Aggregative Behavior is Not Explained by an Allee Effect in the Walnut Infesting Fly, *Rhagoletis juglandis*

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Abstract Component Allee effects are considered to be a driving force in the origin and maintenance of aggregative behavior. In this study, we examine whether a pattern of active host reuse by the walnut fly, *Rhagoletis juglandis* Cresson (Diptera: Tephritidae), involves an Allee effect. We examined how the density of clutches deposited within a fruit, the temporal pattern in which successive clutches are deposited and the spatial distribution of clutches over a fruit's surface influences survival to pupation and pupal size. Within the density range used in this experiment (1 to 7 clutches), increases in larval density strongly reduced pupal weight but not larval survival to pupation. The temporal staggering of clutches into a host strongly reduced offspring survival and, probably owing to competitive release, increased the pupal weight of survivors. Offspring survival and pupal weight were affected relatively little by whether two clutches were deposited within the same oviposition punctures or were evenly spaced. In contrast, in three-clutch treatments offspring survival was higher when clutches were placed within the same oviposition cavity. However, pupal weights did not significantly increase when clutches were placed together and this relatively higher survival rate was not greater than that associated with hosts that contained fewer clutches. The results of the study failed to provide evidence of an Allee effect. We put forward a scenario under which females appear to reuse larval hosts to maximize their own reproductive success, albeit at the expense of the per capita fitness of their offspring.

Key words Allee effect · marking pheromone · offspring fitness · parent-offspring conflict · reproductive trade-offs · *Rhagoletis juglandis* · Tephritidae

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Introduction

In the field of insect ecology, there has been sustained interest in determining the conditions under which insects should avoid the use of resources exploited by conspecifics and when they should actively aggregate at these resources (van Alphen and Visser 1990; Parrish and Edelstein-Keshet 1999; Nufio and Papaj 2001; Prokopy and Roitberg 2001; Wertheim et al. 2005). The avoidance of, or preference for, resources that have been previously exploited by conspecifics can have particularly large fitness consequences when the resources in question are oviposition substrates. Female decisions about whether or not to aggregate clutches at previously exploited oviposition substrates are important because juveniles are often constrained to develop and potentially compete or cooperate with conspecifics at these natal resources (van Alphen and Visser 1990; Wertheim et al. 2005; Guedes et al. 2007). Insects that are known to actively avoid previously exploited larval resources are generally characterized by intense competition and they frequently avoid larval resources associated with conspecifics (Takasu and Hirose 1988; Mappes and Mäkelä 1993), cues that indicate the presence of competitors (Magro et al. 2007; Harris et al. 2006), or marking pheromones (MP) that signal the occupation of a host (Nufio and Papaj 2001). Regardless of the mechanism, the benefits associated with female avoidance of previously occupied larval resources are expected to exceed the potential costs incurred by offspring that would be forced to compete for limiting resources.

Species that actively aggregate brood at larval resources are frequently characterized by the occurrence of a component Allee effect in which an increase in parental or brood density or numbers is associated with a relative increase in per capita fitness (Stephens et al. 1999; Wertheim et al. 2005; Kramer et al. 2009). More specifically, component Allee effects are density-dependent patterns whereby benefits associated with an increase in the number of conspecifics (such as increased survival, developmental rate and body size) set a lower limit to optimal aggregation sizes while the costs associated with competition may set an accompanying upper limit (Stephens et al. 1999; Etienne et al. 2002). In insects, component Allee effects in juvenile stages result when increases in larval densities increase feeding efficiency (Clark and Mangel 1986; Wise et al. 2006) or developmental rates (Klok and Chown 1999; Fletcher 2009) or when they decrease environmental stress (Lockwood and Story 1985) or predation risks (Reader and Hochuli 2003; Codella and Raffa 1995). Species that benefit from a component Allee effect typically utilize aggregation pheromones or other mechanisms to attract and regulate the degree to which a host is exploited by conspecifics (Paine et al. 1997; Prokopy and Roitberg 2001; Wertheim et al. 2002; Lam et al. 2007).

In this paper, we investigate a system involving a tephritid fruit fly, *Rhagoletis juglandis* (a walnut specialist), in which females actively aggregate clutches within hosts (Nufio et al. 2000; Nufio and Papaj 2004a). In this system, females engage in a genus-typical host-marking behavior following the deposition of a clutch into a fruit and they more often reject heavily "marked" fruit than unmarked fruit when selecting hosts for oviposition (Nufio and Papaj 2004b). Yet, despite the deterrent effects of a putatively deposited marking pheromone (MP), *R. juglandis,* like other females in the *R. suavis* group, actively deposits clutches into fruit that have

previously received eggs, often placing their clutches directly within previously excavated oviposition cavities (Papaj 1993; Lalonde and Mangel 1994).

The reuse of oviposition cavities apparently provides female *R. juglandis* with direct benefits including reduced time to deposit clutches (Papaj and Alonso-Pimentel 1997), reduced ovipositor wear (Papaj 1993), and increased access to relatively impenetrable fruit (Lalonde and Mangel 1994). Females might thus reuse fruit because these direct benefits allow them to deposit more clutches over a lifetime and this may more than offset costs in terms of competition suffered by their larvae (Nufio and Papaj 2004a). In this case, eventual rejection of marked fruit may reflect a point at which the benefits to females associated with reuse may be outweighed by the costs associated with larval competition. However, it is also possible that females reuse fruit because their offspring benefit from being deposited into fruit in the company of other larvae. A larval benefit would provide females with a potential benefit that is independent of those associated with the direct reuse of oviposition cavities.

