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Effect of larval host food substrate on egg load dynamics, egg size and adult female size in four species of braconid fruit fly (Diptera: Tephritidae) parasitoids

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ABSTRACT

Life history theory predicts that individuals will allocate resources to different traits so as to maximize overall fitness. Because conditions experienced during early development can have strong downstream effects on adult phenotype and fitness, we investigated how four species of synovigenic, larval-pupal parasitoids that vary sharply in their degree of specialization (niche breadth) and life history (*Diachasmimorpha longicaudata*, *Doryctobracon crawfordi*, *Opius hirtus* and *Utetes anastrephae*), allocate resources acquired during the larval stage towards adult reproduction. Parasitoid larvae developed in a single host species reared on four different substrates that differed in quality. We measured parasitoid egg load at the moment of emergence and at 24 h, egg numbers over time, egg size, and also adult size. We predicted that across species the most specialized would have a lower capacity to respond to changes in host substrate quality than wasps with a broad host range, and that within species, females that emerged from hosts that developed in better quality substrates would have the most resources to invest in reproduction. Consistent with our predictions, the more specialized parasitoids were less plastic in some responses to host diet than the more generalist. However, patterns of egg load and size were variable across species. In general, there was a remarkable degree of reproductive effort-allocation constancy within parasitoid species. This may reflect more “time-limited” rather than “egg-limited” foraging strategies where the most expensive component of reproductive success is to locate and handle patchily-distributed and fruit-sequestered hosts. If so, egg costs, independent of degree of specialization, are relatively trivial and sufficient resources are available in fly larvae stemming from all of the substrates tested.

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1. Introduction

Conditions experienced during early development can have strong downstream effects on the adult phenotype and fitness. When nutritional conditions are good for juvenile stages, animals tend to mature earlier and grow larger (Day and Rowe, 2002; Dmitriew and Rowe, 2011). The physical traits, behaviors and physiology of many adult hymenopteran parasitoids are believed to be adaptive responses to the resources their larvae are able to acquire, and this in turn is determined by the ecological and physiological characteristics of their hosts (Harvey et al., 2004; Jervis and Kidd, 1986; Jervis et al., 2001, 2008; Kaspi et al., 2002). Even for synovigenic parasitoids, that continue to mature eggs during the adult stage and adults may acquire nutrients that can be used for the production of gametes (true for host-feeding parasitoids), larval reserves are the main nutritional resource for eggs during

the first days after emergence and some nutrients necessary for reproduction may only be acquired during this stage (Jervis et al., 2005, 2008; Rivero et al., 2001).

Variability in host nutrient quantity and quality is likely to have significant effects on a number of parasitoid fitness components (Arakawa et al., 2004; Harvey et al., 2001; Jervis et al., 2008; Otto and Mackauer, 1998), and the scope and scale of such tritrophic effects in any particular system could significantly influence parasitoid population dynamics. In the tri-trophic model system investigated here, we examined the effect of fruit species (substrate) in which the fly host larvae developed, on parasitoid egg load (i.e., number of mature eggs), egg size and adult size. These fitness components were chosen because of their fundamental importance to reproductive success and because they may change rapidly depending on nutritional conditions. The evidence for their plasticity and the potential importance of their conditional response to variance in nutrition are briefly reviewed below.

Mean egg loads vary among species of parasitoids and this variability is influenced by the likelihood of encountering a host and the difficulty associated with oviposition. This represents costs of

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reproduction associated to time limitation (Price, 1972; Rosenheim, 1999). Nutritional status is certainly one of the unpredictable factors which could influence parasitoid reproduction (Wheeler, 1996), either through its direct effect on resource allocation to egg production, and/or through its effect on host search or oviposition behavior (Harvey et al., 2001).

Egg size is potentially another adaptive characteristic that females might adjust to suit available resources. Egg size has been a widely evaluated fitness-correlated parameter, especially in vertebrates (Shanbhag et al., 2000; Christians, 2002). In arthropods, variation in egg size is likely to involve a trade-off between the needs of the offspring and the costs of meeting those needs (Rosenheim, 1996). However, there is little information available on the specific factors which influence egg size (Fischer et al., 2002; Fox and Mousseau, 1996), and for offspring fitness related to it (O'Neill and Skinner, 1990). The substantial interspecific differences in mean parasitoid egg sizes among related species attacking similar stages of identical hosts suggests that foraging efficiency and larval competition may be important determinants (Sivinski et al., 2000). While it has been suggested that individuals which develop from eggs of bigger size have higher fitness, this has rarely been investigated, and is not always true (Rosenheim, 1999; Ellers et al., 2000). However, in other insect orders egg size can vary depending on the type of diet (Wallin et al., 1992), temperature (Steigenga et al., 2005), and the quality of the environment (Fox, 2000) which is consistent with a response by females to changing conditions.

