential host plant variation across cages, the leaves within and between cages were all clipped from different 2-4 month-old plants. We visually size and age matched leaf pairs within each cage. Within each cage, the two cabbage leaves were placed side-by-side, with approximately 25 cm of empty space between them. Each leaf was wedged into a water-filled floral stem tube refilled daily to keep the leaf fresh. Each tube was propped up in a small ceramic pot to mimic the position of leaves on a full cabbage plant. The honey solution-soaked sponge was placed in front of and equidistant from the two ceramic pots to prevent location bias.

We provided females with conspecific cues during oviposition to evaluate their response to social cues subsequent to experiencing low or high conspecific density conditions during the larval and adult stages. The conspecific cue we used was dead, pinned “model” conspecific butterflies on one of the two leaves in each experimental cage. Using dead, pinned conspecifics is an established way of providing social cues in insects, including butterflies (P. L. Jones, Ryan, & Chittka, 2015; Otis et al., 2006). We also know, from a previous study, that cabbage whites tend to avoid laying eggs on leaves with these models (Jaumann & Snell-Rood, 2017). The cabbage white butterflies that we used as models were purchased from Carolina Biological Supply as eggs and reared to adults on artificial diet in the lab, to limit variation among models and ensure that all experimental butterflies were equally naïve with regard to models. Model females were immediately frozen upon emergence and stored in the freezer until they were pinned for use as models. To provide a cue relevant for oviposition, female models were pinned in oviposition posture by securing them to a base via a pin through the thorax and curling the abdomen down with two crossed pins. We allowed models to dry for at least one week to ensure that they remained permanently in this posture. Three of these models were then attached to one of the two leaves in each experimental cage before a butterfly was introduced to the cage. Females thus had a choice between an empty leaf and a leaf with three conspecifics during the behavioral assay. To attach a model to a leaf, we removed the crossed pins and attached the remaining pin through the thorax to the leaf by piercing the leaf and securing the pin to the back of the leaf with tape. Tape was similarly attached to the empty leaf in the cage to control for the presence of tape. A model was discarded and replaced with a fresh model if its head, abdomen, or any wings fell off. Models were stored in a shaded area in the lab when not in use to prevent wing color from fading.

Behavioral Assay

We simultaneously tested 10-15 butterflies per run of the assay. For each run, one mated female cabbage white was placed inside each experimental cage and allowed to oviposit on the two leaves inside. We recorded the number of eggs laid on each leaf every 24 hours at approximately the same time of day for three days. We calculated the proportion of eggs laid on the leaf with models each day, and we used the average proportion across the three days of the assay, or the *social attraction score*, as the response variable in our analyses. The higher the score, the more a butterfly tends to lay eggs on leaves with conspecifics and the lower the score, the more they tend to avoid laying eggs on these leaves. Every day, eggs were removed from the leaves after counting, and the leaf pairs in each cage were rotated to another cage so that no butterfly ever experienced a pair of leaves more than once. The same pairs of leaves were used across the three days of each run of the assay because they remained turgid for that amount of time. However, leaves typically began to wilt after approximately five days. We therefore discarded all leaves after each assay, so new leaves from the same original plants were used in each run of the assay.

Abdomen Dissections

Egg load alone can affect how choosy insects are during oviposition (Doak, Kareiva, & Kingsolver, 2006). To verify that egg load did not significantly affect our data, we quantified the number of eggs remaining in the butterflies' abdomens after the oviposition assay. Butterflies that completed the assay were frozen in a sealed container and stored at - 20°C until we were ready to dissect out the remaining eggs. Abdomens were detached from the body and eggs were dissected out in 1x PBS buffer. Eggs were removed through a ventral slice in the abdomen. We counted all mature eggs, defined as eggs with a fully developed chorion- those that were fully yolked, with a yellowish color and striated shell. We used a Leica M165 C dissecting microscope at 12.5x magnification for all dissections. The number of eggs dissected out of the abdomen of each individual was added to the number of eggs it laid in the assay as a measure of fecundity. Female cabbage whites generally only produce eggs for the first week of their adult lives (Scott, 1992). We sacrificed females on the sixth day after adult emergence, so this measure of fecundity is a good approximation of all the eggs they could have laid, had we not sacrificed them.