In walnut flies, there is reason to expect a benefit to larvae that are placed in larger groups. Not only do larvae of different clutches deposited within the same puncture feed together, larvae from clutches laid at different sites on the fruit commonly coalesce into larger feeding aggregations (C. Nufio, *unpubl. obs.*). Larvae also feed not on fresh walnut husk but on a rot that is caused in part by microbes transferred to the fruit by the female during oviposition (Howard et al. 1985; Howard and Bush 1989). Therefore, by reusing fruit, females might provide their offspring with a 'prepared medium' (Hausmann and Miller 1989). Alternatively, greater larval densities might furnish optimal conditions for growth of the microbial flora (Sang 1956; Howard et al. 1985; Courtney et al. 1990), thereby allowing early instars a greater potential for becoming established or for increasing their overall growth rates (Wertheim et al. 2002). Under such a scenario, the deterrent MP may serve to signal the upper limits of optimal aggregations sizes; a point where the costs of competition may begin to exceed the benefits associated with the aggregation of clutches (i.e., Corbet 1973; Paine et al. 1997; Lam et al. 2007).

In the present study, we evaluate whether a component Allee effect is associated with host reuse behavior in the walnut fly *R. juglandis*. We explored this question in relation to 1) the number of clutches deposited into host fruit (i.e. changes in larval density), and 2) the temporal and 3) spatial pattern of successive clutch deposition into the same fruit. In doing so, we were particularly interested in determining whether there is a positive correlation between larval density and fitness (measured as percent survival to pupation and weight at pupation) over a range of fruit infestation levels that is similar to that which occurs in nature (see below). While a previous field study failed to detect a component Allee effect in *R. juglandis*, this field study confounded larval density, time between when clutches were laid and the spatial pattern of clutch deposition on the fruit itself (Nufio and Papaj 2004a). The inability to control for female clutch distribution patterns over time and space may have obscured our ability to detect an Allee effect (Ellis 2008; Kramer et al. 2009). We therefore also sought to determine how the temporal or spatial patterning of clutches in fruit influenced larval survival and weight at pupation.

Methods

Natural History

Rhagoletis juglandis is a member of the walnut-infesting *R. suavis* group (Bush 1966). In southern Arizona, this species is found on the Arizona walnut, *Juglans major* Torr, which is common in montane canyons between 1,200 and 2,700 m. These flies are univoltine and emerge as adults in mid- to late summer prior to the ripening of walnut fruit. Larvae complete development in the husk of the natal fruit, pupate in the soil beneath the natal tree, diapause through the winter and spring and emerge as adults in the following summer. While the number of eggs deposited within a single clutch is ca. 16, within 4–5 days of first being attacked, a fruit may contain ca. 45 eggs, and it is not unusual to find fruit into which multiple females have deposited 80 or more eggs (Nufio et al. 2000; Nufio and Papaj 2004a). Thus the range of infestation levels used in the following experiments (20 to 50 eggs, rarely higher) is comparable to that found in the field.

General Protocol

Adult flies used in the laboratory experiments originated as larvae from fruit collected 1–2 years earlier in Garden Canyon in the Huachuca Mountains in southern Arizona. Until eclosion, pupae were stored at 4°C. When drawn from refrigeration, adults emerged in 4–5 weeks. Upon eclosion, adults were maintained in 3.79-liter plastic containers and provided with ad libitum water, sugar, and slips of a yeast hydrolysate and sugar mixture. Flies were held in a room with a 14:10 h light:dark cycle and a day:night temperature cycle of 32°C:28°C.

Flies used in the laboratory experiments were 12 to 30 days old, post-eclosion. In each experiment, 10 to 15 females and 5 to 7 males were placed into clear 16-fl oz (473-ml) plastic Solo cups, fitted with petri dish lids, which were furnished with water, sugar, and a yeast hydrolysate and sugar mixture. Because males often interfered with female oviposition behavior, they were removed from cages at the beginning of each day on which experiments were conducted. So as to increase the likelihood that females deposited fertile eggs, males were reintroduced into cages at the end of the day (Telang et al. 1996). During each experiment, a walnut fruit was hung from the top of the cage and were females permitted to oviposit into the fruit. After a female initiated oviposition, both fruit and fly were gently removed from the cup. Any other females present on that fruit were removed. The fruit was then hung from the top of an empty cup cage and the female was allowed to complete oviposition. After oviposition was completed, the female was immediately removed from the cup and discarded. Depending on experimental treatment (see below), a fruit was sometimes re-exposed to females. As such, the discarded female was replaced with a new female to keep the number of females per cup constant. The sites of all ovipositions on fruit were circled with a felt-tip marking pen.

For each fruit in each study, we measured the minimum and maximum length in order to estimate the volume of a given host. This was done by assuming that a walnut was spherical in shape, taking the average of the length measurements as an

estimate of sphere diameter, and then computing fruit volume as $4/3\pi\ r^3$, where r is the radius of the sphere.