Adult size is a major constraint on insect fecundity, although this relationship is not always positive (Ellers and Jervis, 2003; Gotthard et al., 2007; Honek, 1993; Leather, 1988; Thorne et al., 2006; Wang and Messing, 2004). But, independent of any correlation with egg load, increases in body length and mass can have several advantages to foraging parasitoids. Such changes may result in a greater flight speed and capacity, and resulting improvements in host searching capacity. For example, across species of tephritid flies, wing shape changes with body size suggesting that larger species are better adapted to long distance flight (Sivinski and Dodson, 1992). If host diet is related to its quality as a parasitoid resource, then parasitoid size and reproductive potential should depend on what and how much food the host has consumed (Ozkan, 2007; Sarfraz et al., 2008; Senravan and Annadurai, 1991; Urrutia et al., 2007).

The present study compares egg load at the moment of adult parasitoid emergence and after 24 h of adult emergence, egg size, adult female size, and female reproductive investment of four species of braconid parasitoids (one exotic and three native) reared on larvae of the polyphagous tephritid fly *Anastrepha ludens* (Loew) that developed in four different substrates. We selected four substrates that we predicted would have different capacities to support *A. ludens* growth and nutritional requirements. These were the exotic mango (*Mangifera indica* L. – Anacardiaceae), the exotic grapefruit (*Citrus paradisi* [Macfad]), the native white sapote (*Casimiroa edulis* [La Llave & Lex]) (both Rutaceae) and an artificial diet. Specifically, we predicted that *A. ludens* larvae reared from grapefruit and white sapote would be more nutritious for parasitoid development than larvae from mango. We based this prediction on the relatedness of grapefruit and white sapote to the main native hosts of *A. ludens*, namely *Casimiroa greggii* (S. Watson) Chiang and *C. edulis* (Plummer et al., 1941). All these are Rutaceae and *A. ludens* has a demonstrated preference for oviposition in rutaceous hosts (Aluja et al., 2009). Additionally, previous studies reported that mango represented a relatively poor diet for *A. ludens* in comparison to grapefruit (Eben et al., 2000). We further predicted that within parasitoid species, host diet would influence parasitoid performance in a hierarchical manner. That is, higher parasitoid quality, in terms of egg number and size, would be

observed according to host size and protein content, which is the most important nutrient used for egg production.

We also chose to compare four parasitoid species because of their potential use in biological control against *A. ludens* attacking different fruit hosts, and because, in nature they share similar host species (all belonging to *Anastrepha* genus), and all can develop successfully in *A. ludens*. Although, these species share hosts in the same genus, they occupy different ecological niches and also have different reproductive strategies (Sivinski et al., 1997). In general, niche breadth is thought to evolve to match environmental variation, with a tendency for specialists to evolve in environments that remain constant and generalists/phenotypic plasticity evolving in variable environments (Kassen, 2002). The four species we chose, although all belonging to the same family (Braconidae), vary sharply in host breadth (López et al., 1999; Sivinski et al., 2000; Aluja et al., 2003), ovipositor and egg size (Sivinski et al., 2001; Sivinski and Aluja, 2003), and foraging strategies (Sivinski et al., 1997; García-Medel et al., 2007) offering us an ideal opportunity to compare resource allocation among closely related congeners under strictly controlled experimental conditions. For example, the more specialized a species (e.g., *Opius hirtus* [Fischer]) the lower the variance it might encounter in host substrate and the less capacity it may require to conditionally respond to resource differences by independently adjusting egg size and number. This should be evidenced by reduced variance in egg numbers and size compared to that of less specialized species. That is, total reproductive investment should be maintained at the expense of investment in total body size (under the argument of a trade-off between reproduction and survival).

