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Resource competition affects developmental outcomes of the acoustic parasitoid fly *Ormia ochracea*

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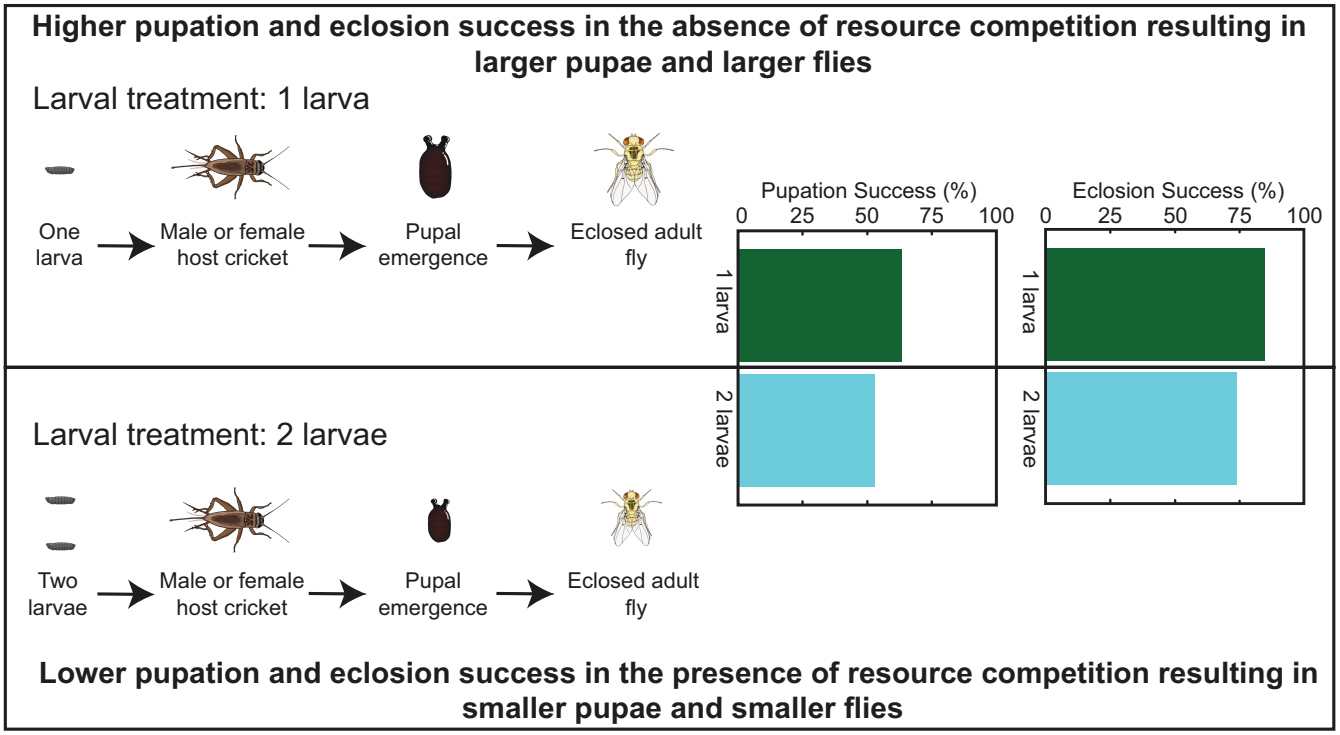
In parasitoid systems, resource competition can significantly impact developmental outcomes. This study investigates how larval competition and host characteristics influence development in the acoustic parasitoid fly *Ormia ochracea*, using the house cricket *Acheta domesticus* as a host. We experimentally manipulated larval load (1 vs. 2 larvae per host) and recorded host sex and size to assess their effects on pupation and eclosion (adult hatching) success, as well as pupal and adult fly size. While double infestations increased total yield (0.78 vs. 0.54 flies per host), larvae developing without competition exhibited higher relative pupation and eclosion success and produced larger pupae and adult flies, indicating greater individual fitness. Although female host crickets yielded larger pupae, resource competition was the dominant factor shaping developmental outcomes. These results highlight the trade-offs between reproductive yield and offspring fitness driven by resource competition and validate the commercially available *A. domesticus* as a viable host.

Keywords: larval development, endoparasite, resource competition, host size

Graphical Abstract



Introduction

In insect parasitoids, competition for limited resources can affect trait expression and life-history evolution (Ode et al. 2022). While adult parasitoids are free-living, their larval young depend on nutritional resources within hosts for growth and development (Godfray 1994). Consequently, the dynamics of resource availability, together with extrinsic competition (among adults for hosts) and intrinsic competition (among immatures within host), can profoundly shape developmental trajectories and fitness outcomes (Harvey et al. 2013, Ode et al. 2022). For example, some parasitoids exhibit preferences for larger hosts and may parasitize such hosts with more eggs, as larger hosts may support the development of a larger number of offspring (Nechols and Kikuchi 1985). Furthermore, the size of the emerging parasitoid is often positively correlated with the size of its host (Kouamé and Mackauer 1991, Cohen et al. 2005). Thus, host size and level of competition may affect resource availability and developmental outcomes of parasitoids.

The tachinid fly *Ormia ochracea* is a larvi- and viviparous endoparasitic dipteran acoustic parasitoid that relies on host field crickets (Gryllidae) for the development of their larval young (Cade 1975, Godfray 1994). Gravid females locate host crickets by homing in on cricket calling songs (Cade 1975, Mason et al. 2001, Müller and Robert 2001). Their auditory system is tuned to the carrier frequency of male cricket calling songs (Robert et al. 1992, 1998, Oshinsky and Hoy 2002, Latham et al. 2024, Hoy 2025, Wikle et al. 2025), and song recognition is based on processing the temporal patterning of sound pulses (Walker 1993, Lee and Mason 2017, Lee et al. 2019, Jirik et al. 2023). Once *O. ochracea* detect suitable host songs (Gray et al. 2007, 2019), they engage in flying phonotaxis (Müller and Robert 2001), land, and continue with walking phonotaxis to the sound source (Mason et al. 2005, Lee et al. 2009), where they deposit planidia (first instar larvae) near or on top of host crickets. These planidia will wave their anterior end in the air, attach to a potential host cricket, and subsequently burrow into the host for development (Adamo et al. 1995a).

Larval development within a host cricket occurs for 6 to 10 d (Adamo et al. 1995a). During the first 3 d of development, the planidia appear to feed on hemolymph while leaving muscle tissue undisturbed. By the fourth day, planidia migrate to the abdomen, molt, attach to the abdominal wall, and form a respiratory funnel, which provides protection from the host immune system and allows the planidia to maintain contact with outside air. The planidia will continue to molt and feed on surrounding tissue. During the last 2 d of parasitization, planidia will feed on the fat body and abdominal and thoracic muscles but will spare the digestive system and the central nervous system. Immediately before emergence from the host, larvae will purge their gut contents inside the host, which ultimately leads to cricket death. The larvae will then exit the host cricket and pupate within a few hours (Adamo et al. 1995a).

In the field, flies can deposit 1 or several larvae on a host cricket (Adamo et al. 1995b). Both male and female crickets are known to be parasitized by *O. ochracea* (Wineriter and Walker 1990), but since *O. ochracea* are attracted to calling songs that are only produced by males, males have been found to be parasitized at a higher rate than compared to females (Zuk et al. 1993, Adamo et al. 1995b). In the field, host crickets parasitized by *O. ochracea* were most often harboring 1 to 2 larvae (Adamo et al. 1995b, Kolluru and Zuk 2001) although sometimes more (Gallagher et al. 2024), but it has not been reported in these studies whether some or all of these larvae are able to successfully develop to eclosion—the hatching of adult flies from pupae. When larviposition behavior was observed in the lab, crickets were never parasitized with more than 2 larvae. However, cricket grooming behavior can help remove larvae before they burrow into the cricket (Vincent and Bertram 2010a).