After fruit received the appropriate number of clutches in the appropriate locations, they were wrapped in parafilm so as to minimize water loss. Fruit were then placed individually into incubators and stored in a growth chamber at a constant temperature of 30°C and 50% humidity. The incubators were 16-fl oz Solo cups with plastic petri dishes inserted onto their wide tops. The original bottoms of the cups were then removed and the cups were then inverted. Infested fruit were placed within the cups, resting on a 4 cm long by 3.5 cm wide PVC tubing inserted into a 3 cm deep bed of mixed vermiculite and sand. The vermiculite/sand layer was kept moist by adding water periodically until Day 15, the earliest day at which larvae emerged from a fruit. Six to seven days after clutch deposition, the parafilm covering a fruit was removed and oviposition cavities were excavated by cutting a cylinder roughly 6 mm long and 8 mm wide around the puncture which contained the oviposition cavity. To keep the fruit from drying out and larvae from prematurely leaving the host fruit, the space previously occupied by the oviposition cylinder was then covered with a piece of parafilm over which was placed a 15 by 20 mm strip of elastic bandage tape. Excavated cavities were stored individually in alcohol in vials and later dissected. The unhatched eggs and egg husks present within each oviposition cavity were counted. The number of egg husks, which are left by individuals that had hatched and migrated into the husk, was used as an estimate of the number of larvae hatching within the fruit.

In order to examine effects of different patterns of reuse on offspring fitness, we calculated total percent survival, as the percentage of eggs deposited into a given fruit that successfully developed to pupation. We also calculated percent survival of hatching eggs to successful pupation. To assess the impact of reuse patterns on offspring size, viable pupae were weighed. The usefulness of pupal weight as a predictor of lifetime female fecundity is documented in Nufio and Papaj (2004a).

Experiment 1: Clutch Density

In the first laboratory experiment, we manipulated the number of clutches within fruit to evaluate a component Allee effect. Egg density was manipulated by distributing walnuts into sets of five that were paired for size and ripeness, then exposing these fruit to females, as described in the general protocol above. Over the course of 1 to 3 h, females were allowed to place a total of 1, 2, 3, 4 or 5 clutches into each of the fruit in a set. Females were free either to deposit clutches into new cavities anywhere on the host fruit or to reuse previously made oviposition punctures. The number of clutches that a given fruit received was a function in part of how many females attempted oviposition. In order to control for among-cage variation in oviposition activity, fruit were often transferred among cages. Finally, in this and all other experiments, we also examined whether females might lay larger clutches into larger fruit determine whether female egg-laying patterns might be influenced by host quality.

After fruit were removed from a cage, they were wrapped in parafilm and stored in a growth chamber, as above, and their cavities were removed after 6–7 days. The cavities were dissected and number of hatched and unhatched eggs. After larvae had

emerged from to fruit and pupated, the number and weight of viable pupae was recorded.

Experiment 2: Temporal Spacing of Clutches

In order to determine whether any component Allee effect depends on time between successive clutch deposition events, we manipulated the temporal spacing of clutches into fruit by matching sets of four fruit for size and ripeness and exposing these fruit to females, as described above. In one of the four fruit, two clutches were deposited on the same day (i.e., within a 1–3 h period) into a single oviposition site. In a second fruit, four clutches were deposited on the same day (i.e., within a 1–3 h period) in sets of two clutches at each of two different sites. In a third fruit, two clutches were deposited into a single puncture on the same day and roughly two days later, two more clutches were deposited into a second site. In a fourth fruit, two clutches were deposited into a single site on the first day and four days later two more clutches were placed into a second site.

In the treatments involving two sets of two clutches, the last two clutches were deposited at a single site roughly 1.5 cm from where the first two clutches were deposited. For replicates that required multiple clutches to be deposited within a single site, gravid females were induced to reuse sites by gently brushing them towards a previously created oviposition puncture or towards pricks made in the fruit with a No. 00 pin roughly 1.5 cm from the first puncture.

After both clutches were deposited in a fruit on a given day, the fruit was wrapped in parafilm and stored in individual "incubators' in a growth chamber as described above. Fruit were than unwrapped either 48 or 96 h later, as determined by the protocol, at which time two additional clutches were deposited. Fruit were then rewrapped in parafilm and placed again into the growth chamber. After 6–7 days, the parafilm was removed, oviposition cavities (once they themselves were 6–7 days old) were excavated as described above, and unhatched and hatched eggs within the cavities counted. We later counted and weighted the viable pupae that emerged from a fruit.

Experiment 3: Spatial Distribution of Clutches

In order to determine whether a component Allee effect depends on whether clutches are placed together, we manipulated the spacing of multiple clutches on fruit. Clutch spacing was manipulated by matching sets of five fruit for size and ripeness and then exposing these fruit to females which were allowed to either lay a single clutch within a fruit, two clutches within the same oviposition puncture, two clutches each placed on opposite sides of the fruit, three clutches within the same puncture, or three clutches spaced equal distance apart around a fruit's perimeter.