2. Materials and methods

2.1. Study insects

2.1.1. Parasitoids

Diachasmimorpha longicaudata (Ashmead) is a relative generalist and, like the other opiines used in this study, a solitary koinobiont larval-prepupal endoparasitoid. It is native to the Indo-Australian region, but has been used around the world in the biological control of *Bactrocera* spp., *Ceratitis capitata* (Wiedemann) and *Anastrepha* spp. (Wharton and Gilstrap, 1983). In Mexico it was introduced in the mid 1950s from Hawaii (Aluja et al., 2008). In Veracruz, Mexico it is now commonly associated to commercial fruit, mainly *Citrus* spp., *M. indica* and *Psidium* spp. It also parasitizes *Anastrepha* spp., larvae in *Spondias* spp., and rarely in *C. edulis* (Aluja et al., 1990; Sivinski et al., 2000). Of all the species studied here, it has the largest ovipositor (Sivinski et al., 2001; Sivinski and Aluja, 2003), the widest host breadth (e.g., Sivinski et al., 2000) and the most plastic foraging behavior (García-Medel et al., 2007).

Doryctobracon crawfordi (Viereck) is a Neotropical native species from Mexico and Central America as far south as Colombia and Ecuador; (Aluja et al., 1998), that principally attacks *A. ludens* larvae in both commercially important host plants like *Citrus* spp., and mango (*M. indica*) (López et al., 1999), and in the native *C. greggii*. Of all the species studied here, it is the one closest in size to *D. longicaudata* and also exhibits flexible foraging behavior (i.e., it can forage for hosts in tree canopies but also on the ground). It directly competes with *D. longicaudata* for resources in nature (López et al., 1999) rendering the current comparison with the latter species of great interest. It also has the largest egg size of all parasitoid species studied here (L. Cicero, unpublished data).

O. hirtus has the narrowest host range of the species that we examined and for the most part is a parasitoid of *Anastrepha cordata* (Aldrich) and *Anastrepha alveata* (Stone) (López et al., 1999) that

occur in Mexican and Central American populations of hog-plum (*Ximenia americana* L.) (Sivinski et al., 2000). It has a great capacity to forage for rare, low density hosts (García-Medel et al., 2007) fitting nicely into the scope of the current work.

Utetes anastrephae (Viereck) is widespread, from Florida to Argentina, and while capable of attacking a number of *Anastrepha* species, is frequently recovered from *Anastrepha obliqua* (Macquart) developing in *Spondias* spp. (López et al., 1999; Sivinski et al., 2000). It has the shortest ovipositor of the four parasitoid species studied here (Sivinski et al., 1997), but exhibits the largest first instar larvae of all species that we compared (M. Aluja, unpublished data).

Based on the above descriptions, it becomes clear that the mix of species selected for this study offers an ideal opportunity to experimentally examine variability in reproductive investment among closely related parasitoid species that nevertheless vary sharply in key attributes such as host breadth, foraging behavior and adult size.

2.1.2. Larval host and quality of fly larvae

The genus *Anastrepha* is endemic to the New World and is restricted to subtropical climates where they typically inhabit highly variable environments in close association with their host plants (Aluja, 1994). The larval host in the present study was *A. ludens*, a polyphagous tephritid fruit fly distributed from the southern USA to Costa Rica (Foote et al., 1993). The hosts of *A. ludens* in nature are mainly those in the family Rutaceae (e.g., *Citrus* spp., *C. greggii* and *C. edulis*), but they can also infest fruit within the family Anacardiaceae (e.g., *M. indica*) (Aluja et al., 2000). The relative quality of *A. ludens* larvae stemming from different substrates was determined by measuring fresh larval weight with an analytical balance (Sartorius CP64) and protein content of third instar larvae. Proteins were determined from crude extracts of *A. ludens* larvae using the Bradford reagent (Sigma) in an ELISA spectrophotometer (standard: bovine serum albumine, Sigma). Data were obtained from a parallel study (L. Cicero, unpublished data) following methods described in Nestel et al. (2003).

2.2. Rearing of hosts and parasitoids

The four parasitoid species were reared following methods described in Aluja et al. (2009). They were maintained under a photoperiod of 12:12 (L/D), at 27 °C and 70% RH in the laboratory of the Red de Manejo Biorracional de Plagas y Vectores (RMBPV) of the Instituto de Ecología, A. C., Xalapa, Veracruz, Mexico.

To evaluate the effect of fly larval diet (host substrate) on parasitoid reproductive investment, larvae of *A. ludens* were reared in different fruit. The treatments were: (1) larvae from a laboratory colony reared on an artificial diet (for more than 300 generations), (2) larvae from the laboratory colony reared on mango (*M. indica* cv. 'Tommy Atkins'), (3) larvae from the laboratory colony reared on pink grapefruit (*C. paradisi* cv. 'Ruby Red'), and (4) wild larvae obtained from field infested white sapote (*C. edulis*). Experiments were conducted from May to November of 2006 and 2007. All fruit used in the experiments, except for white sapote, were obtained from the local market in Xalapa, Veracruz, Mexico.