Statistical Analysis

We analyzed our data in Rstudio version 3.0.2 (R Core Team, 2013) using analyses of variance. Larval and adult density treatments were fixed effects in our linear models, and we looked for effects of these treatments, and their interactions, on social attraction score and our measure of fecundity. In addition, we wanted to see if the mean of each of the four treatment groups was significantly different from 0.5. A mean of 0.5 is what we would expect if all butterflies in a treatment group laid eggs randomly, resulting in an approximately equal number of eggs laid on the leaf with and the leaf without models. For each treatment group, we calculated 95% bootstrap confidence intervals with 1,000 replicates using the boot package in R (Canty & Ripley, 2105) to see if a score of 0.5 was significantly different from the average scores of each group. If the confidence intervals do not include 0.5, then the treatment means are likely to be significantly different from 0.5, or random egg-laying. We also conducted a power analysis using the pwr package in R (Champely, 2015) to assess the probability of detecting an effect with our dataset.

RESULTS

The data were log-transformed to allow for parametric analyses. There were no significant effects of larval ($F(1,45) = 0.0077$, $p = 0.9304$) or adult ($F(1,45) = 0.1709$, $p = 0.6813$) treatment on social attraction score when the larval × adult interaction was not included in the ANOVA (Figure 1). This was also true with the interaction included (larval: $F(1,44) = 0.0076$, $p = 0.9311$, adult: $F(1,44) = 0.1673$, $p = 0.6845$), and the interaction was also not significant ($F(1,44) = 0.0615$, $p = 0.8053$). In addition, a model containing only the intercept was not significantly different from the model with the larval and adult treatment effects ($F(45,47) = 0.0893$, $p = 0.9147$), indicating that the treatments truly had no effects.

Our 95% bootstrap confidence intervals suggest that for social attraction score, each of the four treatment means was significantly different from 0.5, or random egg-laying, since 0.5 was outside the 95% confidence intervals for all four treatments (one larval/one adult: (0.3278, 0.4550), one larval/five adult: (0.3961, 0.4515), five larval/one adult: (0.3372, 0.4611), five larval/five adult: (0.3292, 0.4716), Figure 1). Thus, butterflies in all treatment groups were likely avoiding conspecifics at a low level. The mean of all four groups was around 0.4, where values closer to 0 indicate that butterflies avoid the leaf with models and lay a smaller proportion of their eggs on that leaf.

There were no significant effects of larval ($F(1,45) = 0.1218$, $p = 0.7287$) or adult ($F(1,45) = 0.1415$, $p = 0.7085$) treatment on our measure of fecundity (Figure 2). Again, this was true when the interaction was included in the model (larval: $F(1,44) = 0.1213$, $p = 0.7293$, adult: $F(1,44) = 0.1410$, $p = 0.7091$). The interaction was also not significant ($F(1,44) = 0.8113$, $p = 0.3726$).

The results of a power analysis on the model including the interaction between larval and adult density showed a probability of detection of 0.089 with a significance level of 0.05. For a power of 0.8 with the small effect size we obtained (0.014), the analysis indicated that we would need a sample size of approximately 780 butterflies.

DISCUSSION

We expected females in the high larval/high adult density treatment to invest relatively more in avoiding laying eggs near conspecifics, and consequently have lower fecundity. However, there were no significant differences in fecundity (Figure 2) or social attraction score among treatments (Figure 1), suggesting that cabbage white butterflies do not vary in terms of fecundity or change the extent to which they avoid conspecifics during oviposition, regardless of their previous density experiences. Rather, all treatments had very similar average fecundities and social attraction scores. All social attraction score means were approximately 0.4, indicating that butterflies consistently laid about 60% of their eggs on leaves without conspecific cues in our study. Butterflies reared under different densities all tended to avoid laying eggs near conspecifics at this baseline level of avoidance. This baseline was significantly different from random egg-laying across leaves with and without conspecifics (social attraction score of 0.5).