Ovipositions were obtained generally as described above for egg density. In order to ensure that clutches were distributed around a fruit according to treatment designation we initiated oviposition punctures by lightly pricking the fruit surface with a no. 00 insect pin and females attempting to oviposit into the fruit were lightly brushed to these punctures where they readily deposited their clutches. After a clutch was deposited into a site initiated by a pin prick, the oviposition site was temporarily

covered with tape to prevent further reuse and a new pin prick on the opposite side of the fruit was created before being exposed to the next set of females. All clutches were deposited within the fruit over a 1 to 3 h period. Fruit were then wrapped in parafilm and stored in individual "incubators" in a growth chamber, as above, and their cavities were removed after 6–7 days. Oviposition cavities were dissected after 6–7 days and unhatched and hatched eggs within the cavities counted. We later counted and weighted the viable pupae that emerged from a fruit.

Statistical Analysis

In order to identify factors that influence percent total offspring survival (from deposited egg to successful pupation) in our density experiment, we first conducted an ANOVA to determine whether there was a significant treatment effect (number of clutches) and then conducted Tukey HSD pair wise comparisons between the different treatments. To more precisely measure the effects of egg and larval densities on offspring survival, we also conducted multiple regression analyses with either percent total survival (from deposited egg to successful pupation) or percent larval survival (from egg hatch to successful pupation) as the dependent variables and the number eggs deposited and fruit volume per egg, or in the second case larvae, as the independent variables. While actual percent values are provided in the figures, in all statistical analyses using survival data, percent total and egg-hatch to pupation survival data were arcsine square root transformed to meet the assumptions of normality.

To identify the factors that influenced pupal weight within the density experiment, we first conducted an ANOVA to determine whether there was a significant treatment effect (number of clutches) and conducted Tukey HSD pair wise comparisons between the different treatments. To more precisely measure the effects of larval densities on pupal weight, we then conducted a multiple regression analysis with median pupal weight as the dependent variable and number of larvae that hatched within a fruit and fruit volume per larva as the independent variables. Because pupal weights were not always normally distributed on a per fruit basis, we felt that the median pupal weight of individuals from a given fruit was a more appropriate measure of pupal weights than was the mean; no analyses produced different results when the mean values were used. The median pupal weight recorded for each fruit was normally distributed among fruit and as such parametric statistics were used.

For experiments 2 and 3, we used a general linear model (GLM) to determine whether the number of clutches deposited and either the temporal or spatial placement of the clutches explained percent total survival and median pupal weight. We also conducted pair wise comparisons to highlight difference between treatments. In the temporal spacing experiment, we conducted an ANOVA to compare the within fruit variances associated with the different density and temporal spacing treatments. Because we could not know whether the juveniles emerging and pupating from the temporally staggered fruit were from the first or second sets of deposited clutches, this test was used as an indirect method for accessing the within fruit weight disparities that might exist between clutches deposited simultaneously or delayed by two or four days. In this analysis, within fruit weight variance was log transformed

to meet the equal variance assumption of an ANOVA. In all multiple regressions, ANOVA and GLM analyses we tested for interactions between variables but no interactions were found. All statistical analyses were conducted using JMP-IN statistical software (SAS 2000).

Results

Experiment 1: Clutch Density and Offspring Fitness

Within the larval density range used in the first experiment (Fig. 1a), we found that offspring survival and weight at pupation no point increased with increases in larval density. Although not significant, however, total percent survival (from egg deposition to successful pupation), appeared to decline, not increase, with the number of clutches placed within a fruit ($F_{4,60}=0.47$, $P=0.75$; Figure 1b). A multiple regression analysis showed that neither the number of eggs placed within a fruit ($F_{1,63}=0.34$, $P=0.56$) nor fruit volume available per egg explained offspring survival ($F_{1,63}=0.58$, $P=0.45$). When considering only the percent survival from egg-hatch to successful pupation, neither the number of eggs hatching within a fruit ($F_{1,64}=0.12$, $P=0.29$), nor fruit volume per larva explained larval survival ($F_{1,64}=0.02$, $P=0.86$).

An increase in the number of clutches deposited within a fruit was associated with a reduction in median pupal weight ($F_{4,58}=11.47$, $P<0.0001$, Fig. 1c). A multiple regression analysis found that both the volume of fruit per larva ($F_{1,61}=26.8$, $P<0.001$) and the number of larvae within a fruit ($F_{1,61}=8.79$, $P=0.004$) explained median pupal weight. This suggests that independent of the amount of food available per individual, the more eggs are placed with a fruit, the lower are the pupal weights (Fig. 2). A qualitative examination of the number of clutches placed within a fruit and larval survival and pupal weight did not suggest that intermediate brood densities were associated with greater offspring fitness, but rather that fitness components declined monotonically with increases in brood density (Figs. 1 and 2).

Regardless of the number of clutches deposited within a fruit, we found a significant relationship between fruit volume and the total number of eggs that were deposited into the fruit ($r^2=0.16$, $n=65$, $P=0.001$). This finding was not due to females laying more clutches into larger fruit as average fruit volume ($18.99\pm$SE 0.97 cm^3) did not differ significantly among clutch number treatments ($F_{4,64}=0.29$, $P=0.88$). This pattern, however, appeared to be a result of females depositing larger clutches into larger fruit (relationship between clutch size and fruit volume in 1-clutch fruit; $r^2=0.46$, $n=14$, $P=0.008$).