2.3. Infestation of fruit by *A. ludens* larvae

Fruit were infested in the laboratory by placing 50 reproductively-mature male and 100 mature female *A. ludens* inside seven 30 × 30 × 30 cm acrylic screen cages and then providing them three grapefruits or mangoes every third day. Subsequently, infested fruit were placed in plastic containers labeled with the following information: date of infestation and host (plant species). Fruit were examined 15 and 30 days after infestation to collect

larvae from mango and grapefruit, respectively. Larvae reared on the artificial diet were obtained from the colony maintained in the laboratory, whereas wild larvae (for white sapote) were obtained directly from infested fruit collected in the field at several localities in the states of Veracruz and Puebla, Mexico.

2.4. Oviposition of female parasitoids in infested fruit and diet

Sandwich-type (SD2) oviposition devices, 4.0 cm Petri dish bases covered with two 15 × 15 cm pieces of organdi cloth, were used to expose host fruit larvae to female parasitoids (Aluja et al., 2009). Twenty five *A. ludens* larvae were placed in the oviposition device and exposed to five inexperienced parasitoid females between 4 and 12 days of age inside a 15 × 15 × 15 cm acrylic screened cage. To make the host exposure unit attractive to parasitoids, a piece of mature guava peel was placed on top of oviposition devices. This procedure was followed for each parasitoid species and for each host treatment. Host exposure time varied depending on the parasitoid species, and was based on previous observations of foraging efficacy. Specifically, exposure time was 24 h in the case of *D. crawfordi*, 4 h for *O. hirtus* and *U. anastrephae*, and 1 h for *D. longicaudata*. Exposed larvae were placed in containers with plastic lids and sterile vermiculite that was used as pupation substrate (Aluja et al., 2009). Each container was labeled with the date of exposure, parasitoid species and host treatment.

Ten days after exposure, pupae were weighted and placed individually in 4 cm Petri dishes with vermiculite. Petri dishes were examined daily every 30 min, and females of several ages were obtained: 0, 3, 6, 12 and 24 h. Emergence was monitored until approximately 15 female parasitoids of each age were obtained per host treatment. Female parasitoids were sacrificed and placed in a saline solution (Ringer) to extract the four ovarioles from the abdomen and release the eggs. We used a stereoscope equipped with a 4 – 1.6 × lens and a camera, connected to a computer. Photographs were processed with the Image-Processing Software NIS Elements 3.0 (Nikon). We then conducted mature egg counts (egg load) and egg size measurements (the mean area of the three largest mature eggs per female was used to estimate egg size in μ^2). This software was also used to measure the length of the left hind tibia of each specimen with a 2 – 1.6 × lens.

2.5. Statistical analyses

To evaluate the effect of host substrate on parasitoid initial egg load (EL_0), egg load at 24 h (EL_{24}), egg size and length of tibia of females of each parasitoid species, we applied generalized linear models (GLM) with a logarithmic link function. To select the best approximate model, a manual backward stepwise deletion of variables from a global model was conducted, as recommended by Crawley (2007). Because all models showed some over-dispersion, a quasi-GLM model was fitted by adjusting the scale parameter with a quasi-likelihood function that sets variance increasing as the mean square (family = quasi (link = log, variance = μ^2)) (Crawley, 2007; Zuur et al., 2009). The quasi-likelihood estimation allows us to model the response variable in a regression context without specifying its distribution. It is only needed to specify the link and variance functions to estimate regression coefficients (Wedderburn, 1974; Blough et al., 1999; Blough and Ramsey, 2000). To analyze the effect of host substrate on egg counts over a 24 h period for each species of parasitoid and between species, ANCOVA-type GLMs were used following Otto and Mackauer (1998). In the covariance model, the egg load was specified as response variable, while parasitoid species and host substrate were the main effects (host substrate nested in parasitoid species) and the age of the parasitoid was specified as a covariate.