Several different explanations may account for the lack of any density effects and the fixed level of conspecific avoidance that we observed. The most likely possibility is that a consistent, low level of conspecific avoidance during oviposition may be a sufficient strategy for dealing with natural variation in density in this species, particularly if cues such as conspecific density are not suitable predictors for future levels of competition. There may be too much variation in conspecific density in natural environments for it to reliably predict future density at any given time. A host plant patch for a cabbage white could be anything from a few roadside weeds to a huge crop field where hundreds of individuals congregate. Additionally, adults tend to be highly mobile and do not visit all host plants within a patch (Root & Kareiva, 1984). Thus, females probably visit many patches that vary in larval density within their adult lifetimes, so past experience with conspecific density during any life stage may not be informative about current levels of competition. Unpredictable forces such as larval predation (Molleman, Remmel, & Sam, 2016) or pesticide application by humans (El-Fakharany & Hendawy, 2014; Schlaepfer, Runge, & Sherman, 2002) may add further noise. If no cue reliably predicts future conspecific density, a fixed strategy may be more adaptive than a plastic one (Reed, Waples, Schindler, Hard, & Kinnison, 2010). Thus, females might be more likely to spread eggs across different host plants with a low level of conspecific avoidance, regardless of previous experiences with conspecifics. This pattern is essentially what we observed in our study (Figure 1), with average social attraction scores of the different treatments clustering around 0.4, a low level of conspecific avoidance. Alternatively, it is possible that food availability, rather than the number of conspecific larvae *per se*, is a reliable cue that triggers differences in adult oviposition behavior (Bauerfeind & Fischer, 2005). Food availability often cues dispersal (Bowler & Benton, 2005) and could similarly cue choosy oviposition behavior in adults. We specifically did not manipulate food availability, providing adults and larvae with unlimited food so that no individuals in the high-density treatments were food-deprived. Our aim here was twofold: 1) to reduce variation in mortality across our high-density treatments so that all individuals were consistently exposed to four other conspecifics and 2) to reduce differences in food acquisition between high and low-density treatments that could affect oviposition behavior and confound our results. However, we may not have seen effects on behavior if food availability, or even an abiotic cue like temperature (Papaj, 2000) that might co-vary with conspecific density, is actually the relevant cue.

Several other alternatives might explain our results, though they seem less likely than the idea that conspecific density is an unreliable cue. Several of these alternative explanations revolve around the theme that butterflies may be able cope with the conspecific densities they experience through other means, in which case mothers' oviposition decisions may have little impact. Negative effects of high conspecific density are certainly well-documented at the larval stage in butterflies (Gibbs et al., 2004; Kivela & Valimaki, 2008). Some caterpillars may be able to avoid these effects by leaving the plant on which they hatched and moving to a different host plant, though this is likely not always possible, especially if host plant density is low, given that larvae are relatively immobile (R. Jones, 1977). Individuals that do survive high-density larval conditions may be able to compensate for negative effects on fitness during later life stages (Hyeun-Ji & Johansson, 2016; Metcalfe & Monaghan, 2001), or by investing available resources in other traits that we did not focus on in this study. For example, butterflies and other insects allocate resources differently during and after metamorphosis depending on the nutritional quality of the food they consume as larvae (Boggs & Freeman, 2005; May, Doroszuk, & Zwaan, 2015). Females can also resorb eggs as adults if food resources are scarce (Bell & Bohm, 1975). In general, females with fewer eggs may have stronger wings (Guerra, 2011) or larger eggs (Garcia-Barros, 2000). However, it is notable that variation in density across life stages did not affect female reproductive characteristics such as egg size and fecundity in the butterfly *Bicyclus anynana* (Bauerfeind & Fischer, 2005). If cabbage white butterflies are already capable of coping with high larval densities, to some extent, by altering resource allocation, we might not see strong effects of high larval density on all measures of adult reproductive investment. Indeed, variation in conspecific density across life stages did not have detectable effects on cabbage white conspecific avoidance or fecundity in our study.