Previous work has mainly examined parasitization outcomes in *O. ochracea*'s natural host species (Cade 1975, Adamo et al. 1995a, 1995b), and in alternative host species that are also present in their

natural environment (Thomson et al. 2012, Broder et al. 2023). These studies determined that *O. ochracea* can develop within alternative field cricket species, such as *Gryllodes sigillatus*, *Gryllus bimaculatus*, and *Modicogryllus pacificus* in Hawaii (Broder et al. 2023), and *Gryllus assimilis* in Texas (Thomson et al. 2012). Few studies have looked at fly developmental outcomes utilizing the house cricket *Acheta domesticus* as a host. *A. domesticus* can be easily obtained from commercial suppliers and the ease of access to large supplies of *A. domesticus* can allow for the propagation of thriving laboratory colonies of *O. ochracea*.

In this study, we investigate how host traits (size and sex) and resource competition (single vs. dual larval infestations) influence developmental outcomes in *O. ochracea*. Specifically, we assess how these variables affect pupation and eclosion success, measured both proportionally and in absolute numbers, as well as the pupal size and the size of first-generation (F1) adult flies. To test these effects, we manually parasitize male and female *A. domesticus* of varying sizes and manipulate larval load per host to alter levels of resource competition directly. Our aims are to identify the conditions that best optimize larval development, balance the trade-off between reproductive yield (offspring number and proportion) and individual fitness (fecundity, inferred from pupal and adult body size), and to evaluate the viability of *A. domesticus* as a host.

Methods

Animals

Recently molted adult *A. domesticus* crickets (4 to 6 d post-final molt) were acquired from a supplier (Bug Co., Ham Lake, MN). A total of 600 crickets (300 males and 300 females) were used as host crickets for the study. Ten gravid female *O. ochracea* from a laboratory colony that originated from Gainesville, Florida were used as larval donors.

Animal Care

Crickets

Before being parasitized and individually housed (without a specialized oviposition substrate), crickets were kept in large population containers where they were allowed to mate. After parasitization, *A. domesticus* were housed individually in 3.25 oz sauce cups with perforated lids to allow for respiration. Crickets were kept at approximately 21 °C in a laboratory room with natural lighting. We provided the animals with water and food (Purina Complete alfalfa rabbit feed pellets) ad libitum. Containers were checked daily for fly larval emergence, pupation, and animal deaths.

Flies

Donor *O. ochracea* were reared in temperature-, humidity-, and light-controlled environmental chambers (Power Scientific Inc, model DROS52503, Pipersville, PA) set to a 12 h light/12 h dark cycle at 75% humidity, 25 °C, and provided with butterfly nectar (The Birding Company, MA) ad libitum. Eclosed F1 flies were kept in the same sauce cup container as the cricket they parasitized. Flies were fed butterfly nectar via a cotton stick that was changed daily.

Morphometrics

We measured morphometric traits for host crickets, fly pupae, and eclosed flies (Fig. 1). For host crickets, we recorded pronotum width (Fig. 1A), pronotum length (Fig. 1A), hind femur length (Fig. 1B), body mass, and noted the host cricket sex. For fly pupae, we measured pupal width and length (Fig. 1C). For eclosed flies, we measured mesothorax length (Fig. 1D).

Mass vs. Exoskeletal Dimensions as a Measure of Host Cricket Size

Both body mass (eg Lehmann (2008)) and pronotum length (Judge and Bonanno 2008) can serve as proxies for field cricket size. In parasitoid–host interactions, body mass is widely regarded as a direct measure of the nutritional resources available for parasitoid larvae (Nakamura 1995, Reitz and Adler 1995, Lehmann 2008). This is reflected in the parasitoid-to-host weight ratio, with Ormiini pupae typically weighing 3% to 10% of their host's preinfestation mass (Lehmann 2008).

However, body mass can fluctuate due to changes in hydration and gut contents (MacMillan and Sinclair 2011), making structural size a more consistent metric for assessing host and developmental outcomes. Structural traits, such as fly tibia length, have been shown to correlate with fecundity in other tachinid species, with larger flies producing more eggs (Reitz and Adler 1995, Lauziere et al. 2001). This is particularly relevant for *O. ochracea*, where planidial loads per gravid female can range from 65 to over 500 (Wineriter and Walker 1990). Given these considerations, we used pupal width as our primary developmental metric, as it is less influenced by hydration variability than pupal mass. Additionally, width measurements are more practical for large sample sizes, allowing for rapid and consistent data collection with minimal handling, thereby reducing measurement error.

Imaging

Crickets were prepared for imaging by cold anesthetization in a −20 °C freezer for 10 min. This allowed the experimenter to orient and align the cricket's dorsal aspect upward for imaging the pronotum (Fig. 1A) and the ventral aspect upward for hind femur imaging (Fig. 1B). These images were taken prior to the manual parasitization procedure. After taking the images, crickets were weighed using a digital scale (Sartorius Entris, 124i-1S Balance), given a unique cricket ID, and entered in our data collection log.

Pupal images were taken before eclosion, with pupae positioned to display their maximal length (from between the spiracles to the opposite end) and width (widest perpendicular distance) (Fig. 1C). Eclosed F1 flies were also cold anesthetized in a −20 °C freezer for 10 min to facilitate imaging. For mesothorax length imaging (Fig. 1D), the flies were pinned dorsal aspect upward with Minutien pins on a silicone coated dissection dish.

Images of crickets, pupae, and flies were captured using CellSens Dimension (ver. 3.24) interfaced with an Olympus SZX16 stereomicroscope equipped with a 0.8× objective and a DP80 Digital Camera. The images produced by this setup included a calibrated scale that facilitated measurements of morphological features (Fig. 1A to D). Captured images were imported into ImageJ (ver. 1.53) for morphometric measurements. The 2 mm scale bar in the image was used to calibrate pixel values to real-world size measurements. We then used the line tool to measure the pronotum length and width, and the hind femur length in crickets (Fig. 1A and B); length and width in pupae (Fig. 1C); and mesothorax length in eclosed flies (Fig. 1D).

Parasitizations

Ormia ochracea can be propagated in the laboratory using a manual infestation procedure described by Vincent and Bertram (2010b).