Reproductive Investment (RI) was calculated as the product of the egg load at 24 h by egg average area, and divided by tibial length. Because egg shape for different parasitoid species was not always ovoid, and was very different among species, we refrained from using volume for estimation of RI as suggested by Blackburn (1991). RI represents an estimate of the amount of resources that the female parasitoid allocates to reproduction (considering female size) during its first 24 h of life. A nested ANOVA GLM was used to test for differences in RI, between host substrate within parasitoid species and between parasitoid species. To determine whether a relationship existed between pupal weight at 10 days and tibial length of female parasitoids, Spearman rank correlations were conducted as the data were not normally distributed and could not be normalized by transformation. In all analyses, contrasts were performed to examine for differences among host substrate treatments and parasitoid species, using the “estimable” function of the *gmodels* package for R statistical software.

Differences in variance between egg numbers and size compared to variance in body size were estimated by calculating the coefficient of variation (standard deviation/mean) in all parasitoid species. Third instar larval fresh weights and total amount of protein of *A. ludens* reared in different substrates were compared by means of one way ANOVAs, followed by Tukey's multiple comparison test. Live weight data were transformed (Box-Cox[$y^{\lambda} - 1/\lambda$], using $\lambda = 1.3$) to achieve normality (Crawley, 2007). All statistical analyses were conducted with R software, version 2.11.0 (R Development Core Team, 2010).

3. Results

3.1. Larval host relative quality

Larval weights ($F_{3,156} = 51.854$, $P < 0.0001$) and protein content ($F_{3,76} = 20.306$, $P < 0.0001$) differed significantly among *A. ludens* fruit fly larvae stemming from different substrates (plant hosts). Mango (as predicted) and grapefruit (introduced host) yielded the lightest and less nutritious larvae, while white sapote (native) and artificial diet yielded larger and more nutritious fruit fly larvae (Table 1).

3.2. Initial egg load (EL_0)

Host substrate significantly influenced initial egg load (EL_0) in the exotic species *D. longicaudata* (GLM: $F_{3,102} = 3.29$, $P = 0.024$) (Table 2). Mean *D. longicaudata* EL_0 was highest when the host substrate was grapefruit (24.45 ± 2.17). The smallest mean EL_0 values were recorded when *A. ludens* larvae were reared in white sapote (16.95 ± 2.52) and mango (15.35 ± 2.02). We found no significant differences among host substrate reared on different diets for the more specialized native parasitoids (GLM: *D. crawfordi*: $F_{3,76} = 0.00$, $P > 0.05$; *O. hirtus*: $F_{3,55} = 0.704$, $P = 0.554$; *U. anastrephae*: $F_{3,77} = 1.163$, $P = 0.329$). The observed patterns were not consistent with those of hierarchical host quality.

Table 1
Fresh weight and protein content per individual of *A. ludens* larvae reared in four substrates.

Host substrate	N	Weight (mg)	Protein content (μg)
Artificial diet	20	31.16 \pm 0.812a	2807.04 \pm 107.93a
Grapefruit	20	22.02 \pm 1.116b	2113.44 \pm 75.77b
Mango	20	21.94 \pm 0.819b	2025.6 \pm 144.74b
White sapote	20	31.21 \pm 0.923a	2990.88 \pm 90.54a

3.3. 24 h Egg load (EL_{24})

A significant effect of host substrate on EL_{24} was detected only for *U. anastrephae* (GLM: $F_{3,89} = 3.726$, $P = 0.014$; *D. crawfordi*: $F_{3,79} = 2.145$, $P = 0.101$; *D. longicaudata*: $F_{3,131} = 1.898$, $P = 0.133$; *O. hirtus*: $F_{3,59} = 0.194$, $P = 0.900$). Specifically, *A. ludens* larvae reared on an artificial diet (24.21 ± 1.38), mango (23.74 ± 1.07) and white sapote (25.77 ± 3.50) produced parasitoid females with the greatest mean egg loads. In contrast, hosts reared on grapefruit (19.39 ± 1.03) produced female parasitoids with the lowest mean egg load (Table 2). Again this tendency failed to follow the predicted patterns in putative host quality.

3.4. Egg counts over a 24 h period

For all four parasitoid species, females had at least one mature egg at the time of emergence. Egg load increased in all parasitoid species over the 24 h period (GLM: *D. crawfordi*: Slope = 0.06, $P < 0.0001$; *D. longicaudata*: Slope = 0.036, $P < 0.0001$; *O. hirtus*: Slope = 0.035, $P < 0.0001$; *U. anastrephae*: Slope = 0.031, $P < 0.0001$), which confirms that all these parasitoid species are synovigenic. Nonetheless, within species, the egg counts over a 24 h period were similar among host treatments for all parasitoid species (i.e., no significant differences in slopes were observed).