It is also possible that cabbage whites *do* respond to high densities with flexible conspecific avoidance behavior during oviposition, but that our experiment was unable to detect this response. However, this again seems unlikely given our small effect size and the result that we would need 780 butterflies to detect a significant effect with a power of 0.8. Our density treatments may have been too extreme or not extreme enough. The latter is unlikely. Because our adult butterflies were housed in relatively small cages, our adult high-density treatments had higher densities than those experienced naturally by butterflies in the population we tested. For our larval high-density treatments, a density of 5 caterpillars per cup facilitates more social contact than the larvae would likely encounter outside of the laboratory, due to the small space of a 5-oz cup. We avoided larval densities higher than 5 caterpillars per cup because we did not want to confound our behavior measurements by inducing differences in survival or fitness in the larval stage. The density we chose was the maximum possible before caterpillars become diseased at high rates and emerging adults have wing deformities upon emergence due to lack of space (personal observations). One other possibility is that butterflies are not motivated to be choosy when they only have two possible oviposition sites to choose from, as in our experiment. We wanted to simplify the experiment and eliminate as many confounds as possible by providing each butterfly with two host options per day. Two-choice tests are common in oviposition assays in butterflies and moths (e.g. Bossart, 2003; Cahenzli, Wenk, & Erhardt, 2015; Groeters, Tabashnik, Finson, & Johnson, 1992; Nylin, Bergström, & Janz, 2000; Tabashnik, Wheelock, Rainbolt, & Watt, 1981). However, with so few options, being less choosy by accepting all available sites may have been a better strategy for the butterflies. We attempted to avoid this issue by providing each female with six options over the span of three days. It is possible that butterflies do not integrate or remember information about individual host plants over multiple days, although there is evidence to suggest that they can remember the color of flowers rewarded with sucrose solution for the same period of time (Kandori & Ohsaki, 1996). Regardless, if few host options contributed to low choosiness during oviposition, such a phenomenon would still be consistent with the idea that current conditions, and not past experience, is largely what determines conspecific avoidance behavior in these butterflies.

CONCLUSIONS

Broadly, our results show that fecundity and conspecific avoidance behavior in cabbage whites does not depend on conspecific density experienced during larval and adult life stages. Rather, butterflies adopted a relatively fixed strategy of moderate avoidance behavior during oviposition, regardless of the level of larval or adult conspecific density they had previously experienced. The level of conspecific density prior to oviposition may not be a reliable cue for predicting the level of competition a female's offspring will face, and thus it may not lead to changes in life history traits. A "rule of thumb" of consistently low conspecific avoidance may be sufficient for dealing with the range of conspecific density this species naturally encounters. However, *P. rapae* is somewhat unique in that it is an especially widespread species (Scott, 1992), so it could also be informative to explore the behavior of rarer species that might benefit more by adopting a flexible strategy when making decisions during oviposition. This is especially relevant given that humans are causing changes in demographic factors like density through processes such as laboratory rearing, habitat fragmentation, land use change due to agriculture, and climate change.

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CONFLICTS OF INTEREST

The authors have no competing interests.

FIGURE CAPTIONS

Figure 1. Average proportion of eggs laid on the leaf with conspecific models, a measure of choosiness, of females exposed to low or high density conditions during the larval and adult stage. Low-density conditions consisted of a single female, and high-density conditions consisted of one female plus four other individuals (total of five individuals). The first word in each treatment label corresponds to the larval density of that treatment, and the second word, after the hyphen, corresponds to the adult conspecific density. Choosiness scores close to 0.5 (dashed line) indicate random egg-laying, and are therefore associated with females that were less choosy. Scores closer to zero are associated with higher choosiness. There were no significant differences among the four treatments. Bars show 95% bootstrap confidence intervals.

Figure 2. Average fecundity of females exposed to low or high density conditions during the larval and adult stage. Low-density conditions consisted of a single female, and high-density conditions consisted of one female plus four other individuals (total of five individuals). The first word in each treatment label corresponds to the larval density of that treatment, and the second

word, after the hyphen, corresponds to the adult conspecific density. Fecundity included the number of eggs laid in the behavioral assay and the number of eggs in the abdomen. There were no significant differences among the four treatments. Bars denote standard error.