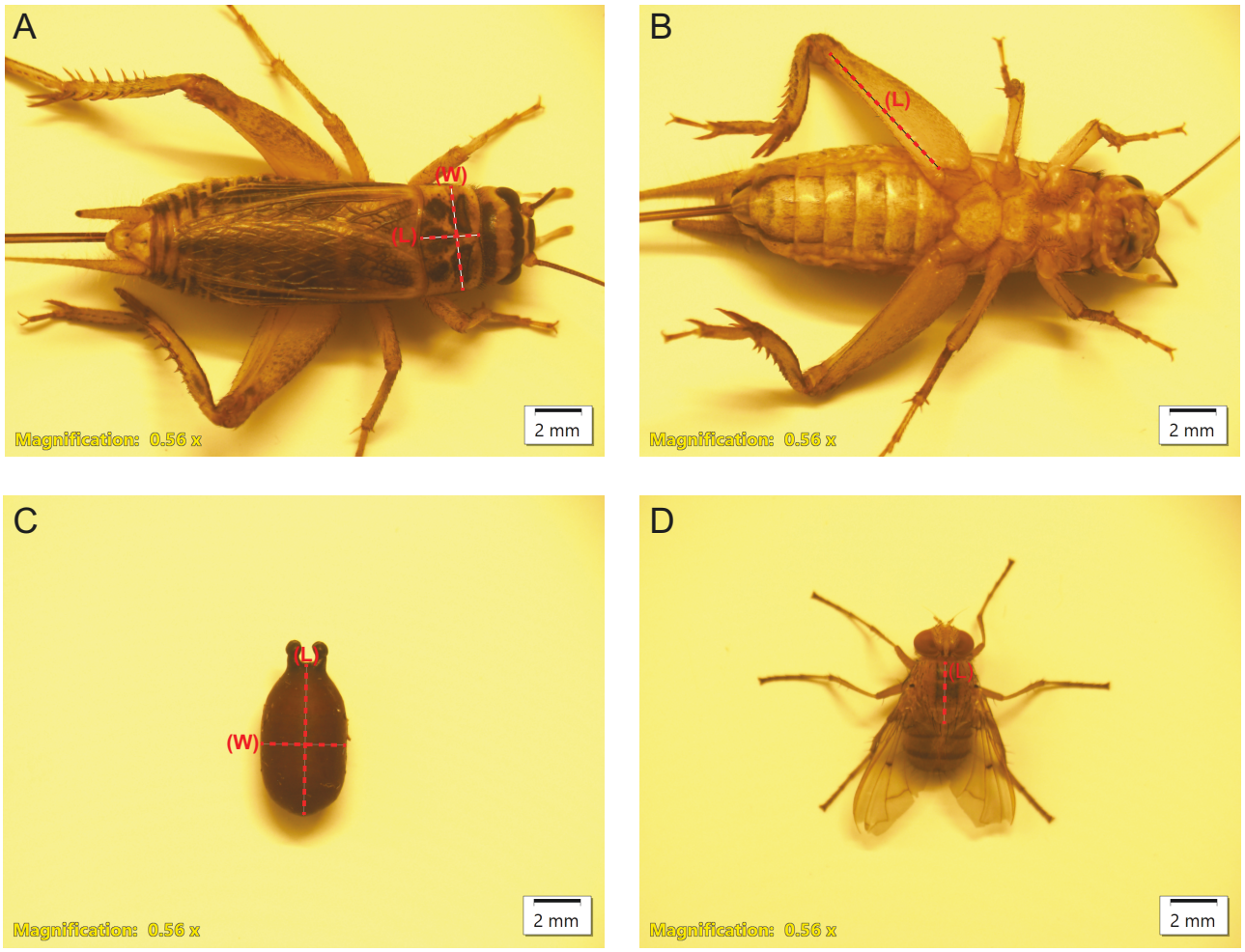


Fig. 1. Imaging and measurement of crickets, pupae, and eclosed flies. A) Dorsal view of an *A. domesticus* cricket host showing pronotum length (L) and pronotum width (W). B) Ventral view of a cricket with hind femur length (L) indicated. C) Emerged *O. ochracea* pupa positioned to display maximal length between spiracles (L) and width (W). D) Dorsal view of an eclosed F1 (first generation) fly showing mesothorax length (L). Crickets, pupae, and flies were imaged using an Olympus SZX16 stereomicroscope and measured using ImageJ. Dashed lines indicate measured morphological features. Scale bars represent 2 mm.

Manual parasitizations are performed by extracting larvae from freshly dispatched donor flies. This involves dissecting the abdomen of a fly and spreading the larvae contained in its reproductive tract (Henderson et al. In press) on a petri dish lined with filter paper. Freshly extracted larvae move around on the petri dish and often stand on their posterior ends and move their anterior end in a wave-like motion. On a piece of damp filter paper, larvae are most active within the first 2 h after removal from the gravid fly but can potentially survive for 7 to 8 h (Beckers et al. 2011). In this state, larvae can easily attach to a wooden probe. Once attached to the probe, an individual larva can be placed on top of the articular sclerite, an area of soft tissue located anterior to the cricket's thorax and directly underneath the pronotum.

Larvae from 10 gravid female flies were used to infest 600 *A. domesticus* crickets, evenly split between females and males. Half of the crickets in each sex group were infested with 1 larva and the other half with 2 larvae, to test for resource competition. Per donor fly, 60 crickets were infested, with 15 females and 15 males receiving 1 larva, and another 15 females and 15 males each receiving 2 larvae. In total, 150 females and 150 males were infested with 1 larva, and another 150 females and 150 males with 2 larvae, totaling 900 larvae across 600 crickets.

Data Analysis

Statistical analyses were conducted in R (ver. 4.4.2) using RStudio. We visually inspected Q–Q plots and used the Shapiro–Wilk test to assess whether different morphometric data were derived from normally distributed populations. We found that male pronotum width, hind femer length, and mass, as well as female pronotum width and hind femur length, all violated normality assumptions. Therefore, we used the Spearman's Rank Correlation to examine the correlation structure between different morphological traits. The correlation matrix showed strong positive associations among pronotum length, width, and mass (r = 0.673 to 0.685), suggesting proportional body size scaling. In contrast, hind femur length exhibited weaker correlations (r = 0.287 to 0.421), suggesting greater independence from overall body size. High intercorrelations among pronotum length, width, and mass raised multicollinearity concerns.

To assess multicollinearity among predictor variables, we calculated variance inflation factors (VIF) using a linear model with pupation success as the response variable and cricket sex, pronotum length, body mass, and larval load as predictors. VIF values were computed using the VIF function from the *car* (ver 3.1.3) package in R (Fox and Weisberg 2019). Predictor variables with VIF values exceeding 5 are suggestive of moderate to high levels of multicollinearity

among predictor variables. In our analysis, none of the predictors of pupation success exceeded a VIF of 5, thus the predictors are not highly correlated with each other and that multicollinearity would be unlikely to distort our statistical analyses.

We used 2 separate generalized linear mixed models (GLMMs) to examine factors influencing pupation success, defined in 2 ways: (i) whether pupation occurred at all, regardless of the number of resulting pupae (cricket-level analysis), and (ii) the proportion of individual larvae that successfully pupated (larval-level analysis). Both models used a binomial distribution with a logit link function. Fixed effects included cricket sex (male or female), pronotum length, cricket mass, and larval load (the number of larvae per host) as a measure of resource competition. Donor fly was included as a random effect to account for variability between individual flies. To assess the effect of larval load on fly hatching success, we fitted a GLMM with a binomial distribution and a logit link function. The model included larval load (number of larvae per host) as a fixed effect and donor fly as a random effect. Model fitting was performed using maximum likelihood estimation with the Laplace approximation. All analyses were conducted using the *lme4* package (ver. 1.1.35.5) in R (Bates et al. 2015, 2025).

We evaluated the relationship between pupal width and multiple morphological and biological factors by fitting and comparing a series of linear models (Table 1). Specifically, we tested 8 candidate models to determine the best predictors of pupal width. Our models included: (i) a model with pronotum width, pronotum length, larval number treatment, and cricket sex as predictors; (ii) a model replacing pronotum width with hind femur length; (iii) a model replacing hind femur length with host mass; (iv) a model including an interaction between pronotum length and larval number treatment; (v) a model including an interaction between mass and larval number treatment while also accounting for pronotum length and cricket sex; (vi) a model including an interaction between pronotum width and larval number treatment; (vii) a model including an interaction between hind femur length and larval number treatment; and (viii) a full interaction model including pronotum length, pronotum width, hind femur length, and mass, each interacting with larval number treatment, as well as cricket sex. We compared model performance using Akaike's Information Criterion (AIC), assessed model fit using adjusted R^2 values, and examined residuals for normality and homoscedasticity. This rigorous model selection approach allowed us to identify several competitive linear models with important predictors of pupal width.