By contrast, comparisons of egg counts over time showed significant differences across parasitoid species ($F_{3,1567} = 2.5602$, $P = 0.05$). The egg counts over a 24 h period for the native species *D. crawfordi* differed significantly from those of *D. longicaudata*, *O. hirtus* and *U. anastrephae* (Fig. 1).

3.5. Egg size

Egg size differed significantly among host fruit treatments for all of the studied parasitoid species (GLM: *D. crawfordi*: $F_{3,202} = 4.523$, $P < 0.004$; *D. longicaudata*: $F_{3,278} = 17.565$, $P < 0.001$; *O. hirtus*: $F_{3,215} = 14.001$, $P < 0.001$; *U. anastrephae*: $F_{3,286} = 5.484$, $P = 0.001$) (Fig. 2, Table 2). For most parasitoid species, the smallest eggs were from female parasitoids reared in *A. ludens* larvae fed with mango and those that developed in artificial diet. The largest eggs were observed in parasitoids reared in *A. ludens* larvae fed with grapefruit and white sapote, except for *U. anastrephae*, for which larger eggs were observed in females reared on hosts fed with grapefruit and artificial diet.

3.6. Reproductive investment (RI)

Significant differences in RI were found among parasitoid species (nested ANOVA, parasitoid species: $F_{3,991} = 66.458$, $P < 0.001$; host treatments: $F_{12,979} = 2.887$, $P < 0.001$), but within host substrate differences were significant only for the exotic parasitoid *D. longicaudata* ($F_{3,278} = 6.852$, $P < 0.001$) (Fig. 3). For this species, RI values were highest in the grapefruit substrate and lowest in the mango substrate. *D. longicaudata* (762.54 ± 23) and *U. anastrephae* (851.44 ± 24.31) invested the most in reproduction (i.e., higher RI), whereas *D. crawfordi* (512.93 ± 21.67) and *O. hirtus* (501.95 ± 16.36) invested the least.

3.7. Tibia length

Significant differences in tibia length were detected among host substrates for all parasitoid species (GLM: *D. crawfordi*: $F_{3,337} = 19.363$, $P < 0.001$; *D. longicaudata*: $F_{3,570} = 22.630$, $P < 0.001$; *O. hirtus*: $F_{3,286} = 17.427$, $P < 0.001$; *U. anastrephae*: $F_{3,371} = 17.993$, $P < 0.001$) (Fig. 4, Table 2). Overall, the smallest parasitoids developed in hosts reared on grapefruit, and for *U. anastrephae*, grapefruit and white sapote, whereas *A. ludens* larvae

Table 2

Generalized linear model results for the effect of host substrate on initial egg load (EL₀), egg load at 24 h (EL₂₄), egg size and tibial length of female parasitoids of *D. crawfordi*, *D. longicaudata*, *O. hirtus* and *U. anastrephae*.

Parasitoid species	Response variable	Df	Deviance	Resid. Dev.	F	P
<i>D. crawfordi</i>	EL ₀	3,76	0.00	41.46	0.000	1.000
	EL ₂₄	3,79	1.48	23.48	2.145	0.101
	Egg size	3,202	0.19	2.86	4.523	0.004
	Tibia length	3,337	0.33	1.94	19.363	<0.001
<i>D. longicaudata</i>	EL ₀	3,102	3.65	51.41	3.293	0.024
	EL ₂₄	3,131	0.59	18.20	1.898	0.133
	Egg size	3,278	0.55	3.01	17.565	<0.001
	Tibia length	3,570	0.52	4.52	22.630	<0.001
<i>O. hirtus</i>	EL ₀	3,55	1.21	28.61	0.704	0.554
	EL ₂₄	3,59	0.08	13.13	0.194	0.900
	Egg size	3,215	0.54	2.73	14.001	<0.001
	Tibia length	3,286	0.30	1.70	17.427	<0.001
<i>U. anastrephae</i>	EL ₀	3,77	0.83	18.65	1.163	0.329
	EL ₂₄	3,89	1.00	8.92	3.726	0.014
	Egg size	3,286	0.22	4.03	5.484	0.001
	Tibia length	3,371	0.23	1.61	17.993	<0.001