Experiment 2: Temporal Spacing of Clutches and Offspring Fitness

Changes in the number of days between when clutches were deposited within a fruit significantly affected both larval survival and pupal weight, although clutches deposited simultaneously did not experience both higher survival and pupal weights. Mean total percent survival differed among treatments ($F_{3,80}=15.12$, $P<0.0001$; Figure 3b) with the two clutch treatment having significantly higher survival than the four clutch treatments. Within the four clutch treatments, the treatment in which pairs

Fig. 1 Effects of offspring density within a walnut host on offspring survival and weight. **a** Mean number of eggs (± SE) deposited within each treatment. **b** Percent survival (± SE) of offspring within each treatment. **c** Median pupal weight (± SE) of offspring emerging from a fruit and successfully pupating. The number of replicates for each treatment range from 11 to 14. Bars sharing same letters are not significantly different (Tukey HSD, $P<0.05$)
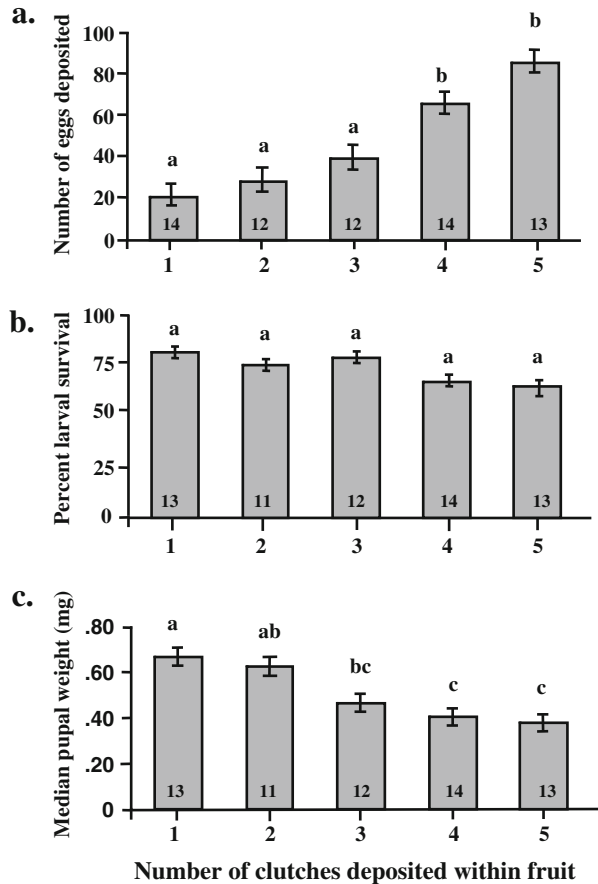
of clutches were separated by four days had the lowest percent total survival (Fig. 3b). A GLM using the four clutch treatment data only, showed that differences in total survival were related to treatment effects (whether the four clutches were separated by 0, 2 or 4 days) ($F_{2,62}=9.10$, $P=0.0003$) and not by the amount of fruit volume available per deposited egg ($F_{1,62}=2.25$, $P=0.14$). Specifically, additional clutches placed into a fruit four days after the initial clutches were deposited appear to have lower per-capita survival than clutches deposited earlier, although survival was still greatest in the two clutch treatment (Fig. 3b).

Median pupal weight differed significantly among treatments ($F_{3,88}=25.90$, $P<0.0001$, Fig. 3c). While percent survival declined as clutches were separated by greater amounts of time, a GLM showed that median pupal weight progressively increased from when clutches were non-staggered to staggered-two-days to staggered-four-days ($F_{2,63}=17.76$, $P<0.0001$) and that this increase in weight was not explained by differences in the amount of available resources per hatched larva ($F_{1,62}=2.25$, $P=0.14$). The significant difference in median pupal weight between the non-staggered and two-days-staggered treatments is not so readily explained, because total percent survival did not differ significantly between those two

Fig. 2 a Median pupal weight (± SE) as a function of the number of eggs that hatched within a fruit. b Median pupal weight (± SE) as a function of the available fruit volume per egg that hatched
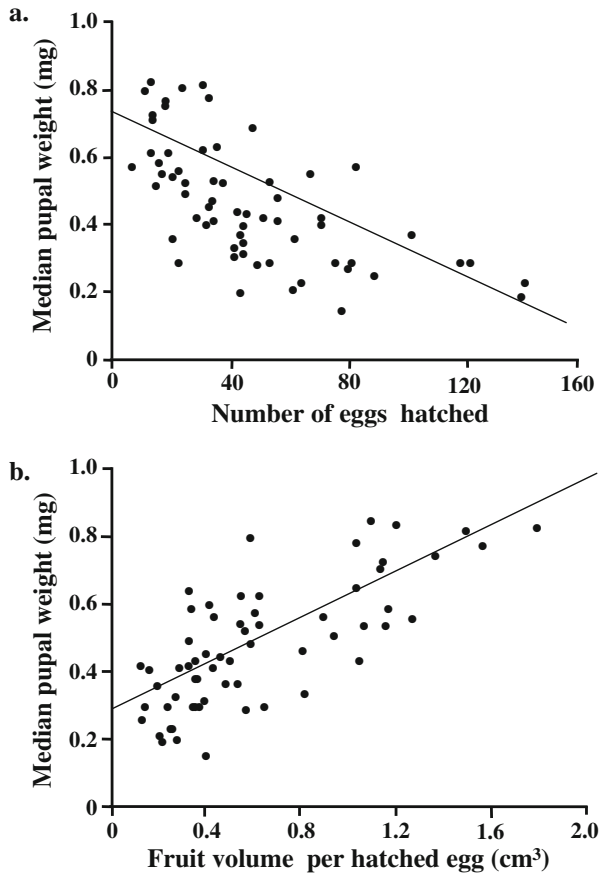

treatments, although the non-staggered treatment did have marginally more eggs deposited within the fruits than the other temporally staggered treatment (see below). Finally, the within fruit pupal weight variance significantly differed between treatments ($F_{3,84}=9.19$, $P<0.0001$; Figure 4) and, among the four clutch treatments, this variance increased with increases in the temporal spacing of clutches.