To assess the relationship between pupal width and eclosion success, we performed a binomial logistic regression using pupal width as a continuous predictor and hatching success (eclosed = 1, failed = 0) as the response variable. The model was fitted using the GLM function in the *stats* package (ver. 4.4.1) with a binomial error distribution and logit link function. Model estimates were reported with standard errors, and significance was assessed using Wald *z*-tests. To facilitate interpretation, we calculated predicted probabilities of eclosion across the observed range of pupal widths along with 95% confidence intervals.

To examine whether pupal width predicted F1 adult fly size (mesothorax length), we fitted a linear mixed-effects model using the lmer function from the *lme4* package (Bates et al. 2015, 2025). The model included pupal width, larval competition level (number of larvae per host), and their interaction as fixed effects. The donor fly ID was included as a random intercept to account for potential variation among individual donor flies. Model estimates were obtained using restricted maximum likelihood, and statistical significance of fixed effects was assessed using Satterthwaite's approximation

Table 1. Model selection summary for predictors of pupal width. Linear models were tested to evaluate the effects of cricket morphological traits (pronotum width, pronotum length, hind femur length, and mass) and other biological factors (cricket sex, level of resource competition) on pupal width. Best models were selected based on adjusted R^2, AIC, and predictor statistical and biological significance. Asterisks (*) indicate statistically significant factors ($P < 0.05$) and daggers (†) indicate borderline significance.

Model	Predictors	AIC	R^2 (Adjusted)	*F*-statistic (df)	*P* value	Significant predictors
i	Pronotum width + Pronotum length + Larval number treatment + Cricket sex	51.66843	0.1205	8.875 (4,226)	**<0.0001**	Larval number treatment*, Cricket sex†
ii	Hind femur length + Pronotum length + Larval number treatment + Cricket sex	51.55999	0.1209	8.905 (4,226)	**<0.0001**	Larval number treatment*, Cricket sex†
iii	Mass + Pronotum length + Larval number treatment + Cricket sex	51.83619	0.1198	8.827 (4,226)	**<0.0001**	Larval number treatment*, Cricket sex†
iv	Pronotum length* Larval number treatment + Cricket sex	51.50512	0.1211	8.921 (4,226)	**<0.0001**	Cricket sex
v	Mass* Larval number treatment + Pronotum length + Cricket sex	52.56166	0.1208	7.318 (5,225)	**<0.0001**	Larval number treatment*, Cricket sex
vi	Pronotum width* Larval number treatment + Pronotum length + Cricket sex	53.18793	0.1195	7.245 (5,225)	**<0.0001**	Cricket sex
vii	Femur length* Larval number treatment + Pronotum length + Cricket sex	52.72089	0.1200	7.398	**<0.0001**	Cricket sex
viii	Pronotum length* Larval num treatment + Pronotum width* Larval number treatment + Femur length* Larval num treatment + Mass* Larval number treatment + Cricket sex	56.93803	0.1180	6.982 (8,222)	**<0.0001**	Pronotum width, Mass, Cricket sex

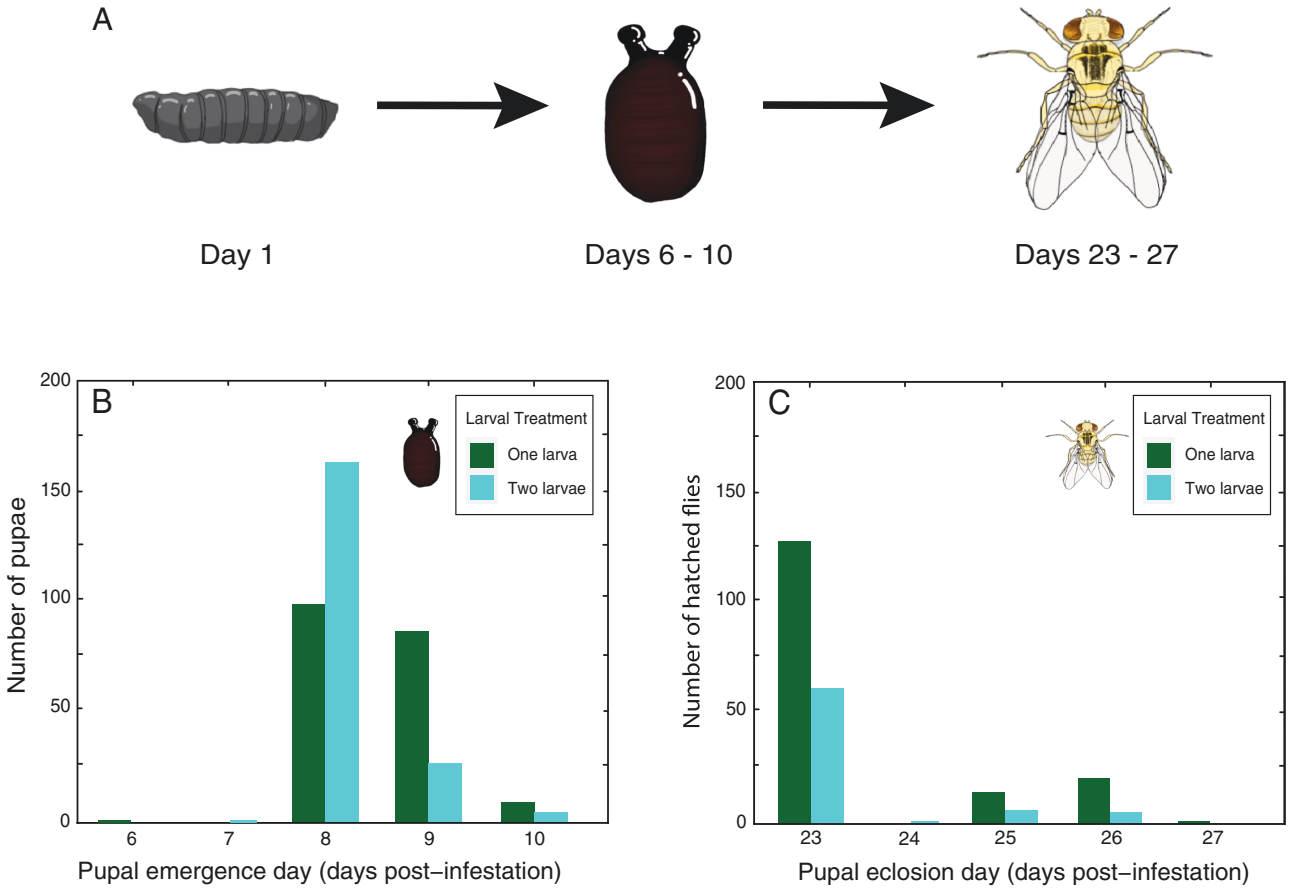


Fig. 2. Timeline of larval development, pupation, and eclosion. A) Schematic of the *O. ochracea* life cycle with observed timings of larval parasitization (day 1), pupation following development inside host *A. domesticus* (days 6 to 10), and adult fly eclosion (days 23 to 27). B) Number of pupae recorded per day of emergence post-infestation, separated by larval parasitization treatment (1 larva vs. 2 larvae). Across treatments, most larvae emerged and pupated on day 8 (72.33%, 366 of 506 pupae). C) Number of flies that successfully eclosed per day postparasitization, with peak eclosion occurring on day 23. Bars represent counts of individuals from the 1-larva (dark green) and 2-larvae (cyan) treatments.

for degrees of freedom, as implemented in the *lmerTest* package (Kuznetsova et al. 2017). Model residuals were visually inspected to confirm normality and homoscedasticity.