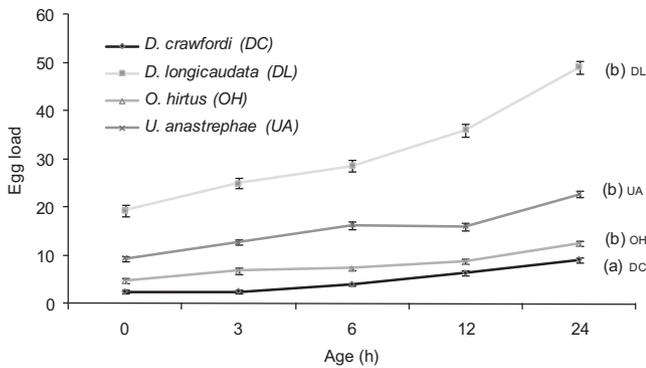


Fig. 1. Egg load (mean ± SE) of female parasitoids of four parasitoid species (*D. crawfordi*; *D. longicaudata*; *O. hirtus* and *U. anastrephae*) at five different ages (0, 3, 6, 12 and 24 h) reared in *A. ludens* larvae. Different letters indicate significant differences among species ($P < 0.05$).

reared in artificial diet gave rise to the largest individuals for all species.

The correlation between tibia length and fly-puparium + parasitoid pupal weight was significant for all parasitoid species (*D. crawfordi*: $r = 0.838$, $P < 0.001$, $n = 342$; *D. longicaudata*: $r = 0.714$, $P < 0.001$, $n = 518$; *O. hirtus*: $r = 0.736$, $P < 0.001$, $n = 291$; *U. anastrephae*: $r = 0.682$, $P < 0.001$, $n = 376$).

3.8. Coefficient of variation (CV)

The coefficient of variation was greater in egg load compared to tibial length (body size) and egg size in all parasitoid species (Fig. 5). In general, the way in which variation varied in magnitude across parameters was similar among parasitoid species and host substrates. In all cases, the greatest CV was observed in the egg load parameter, followed by egg size, and finally body size.

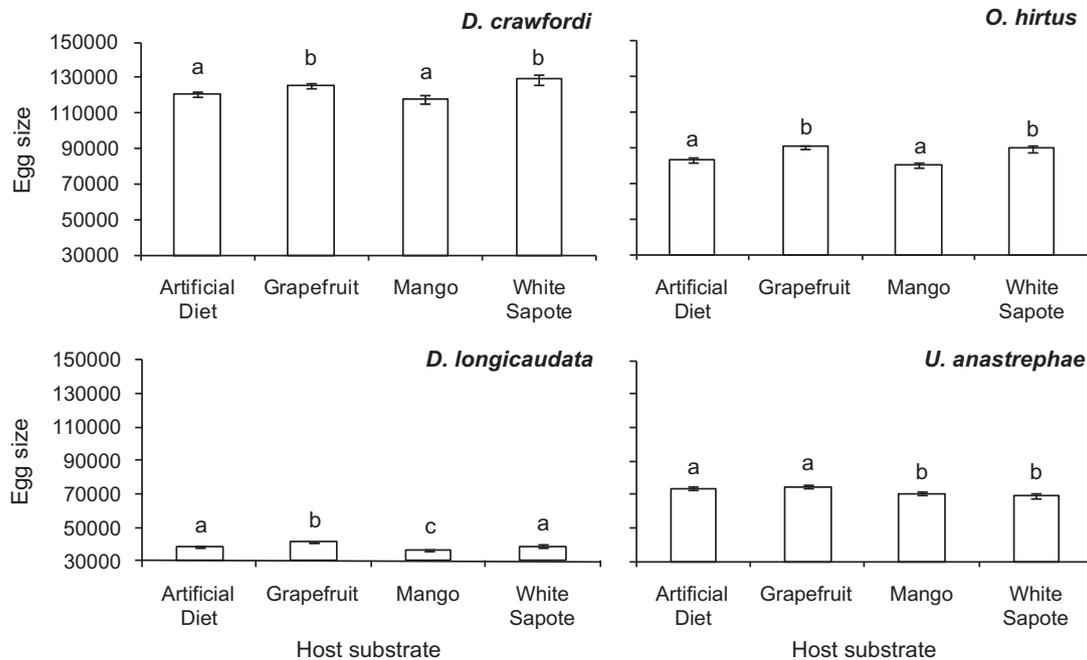


Fig. 2. Egg size (mean ± SE) of female *D. crawfordi*, *D. longicaudata*, *O. hirtus* and *U. anastrephae* parasitoids reared in *A. ludens* larvae from a laboratory colony fed with an artificial diet, mango and grapefruit, or *A. ludens* fed with white sapote. Different letters indicate significant differences among host food substrate treatments ($P < 0.05$).