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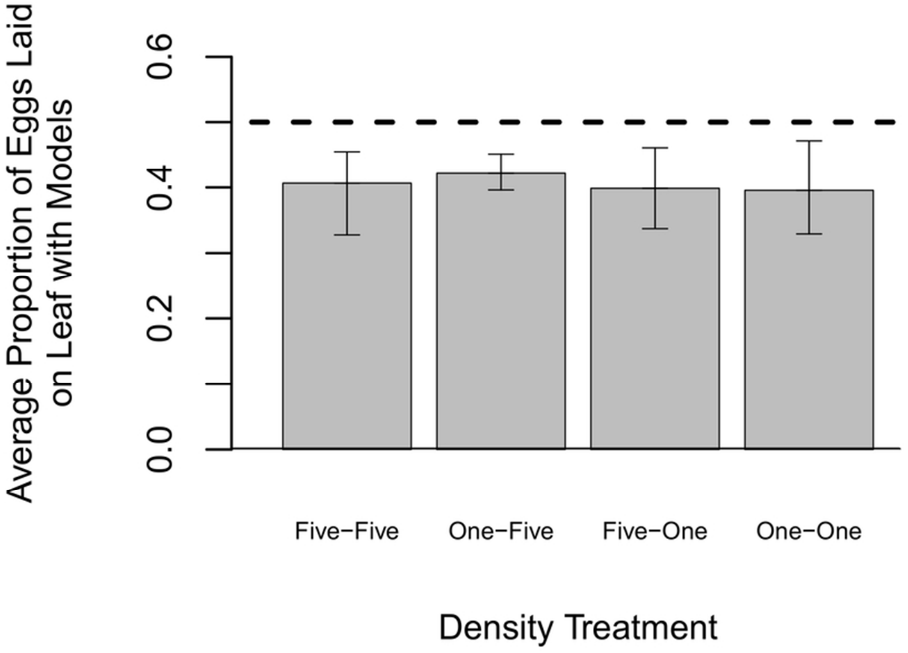

Figure 1. Average proportion of eggs laid on the leaf with conspecific models, a measure of choosiness, of females exposed to low or high density conditions during the larval and adult stage. Low-density conditions consisted of a single female, and high-density conditions consisted of one female plus four other individuals (total of five individuals). The first word in each treatment label corresponds to the larval density of that treatment, and the second word, after the hyphen, corresponds to the adult conspecific density. Choosiness scores close to 0.5 (dashed line) indicate random egg-laying, and are therefore associated with females that were less choosy. Scores closer to zero are associated with higher choosiness. There were no significant differences among the four treatments. Bars show 95% bootstrap confidence intervals.

76x57mm (300 x 300 DPI)

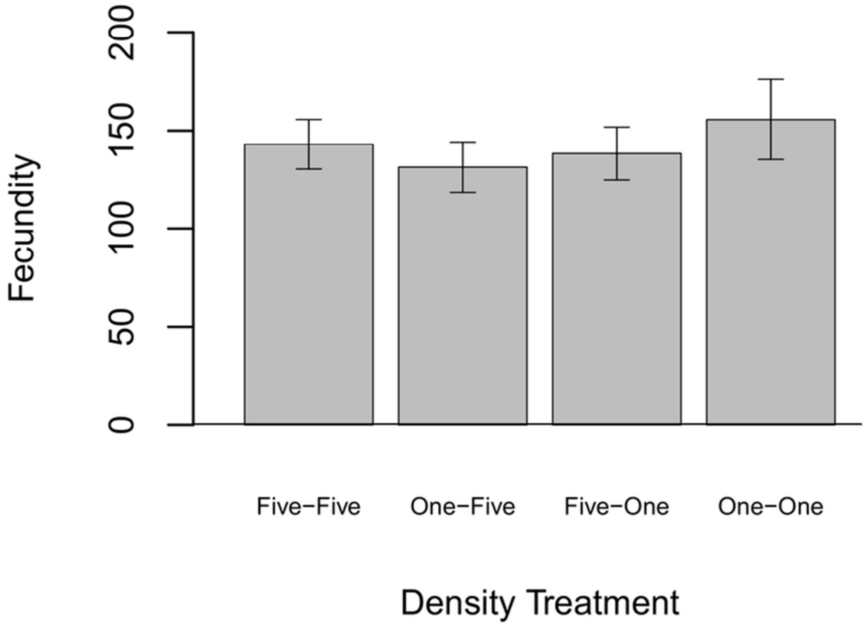

Figure 2. Average fecundity of females exposed to low or high density conditions during the larval and adult stage. Low-density conditions consisted of a single female, and high-density conditions consisted of one female plus four other individuals (total of five individuals). The first word in each treatment label corresponds to the larval density of that treatment, and the second word, after the hyphen, corresponds to the adult conspecific density. Fecundity included the number of eggs laid in the behavioral assay and the number of eggs in the abdomen. There were no significant differences among the four treatments. Bars denote standard error.

76x57mm (300 x 300 DPI)