To assess whether F1 mesothorax length differed among infestation outcomes, we conducted a Kruskal–Wallis test, a nonparametric alternative to ANOVA, to account for potential deviations from normality and unequal variances. Following a significant Kruskal–Wallis result, we performed posthoc pairwise comparisons using Dunn's test with Bonferroni correction to adjust for multiple comparisons. These analyses were conducted in R using the Kruskal–Wallis test that is part of the native stats package and the Dunn's Kruskal–Wallis multiple comparisons test from the *FSA* (Fisheries Stock Assessment: ver. 0.9.5) package (Ogle et al. 2025).

Results

Timeline of Larval Development, Pupation, and Eclosion

Within 30 min of parasitization, the larva pierced through the prothoracic-tergal membrane (articular sclerite) and burrowed into the cricket (Supplementary Video 1). Larvae emerged from host crickets 6 to 10 d later (Fig. 2A and B) and pupated within several hours after emergence. Emergence and pupation began on day 6 post-parasitization, with 1 larva (0.02% of total successful pupations) successfully emerging and pupating on this day (Fig. 2B), while the remaining larvae did so by day 10 (day 9: 23.52%, 119/506; day 10: 2.57%, 13/506) (Fig. 2B). Flies eclosed as adults approximately 23 to 27 d post-parasitization (Fig. 2C, Supplementary Video 2). Most of the pupae (83.04%, 328/395) eclosed 23 d post-infestation. In the following days, 0.05% eclosed on day 24 (2/395), 7.34% (29/395) eclosed on day 25, and 9.11% (36/395) eclosed on day 26 after infesting (Fig. 2C).

Effect of Resource Competition on Pupation and Eclosion Success

First, when pupation success was defined as whether emergence and pupation occurred at all (regardless of the number of resulting pupae) after parasitizing a cricket (cricket-level analysis), we found that pupation success was not strongly driven by cricket size, sex, or the level of resource competition (Fig. 3A). Larvae developing in male and female crickets were equally likely to pupate ($\beta = -0.08$, SE = 0.24, $P = 0.745$). Similarly, cricket size, as defined by pronotum length ($\beta = -0.28$, SE = 0.499, $P = 0.58$) or cricket mass ($\beta = 1.99$, SE = 1.68, $P = 0.238$), did not affect pupation success. The number of larvae per host also had no significant effect on pupation success ($\beta = -0.03$, SE = 0.17, $P = 0.842$). Of the 300 crickets infested with a single larva, 63.33% (190/300) yielded pupation, while among the 300 crickets infested with 2 larvae, 62.33% (187/300)

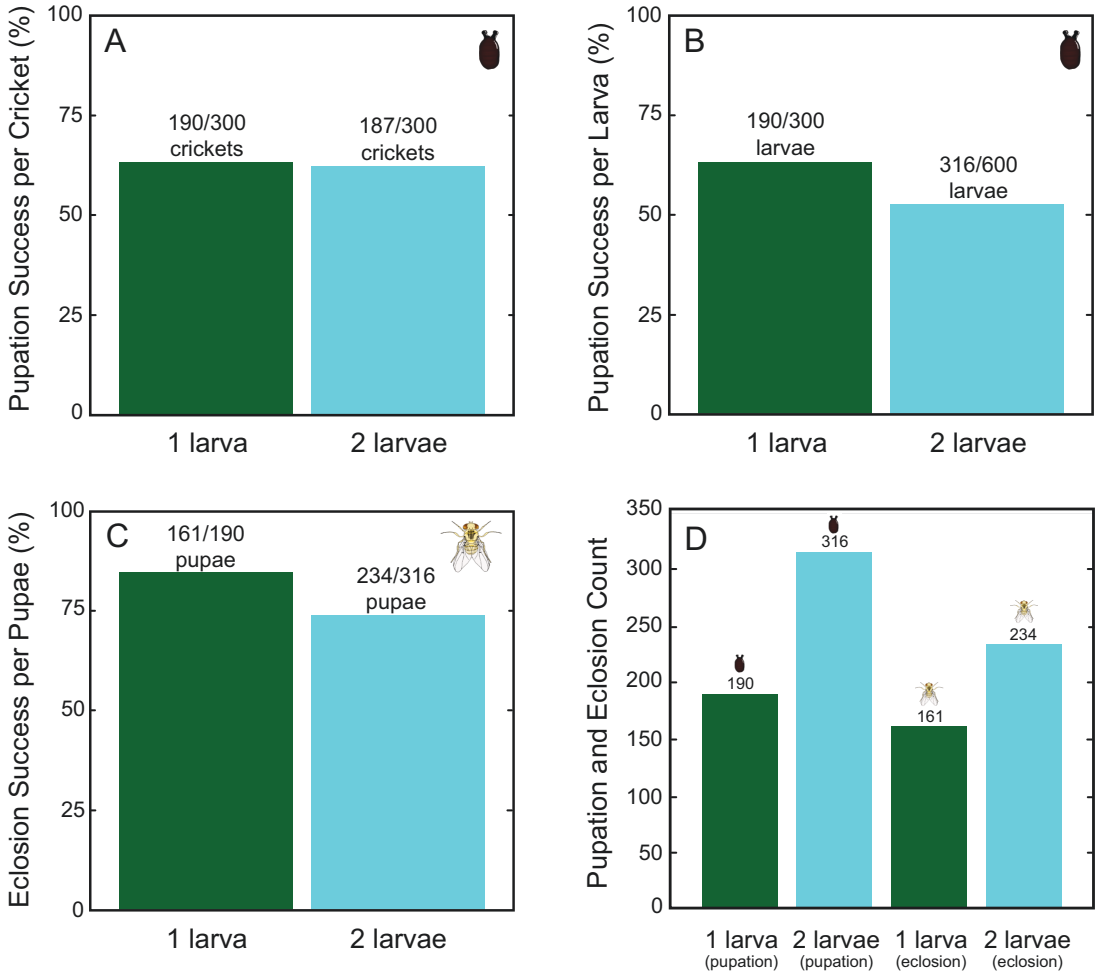


Fig. 3. Effect of resource competition on pupation and eclosion success. A) Pupation success per *cricket*, the proportion of crickets yielding at least 1 pupa, did not differ between treatments. B) Pupation success per *larva*, the proportion of individual larvae that successfully pupated, was significantly lower under competition. C) Eclosion success per *pupa*, the proportion of pupae that successfully hatched into adult flies, was also reduced under competition. D) Absolute counts of pupae and eclosed flies per treatment. Despite lower per-larva pupation and per-pupa eclosion success (B, C), total pupae and adult flies were higher in the 2-larvae treatment due to the greater initial number of larvae per host. This resulted in an average reproductive output of 0.78 flies per cricket under double infestation versus 0.54 in the 1-larva treatment. Dark green bars represent the 1-larva treatment, and cyan bars represent the 2-larvae competitive treatment.

pupated (Fig. 3A). However, when pupation success was defined as the proportion of individual larvae that successfully pupated, pupation success was significantly impacted by resource competition (Fig. 3B). Greater resource competition strongly reduced larval pupation success ($\beta = -1.59$, SE = 0.14, $P < 0.001$). In contrast, cricket sex ($\beta = -0.05$, SE = 0.16, $z = -0.32$, $P = 0.749$), pronotum length ($\beta = -0.09$, SE = 0.32, $P = 0.781$), and mass ($\beta = 1.27$, SE = 1.11, $P = 0.254$) did not significantly influence larval pupation success. Among the 900 larvae used, 63.33% pupated when developing individually in a cricket, compared to 52.67% (316/600) of larvae that developed in competition (Fig. 3B). These results suggest that while cricket characteristics do not determine pupation success, resource competition decreases larval survival.