While the number of eggs deposited in the four-simultaneous-clutches treatment was higher than that of the four-clutches-staggered treatments, the difference was only marginally significant (Fig. 3a; $F_{2,67}=2.88$, $P=0.06$). The mean fruit volume, (24.06 ± 0.29 cm^3), however, did not differ significantly among all treatments ($F_{3,102}=1.16$, $P=0.33$). Finally, similar to the density experiment above, among the pooled four clutch treatments, there was a significant relationship between fruit volume and the number of eggs that were deposited into the fruit ($r^2=0.07$, $n=68$, $P=0.03$).

Experiment 3: Spatial Distribution of Clutches and Offspring Fitness

The spatial distribution of clutches experiment showed that both changes in offspring density and the spatial placement of clutches impacted offspring fitness. Mean total

Fig. 3 Effects of offspring density and temporally spacing clutches within a walnut host on offspring survival and weight. **a** Number of eggs (± SE) deposited within each treatment. **b** Percent survival (± SE) of offspring within each treatment. **c** Median pupal weight (± SE) of offspring emerging from a fruit and successfully pupating. The number of replicates for each treatment range from 21 to 24. Bars sharing same letters are not significantly different (Tukey HSD, $P<0.05$)
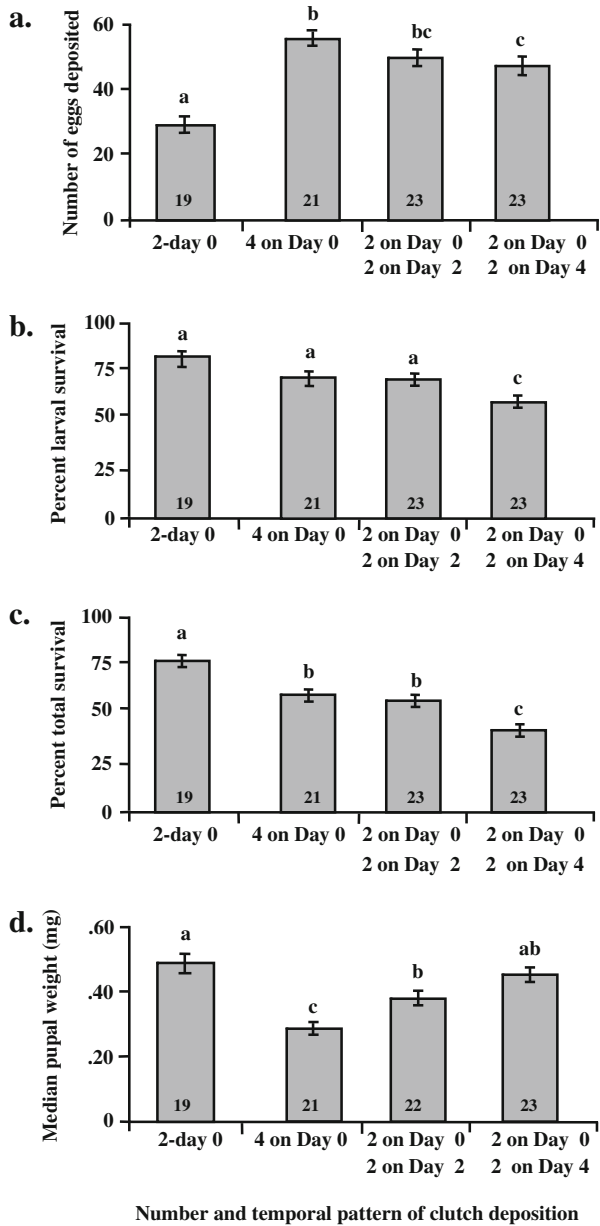
percent survival differed significantly among the two and three clutch treatments ($F_{1,48}=2.82$, $P=0.04$) (Fig. 5b). A GLM, using two-clutch and three-clutch treatment data only, showed that the differences in total survival were marginally related to clutch spacing (whether clutches were placed together or separately along a fruit) ($F_{1,43}=3.60$, $P=0.06$) and not by clutch number ($F_{1,43}=0.04$, $P=0.55$). This finding was largely due to the separated three clutch treatment experiencing a lower survival rate.