Eclosion success was significantly influenced by resource competition. Increasing the larval number per host reduced the proportion of pupae that successfully eclosed (β = −0.68, SE = 0.24, $P = 0.0046$; Fig. 3C). In the absence of competition, 84.74% (161/190) of the pupae successfully eclosed (Fig. 3C, left bar). Eclosion success rate decreased to 74.05% (234/316) when 2 larvae were competing for resources within the same host cricket (Fig. 3C, right bar).

In terms of absolute counts (Fig. 3D), rather than proportions (Fig. 3A to C), parasitizing each cricket with 2 larvae resulted in a higher number of pupae and successfully eclosed F1 flies compared to single-larva infestations. Among the 300 doubly infested crickets, a total of 316 pupae emerged, significantly more than the 190 pupae produced from 300 singly infested hosts ($\chi^2(1) = 31.38$, $P < 0.001$). Likewise, double infestation led to 234 eclosed F1 flies, significantly exceeding the 161 adults obtained from the single-larva treatment ($\chi^2(1) = 13.49$, $P < 0.001$). This translates to an average reproductive output of 0.78 flies per cricket under double infestation, compared to 0.54 flies per cricket with a single larva.

Effects of Resource Competition and Cricket Sex on Pupal Width

In each of the top 3 linear models predicting pupal width, level of competition (ie larval number treatment) was a significant predictor, while cricket sex was marginally significant (Table 1). As the number of larvae increased, pupal width decreased (Fig. 4A), suggesting a resource-limiting effect where higher infestation levels constrain larval growth. Cricket sex showed a borderline effect, with males tending to produce slightly smaller pupae than females (Fig.

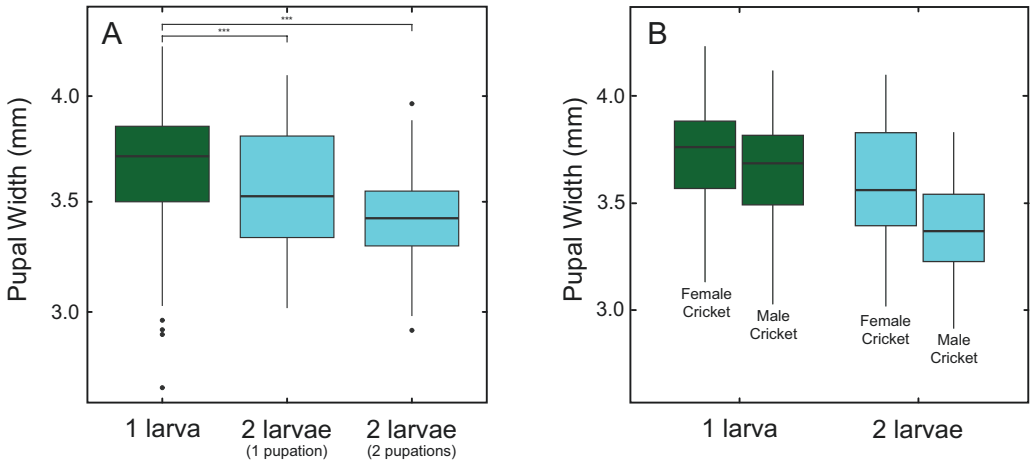


Fig. 4. Effects of resource competition and host sex on pupal size. A) The size (width) of pupae was significantly reduced under resource competition. Pupae from singly parasitized crickets were larger than those from double infestations, whether both larvae pupated or only 1 did. Pupae from single-pupation double infestations (18.35% of pupae from the competitive treatment) remained larger than those from double pupations, though this difference was not significant. B) Pupae from male crickets were typically smaller than from female crickets, regardless of larval treatment. Dark green represents the 1-larva treatment, and cyan represents the 2-larvae competitive treatment. Box plots depict the first and third quartiles, median values, with the length of the whiskers showing no less (lower) or more (upper) than 1.5 times the interquartile range.

4B). Although this effect was not strongly significant ($P = 0.055$ to 0.086), it suggests potential sex-based differences in host suitability.

Analysis of pupal width across pupation outcomes revealed that pupae developing without competition (ie from crickets parasitized by a single larva) were significantly larger than those that developed under competitive conditions (Kruskal–Wallis test, $\chi^2(2) = 104.6$, $P < 0.001$). Posthoc Dunn's tests indicated that pupae from single-larva infestations were significantly larger than both those from crickets that hosted 2 larvae but only produced 1 pupa (18.35%, 58/316) ($P < 0.001$) and those from crickets where both larvae successfully pupated ($P < 0.001$; Fig. 4A). However, among crickets that hosted 2 larvae, the pupal width of the single surviving pupa was slightly, but not significantly, larger than the pupae from cases where both larvae pupated ($P = 0.064$). This pattern suggests that when 2 larvae compete within a host, the constraint on resources reduces overall pupal size, but the surviving larva may still gain a slight advantage over cases where both larvae survive to pupation.

The best-fitting linear model included pronotum width, pronotum length, level of competition, and cricket sex as predictors ($R^2 = 0.12$, $P < 0.001$). Level of competition had a significant negative effect on pupal width ($\beta = -0.19$, SE = 0.04, $P < 0.001$), further supporting the role of larval competition in determining pupal size. Cricket sex was marginally significant ($\beta = -0.08$, SE = 0.05, $P = 0.0748$), reinforcing the possibility of sex-based host differences. Pronotum width ($\beta = 0.04$, SE = 0.10, $P = 0.6477$) and length ($\beta = 0.11$, SE = 0.13, P = 0.4030) were not significant predictors, suggesting that overall body size of the cricket does not strongly influence pupal width. While the model highlights the importance of larval competition and suggests potential sex-based effects, the overall weak fit ($R^2 = 0.12$) indicates that there are likely additional, unaccounted-for factors influencing pupal size.

Pupal Width as a Predictor of Eclosion and Fly Size

As resource competition negatively impacted pupal width, we examined whether this also impacted eclosion success. In a binomial logistic regression, using pupal width as a predictor of hatching success, we found that pupal width was a significant predictor of eclosion success in crickets infested with 2 larvae ($\beta = 1.45 \pm 0.45$ SE, $P = 0.001$). Larger pupae had a significantly higher probability of successful eclosion (Fig. 5A). Predicted probabilities from the model indicated that at a pupal width of 2.50 mm, the probability of eclosion was approximately 45% (95% CI: 0.28, 0.65), whereas at 2.75 mm, the probability increased to 54% (95% CI: 0.40, 0.68). This suggests that even within a narrow size range, small increases in pupal width are associated with improved survival outcomes.

We next examined whether pupal width predicts F1 adult fly size (mesothorax length). A linear mixed-effects model, accounting for variation among donor flies, revealed that pupal width was a significant predictor of mesothorax length ($\beta = 0.61$, SE = 0.15, $P < 0.001$), indicating that larger pupae tend to develop into larger adult flies (Fig. 5B). However, resource competition did not significantly predict adult fly size ($\beta = -0.06$, SE = 0.39, $P = 0.878$), nor was there a significant interaction between pupal width and level of competition ($\beta = 0.004$, SE = 0.11, $P = 0.972$). This suggests that while competition during larval development strongly influences pupal size (Fig. 4A), its direct effect on adult body size is less pronounced, with pupal width remaining the primary determinant of mesothorax length. The random effect of fly ID accounted for a small proportion of variance ($\sigma^2 = 8.19 \times 10^{-5}$), indicating that individual differences among donor flies had minimal influence on the relationship between pupal width and adult size.