Fig. 4 Within fruit variance in pupal mass (mean±SE) associated with pupae emerging from fruit that vary in number of clutches (2 vs. 4) and the temporal spacing of clutches (4 clutches spaced 0, 2 and 4 d apart). Bars sharing same letters are not significantly different (Tukey HSD, $P<0.05$)
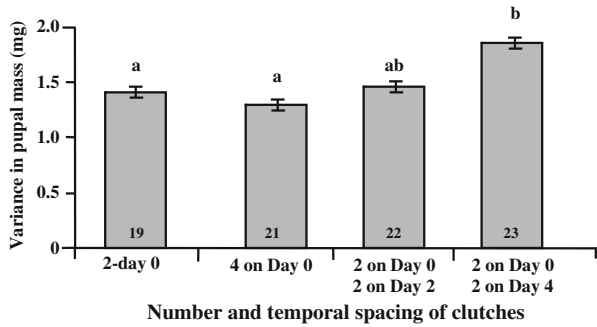
A GLM, using two-clutch and three-clutch treatment data only, showed that pupal weights were explained by the number of clutches deposited within a fruit ($F_{1,48}=9.30$, $P=0.003$) and not by whether the clutches were placed together or apart ($F_{1,48}=1.93$, $P=0.17$). Still, the lower survival of the separated three clutch treatment led surviving larvae in this treatment to experience a relative increase in pupal weight, such that their

Fig. 5 Effects of offspring density and spatial distribution of clutches within a walnut host on offspring survival and weight. **a** Mean number of eggs (± SE) deposited within each treatment. **b** Percent survival (± SE) of offspring within each treatment. **c** Median pupal weight (± SE) of offspring emerging from a fruit and successfully pupating. Number of replicates for each treatment range from 11 to 14. Bars sharing same letters are not significantly different (Tukey HSD, $P<0.05$)
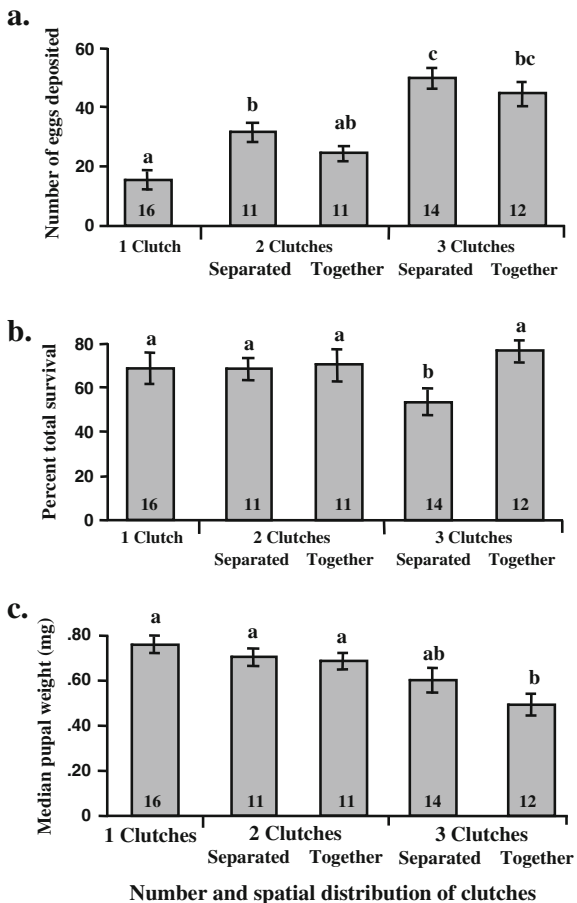
weights were no different from those achieved in the one and two clutch treatments (Fig. 5c).

While the number of eggs deposited within fruit differed among treatments ($F_{2,59}=32.9$, $P<0.001$), the number of eggs deposited within fruit did not differ within the two or three-clutch treatments (Fig. 5a). Mean fruit volume (overall, 25.33 ± 1.05 cm^3) did not differ among all treatments ($F_{4,60}=0.94$, $P=0.45$) and in this experiment we did not find a relationship between fruit size and the number of eggs that were deposited into the fruit ($r^2=0.003$, $n=60$, $P=0.68$). We did not find such a relationship when looking within 1, 2 and 3 clutch treatments, nor when we controlled for clutches being placed together or apart ($P<0.05$).

Discussion

In this study, we found no component Allee effects associated with increases in larval densities. While percent total survival and survival from egg hatch to successful pupation was surprisingly little impacted by an increase in the number of offspring deposited within a fruit, increasing larval densities were consistently accompanied by reduced pupal weight. Given that weight at pupation was always highest when fewer clutches were deposited within a host, reuse of hosts in this system is consistent with a model of competition and not with special benefits enjoyed by offspring that are placed together. The effects of density were modified by different temporal and spatial clutch distribution patterns but in no instance were both offspring survival and weight higher when more clutches were deposited in a fruit. The results of our study are consistent with the hypothesis that host reuse behavior in female walnut flies is associated with benefits enjoyed by females (see below) and not by the offspring that are forced to compete with conspecifics (Nufio and Papaj 2004a). In turn, the eventual rejection of "heavily" marked fruit may reflect a point at which the benefits to females associated with reuse may be outweighed by the costs associated with larval competition (Nufio and Papaj 2004b). In the following sections, we briefly discuss the findings of each larval rearing experiment and discuss the conditions under which females may be selected to aggregate offspring even at the expense of those offspring's fitness.