Flies that developed in the absence of competition were significantly larger than those that developed under competitive conditions (Fig. 5C). The Kruskal–Wallis test revealed a significant overall effect of infestation outcome on mesothorax length ($\chi^2(2) = 16.64$, $P < 0.001$), indicating that resource competition during development influences adult body size. Dunn's posthoc test showed that mesothorax length was significantly greater in flies that developed from a single larva in the absence of competition, compared to flies that developed from co-infestation where the other larva failed to emerge ($Z = 4.56$, $P < 0.001$), and compared to those that developed under competition where both larvae pupated ($Z = 7.92$, $P < 0.001$). Among flies from a competitive treatment, those that developed from a co-infested larva where the other larva failed to pupate (17.52%, 41/234) were larger but not significantly so ($Z = 1.83$, $P = 0.20$). These results suggest that competition during development imposes

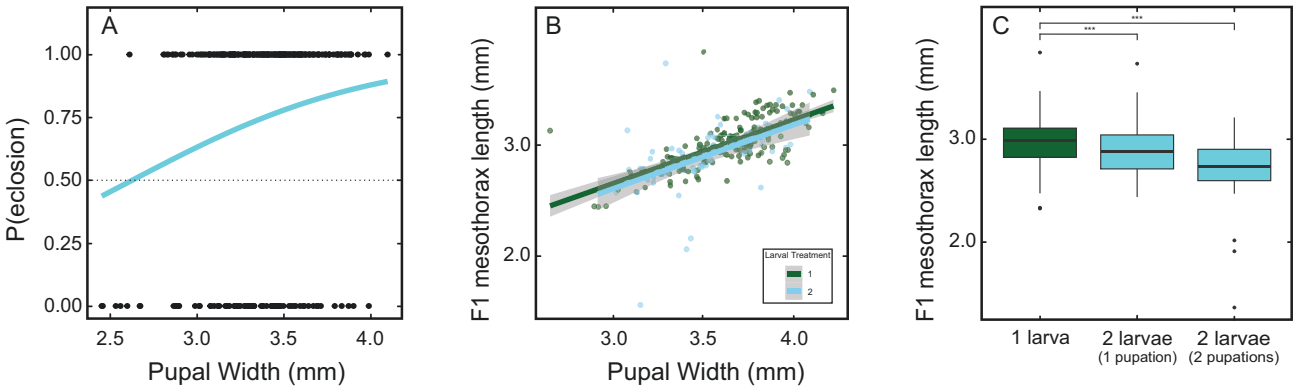


Fig. 5. Effect of pupal size and resource competition on adult fly size. A) Larger pupae had a significantly higher probability of successful eclosion. Fitted line (cyan) from a generalized linear model with a binomial logit link predicts eclosion success. Dotted line depicts chance level (50%) success. Black data points indicate individual successful eclosion events (top), and unsuccessful eclosion events (bottom). B) Pupal width strongly predicted eclosed fly size (mesothorax length), but did not depend on the level of competition. Lines of best fit and data points for 1 larva versus 2 larvae per host are indicated in green and cyan, respectively. C) Adult fly size was significantly affected by resource competition. Flies that had developed in the absence of competition were significantly larger than those from a competitive treatment regardless of whether 1 or both larvae pupated. Among flies with a history of resource competition, those from a larva where the other larva failed to pupate were larger, but not significantly so. Dark green represents the 1-larva treatment, and cyan represents the 2-larvae competitive treatment. Box plots depict the first and third quartiles, median values, with the length of the whiskers showing no less (lower) or more (upper) than 1.5 times the interquartile range.

constraints on growth, likely due to resource limitation. Furthermore, the lack of a significant difference between the 2 competitive groups suggests that once competition is present, additional crowding does not further reduce body size significantly. These findings support the view that larval competition negatively impacts adult body size, with potential consequences for fitness and survival.

Discussion

In this study, we investigated the developmental outcomes of *O. ochracea* larvae in the presence and absence of resource competition while developing within *A. domesticus* as the host cricket. Although the likelihood of at least 1 larva pupating did not differ between hosts with 1 or 2 larvae (Fig. 3A), individual larvae in co-infested hosts exhibited reduced pupation (Fig. 3B) and eclosion success (Fig. 3C), indicating strong within-host competition. Competition also led to smaller pupal size (Fig. 4A), which was associated with lower eclosion probability (Fig. 5A). Additionally, pupae tended to be smaller when developing in male versus female crickets (Fig. 4B). Finally, pupal size positively correlated with adult body size (Fig. 5B), and the largest flies developed from larvae that did not experience competition (Fig. 5C). These findings highlight the roles of host characteristics and larval competition in shaping developmental outcomes.

Despite the negative effects of resource competition, we found that 2-larva infestations resulted in significantly higher overall yield (Fig. 3D). From the same number of hosts (300 crickets), double infestations produced 234 adult flies compared to 161 under single infestation, averaging 0.78 vs. 0.54 flies per host. Thus, infesting with 2 larvae per host led to a greater absolute number of offspring. In fact, across studies, the mean number of larvae that tachinid flies deposit per host is nearer to 2 than to 1: 1.7 ± 1.0 in Adamo et al. (1995b), 1.8 ± 1.2 in Kolluru and Zuk (2001), 2.13 ± 0.18 and 1.92 ± 0.09 in Lehmann (2008), and 2.28 ± 0.22 and 2.90 ± 0.19 in Reitz and Adler (1995). Interestingly, this is despite maximum reproductive yield being achieved with an even larger clutch size of 4 to 5 larvae (Adamo et al. 1995b, Welch 2006, Lehmann 2008), prompting the conclusion that there "*must be some ecological advantage*" to infesting with approximately 2 larvae rather than 4 or 5 (Welch 2006).

Previous studies have shown that as clutch size increases from 1 to 4 larvae, the reproductive yield rises steadily while fitness (pupal size) consistently declines (Adamo et al. 1995b, Nakamura 1995, Lehmann 2008). Lehmann (2008) determined that as clutch size increased, pupal weight and the proportion of pupae hatching into adult flies decreased, and that over time smaller adult Ormiini flies may impact life-history traits or potentially the ability of parasitoid flies to locate hosts. Although our study tested only up to a 2-larvae regime, our results followed the same trend, with yield increasing (Fig. 3D) and fitness (pupal and adult size) declining with competition (Figs. 4A and 5C). However, among the flies that developed under 2-larvae conditions, we identified a subset (17.52%) that emerged from hosts in which the other larva failed to pupate. These flies were larger than those from hosts in which both larvae pupated. These findings not only raise the possibility of competitive exclusion (Feener and Brown 1997) but also suggest that this subset may help offset the average fitness costs of 2-larvae infestations—offering a conceivable explanation for why natural clutch sizes tend toward 2. However, the subset of 2 larvae (1 pupation) flies were not significantly larger.

Our study demonstrates clear advantages to avoiding resource competition through single parasitization. Larvae developing alone exhibited significantly higher pupation success per larva (Fig. 3B) and eclosion success per pupa (Fig. 3C). Consistent with previous findings (Adamo et al. 1995b, Lehmann 2008), singly infested larvae also produced significantly larger pupae (Fig. 4A), which were more likely to eclose (Fig. 5A) into larger adult flies (Fig. 5BC). In tachinids, such increases in size are closely linked to higher fecundity (Nakamura 1995, Reitz and Adler 1995, Lauziere et al. 2001, Ho et al. 2011). These results therefore suggest that a single-larva parasitization strategy may offer the most effective balance between reproductive yield and offspring fitness.