Density Effects

In the first experiment, using a larval density range that was similar to those found in nature, we did not detect a relationship between pre- and post-hatching survival and the number of clutches deposited within a fruit. However, pupal weight (a strong predictor of lifetime female fecundity [Nufio and Papaj 2004a]) declined significantly both with increases in larval densities and with decreases in fruit volume per larva. Increases in larval densities were associated with reductions in pupal weight independent of how densities affected the availability of larval resources (Fig. 2). Given that increases in larval densities do not affect survival but strongly impact weight at pupation, it appears that scramble competition for limited food resources explains the dynamics among these same aged conspecifics better than an exploitative model of competition (Guedes et al. 2007). In short, for the

components of larval fitness estimated here (survival and weight at pupation), no component Allee effects were detected with increases in offspring density.

Temporal Spacing of Clutches

As with the density experiment, results of the temporal spacing experiment failed to provide evidence of a component Allee effect. In particular, survival and pupal weight in 4-clutch treatments was never higher than that of the 2-clutch treatment (Fig. 3). Moreover, the temporal spacing of clutches also affected total survival and pupal weight of progeny in a manner wholly consistent with a pattern of competition between clutches. Percent survival in this experiment was greatest when only two clutches were deposited in a fruit and lower in all other treatments receiving four clutches (Fig. 3b). Within the four clutch treatments, a delay of four days significantly reduced overall survival. The lower survival, presumably of the later laid clutches in the four day delayed treatment could be a result either of a competitive superiority experienced by earlier clutches (Averill and Prokopy 1987; Dukas et al. 2001), by early clutches simply consuming available resources, and/or by a decline in fruit quality being caused by larval infestation (Huang et al. 1971). The lower survival in this later treatment is consistent with the finding that the pupal weight of surviving progeny was similar to that in the treatment that only contained two clutches. This result could not be obtained if the later laid clutches made up most of the survivors because these later clutches would have fewer available resources for development; resources that would have been at least partially consumed by the earlier brood. In addition, increases in the within fruit pupal weight variance were associated with an increase in the temporal staggering of clutches. This pattern in pupal weight variance suggests that the age difference between the earlier and later laid clutches heightened the competitive advantage of earlier clutches that consequently attained larger pupal mass than survivors of the later laid clutches (Dukas et al. 2001).

Spatial Distribution of Clutches

As with the density and temporal spacing experiments, results of the spatial distribution experiment failed to provide evidence of an Allee effect. In this experiment, we found that clutches placed in the same oviposition cavity sometimes performed better than clutches spaced uniformly around the fruit. That is, whether clutches were placed together or spaced apart did not seem to affect total survival or median pupal weight of the two-clutch treatments, although within the three clutch treatments offspring survival was greatest when the clutches were placed together (Fig. 4b). Perhaps survival is greater when larvae are placed together because this increases their developmental rates or because a localized and moving larval front degrades the host at a higher rate. Still, while reuse of oviposition punctures may lessen larval mortality, offspring are not experiencing a correlated gain in pupal weight that would be expected if clutches placed together experience an overall fitness benefit. In turn, pupal weights were similar or lower, but never higher, when more clutches were placed into a fruit and survival was never greater (Fig. 4b & c).

The absence of a component Allee effect and the presence of competition associated with increases in larval infestation levels is consistent with the results of a previous field study that also detected a negative relationship between infestation levels and offspring fitness (Nufio and Papaj 2004a). In the field study, however, a fruit harboring a relatively high density of eggs was also a fruit in which the time between first and last clutches was nearly a week long (Nufio and Papaj 2004a) and oviposition punctures, while often aggregated in close proximity, could also be spaced apart along the fruit surface (C. Nufio, *unpubl. obs.*). While the present laboratory study failed to detect a component Allee effect associated with different reuse patterns (i.e. through an increase in survival and/or weight at pupation), it did show that larval density and the spatial and temporal spacing of clutches could have significant impacts larval fitness. That is, females may lessen the effects of larval competition not only by placing fewer clutches into fruit, but also by placing clutches into fruit in close temporal and spatial proximity. In turn, in the density and temporal spacing experiments we found a significant relationship between fruit size and the number of eggs deposited within. This suggests that females may also minimize competition between progeny and clutches, by allocating more eggs to fruit that may provided greater resources.

Why Do Females Reuse Hosts?

If reuse of larval hosts reduces offspring survival and weight at pupation, why do *R. juglandis* females reuse hosts as much as they do (Papaj 1993; Nufio et al. 2000; Nufio and Papaj 2004a)? In regards to the evolution of aggregative behavior, such behavior can evolve not simply as a result of its positive impacts on offspring fitness (through a component Allee effect), but also as a result of the benefits incurred by females that are able to deposit more clutches over a lifetime (Rausher 1980; Nylin et al. 1996; Scheirs and De Bruyn 2002). While this study suggests the latter for *R. juglandis*, future studies should explore whether host reuse patterns in this system might actually lead to a demographic and/or component Allee effect when females themselves are found at higher densities (Courchamp et al. 1999; Stephens et al. 1999). In such a case, an increase in the number of available females may increase the ability of females to access host resources (Prokopy and Duan 1998; Diaz-Fleischer and Aluja 2003) or find potential mates (Gascoigne et al. 2009) and these benefits, which can increase the number of progeny produced over a lifetime, may exceed the fitness costs (in terms of survival probability and size at pupation) incurred by their progeny.

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