Indeed, 1-larva parasitization appears to be the most common (although not the mean-average) strategy employed by Ormiini flies. Adamo et al. (1995b) dissected *Gryllus* hosts (*G. texensis*, formerly *G. integer*, and *G. rubens*) naturally parasitized by *O. ochracea*, and found that single-larva infestations predominated—occurring twice as often as the next most frequent clutch size of 2. A similar pattern

was reported by Kolluru and Zuk (2001), with nearly twice as many single-larva infestations as double infestations in field-collected *Teleogryllus oceanicus* in Hawaii, also parasitized by *O. ochracea*. In another field study, Lehmann (2008) compared 2 European species of bushcricket (also known as katydids), *Poecilimon mariannae* and *Poecilimon thessalicus*, which are parasitized by the Ormiini fly *Therobia leonidei*, and found that *T. leonidei* mostly parasitized with a single larva, with over 50% of *P. mariannae* and 38% of *P. thessalicus* harboring just 1 larva. Most frequently infesting a host with a single larva therefore appears to be a conserved strategy across diverse host taxa and body sizes, in both natural and non-native hosts, suggesting it may be an inherent or adaptive trade-off for balancing yield and fitness.

If other variables such as host size also influence yield or fitness, then Ormiini flies might be expected to modulate their clutch size accordingly. This strategy is known as clutch size adjustment (Lack 1947) and has been demonstrated in parasitoid wasps, where females lay more eggs in larger hosts to maximize reproductive return (Hardy et al. 1992) whilst presumably maintaining offspring fitness. These findings align with evidence that parasitoid fitness scales with host size: Cohen et al. (2005) reported larger hosts yielding correspondingly larger wasp parasitoids, while Lehmann (2008) found that the larger bushcricket species, *P. mariannae*, resulted in heavier pupae and positively influenced adult fly eclosion. In our study, larvae developing in female crickets tended to form larger pupae than those from males (Fig. 4B), likely reflecting size differences between host sexes.

However, despite the significant *between-species* effect observed by Lehmann (2008), the study found no significant *within-species* effect of host size on pupal size. Consistent with this finding, none of our models showed that host size—whether measured as pronotum length or mass—significantly influenced pupal width. Host sex was only borderline significant across the best models. In contrast, larval number consistently emerged as a strong predictor of pupal size (Table 1). Moreover, multiple studies on tachinid flies have shown that variations in host size do not influence the number of larvae deposited. *O. ochracea* did not increase larval number with larger *Gryllus* or *Teleogryllus* hosts (Adamo et al. 1995b, Kolluru and Zuk 2001), *T. leonidei* showed no significant difference in brood size between large and small *Poecilimon* species (Lehmann 2008), and *Ormia lineifrons* exhibited no difference in parasitoid load across 4 *Neoconocephalus* bushcricket species of varying sizes (Rogers and Beckers 2023). Together, these findings suggest that, for tachinid flies, the optimal number of larvae per host is constrained to a narrow range, rather than being contextually modulated via clutch size adjustment to exploit variation in host quality.

Given the limited influence of host size on developmental outcomes, this may afford *O. ochracea*—which is known to parasitize a range of host species (Wineriter and Walker 1990, Walker 1993, Gray et al. 2007, 2019, Sakaguchi and Gray 2011)—increased plasticity in host switching. *O. ochracea* is native to the continental US, where geographically separated populations parasitize at least 17 species of field cricket, with certain populations exhibiting host specialization by preferentially targeting the locally predominant cricket species (Gray et al. 2007, 2019). In Hawaii, *O. ochracea* was introduced without its ancestral hosts, and its behavioral plasticity in host choice has been evident from its successful exploitation of the Pacific field cricket, *Teleogryllus oceanicus*, a non-native cricket that has become its preferred host (Broder et al. 2023, Wikle et al. 2025). More recently, rapid ongoing changes in host acoustic traits in Hawaii, including the emergence of multiple morphs of *T. oceanicus* that produce more cryptic songs, have dramatically altered the host signal landscape (Zuk et al. 2006, Tinghitella et al. 2018, 2021, Rayner et al. 2019, Broder et al. 2022), potentially selecting for Hawaiian *O. ochracea* with more sensitive hearing (Hoy 2025, Wikle et al. 2025), and prompting host switching to the alternative cricket species in Hawaii (Broder et al. 2023).

Here, we demonstrate that another nonancestral host, *A. domesticus*, is viable for propagating *O. ochracea*, in the laboratory. Although few studies have examined its suitability, *A. domesticus* has previously been successfully used to rear laboratory populations (Paur and Gray 2011). Wineriter and Walker (1990) described *A. domesticus* as "*less satisfactory*", but their findings were influenced by cricket mortality unrelated to larval development, possibly due to housing conditions. To assess host viability, Thomson et al. (2012) manually infested three species of *Gryllus* with 2 *O. ochracea* larvae per cricket and observed the highest larval emergence (pupation) success of 54% to 61% in the natural host *G. texensis*. Using the same methodology with two larvae, we recorded a comparable success rate of 62.33% in *A. domesticus* (Fig. 3A).

Conclusions

Our results confirm that *A. domesticus* is a suitable and commercially available host for maintaining laboratory colonies of *O. ochracea*. We identify resource competition as a key factor influencing larval development. Although 2-larvae infestations increase overall offspring yield, we recommend a protocol of 1 larva per host, preferably females, for colony maintenance, to maximize individual fecundity. Finally, our findings rely on manual parasitizations. The ability of *O. ochracea* flies to naturally parasitize *A. domesticus* warrants investigation.

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Author contributions

Jimena Dominguez (Conceptualization [supporting], Data curation [equal], Formal analysis [supporting], Investigation [lead], Methodology [supporting], Visualization [equal], Writing—original draft [equal], Writing—review & editing [supporting]), Brendan Latham (Conceptualization [supporting], Data curation [equal], Formal analysis [supporting], Investigation [supporting], Methodology [supporting], Visualization [equal], Writing—original draft [equal], Writing—review & editing [supporting]), Lauren Bitner (Data curation [supporting], Formal analysis [supporting], Validation [equal], Visualization [equal], Writing—review & editing [supporting]), Laura Mongui (Investigation [supporting]), Addie Rossinow (Investigation [supporting]), Yeng Xiong (Conceptualization [supporting]), Briella Schmidt (Investigation [Supporting]), Quang Vu (Conceptualization [Supporting]), Blanca Torres-Lopez (Investigation [supporting]), Parker Henderson (Investigation [supporting]), Andrew Mason (Conceptualization [supporting], Formal analysis [supporting], Methodology [supporting], Writing—review & editing [supporting]), and Norman Lee (Conceptualization [Lead], Data curation [Equal], Formal analysis [lead], Funding acquisition [lead], Investigation [supporting], Methodology [lead], Project administration [lead], Resources [lead], Supervision [lead], Validation [lead], Visualization [equal], Writing—original draft [equal], Writing—review & editing [lead])

Supplementary material

Supplementary material is available at *Annals of the Entomological Society of America* online.

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Conflicts of interest. None declared.

Data availability

Data from this study is available from the Mendeley Data repository: Dataset DOI. 10.17632/bc2wjyh7hy.1 Dominguez et al., 2025.

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