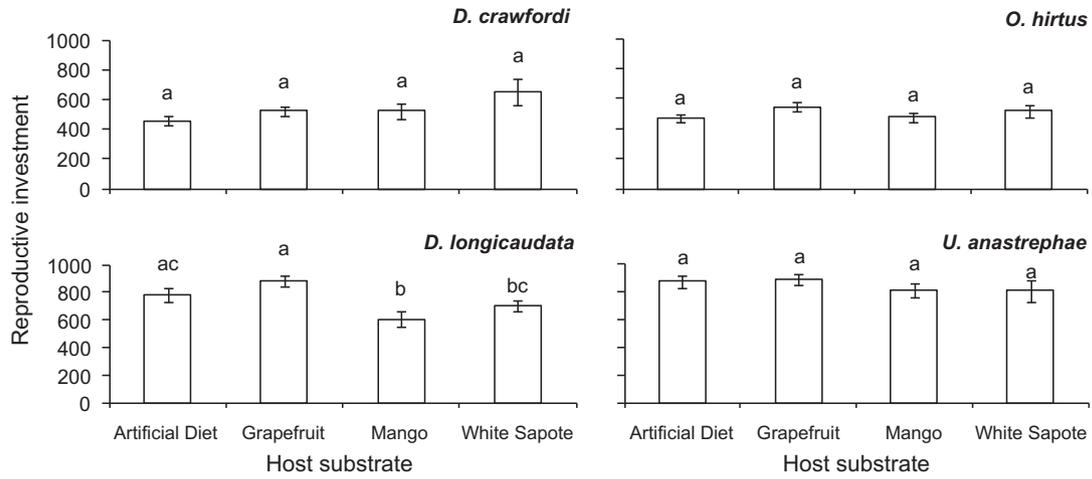


Fig. 3. Reproductive Investment (mean egg size * egg load/tibia length ± SE) of female parasitoids reared in *A. ludens* larvae reared in four different host fruit 24 h after emergence. Different letters indicate significant differences among host food substrate (data are not transformed) ($P < 0.05$).

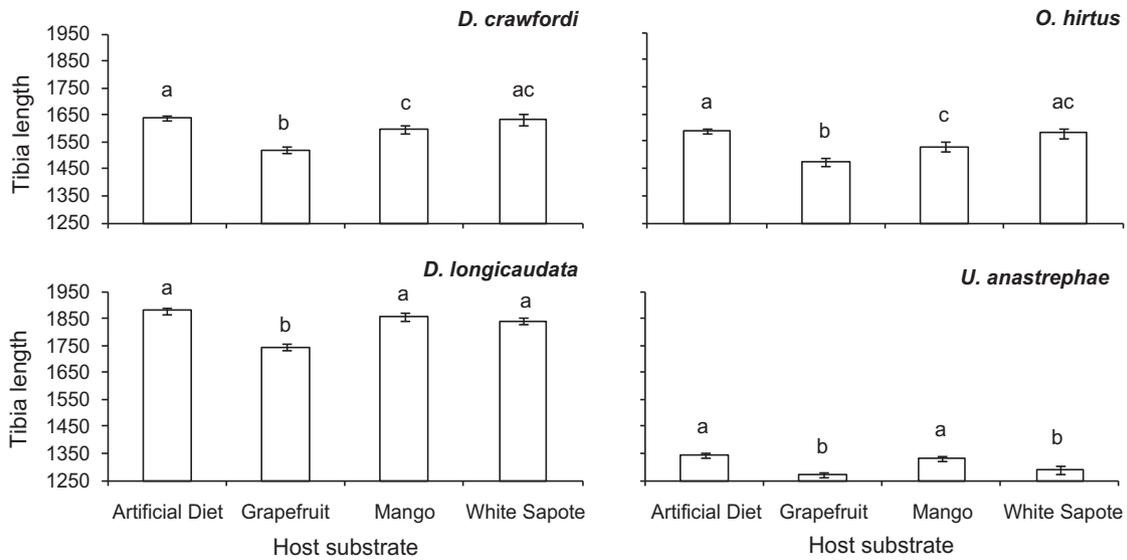


Fig. 4. Left hind tibia length (mean ± SE) of female *D. crawfordi*, *D. longicaudata*, *O. hirtus* and *U. anastrephae* parasitoids reared in *A. ludens* larvae from a laboratory colony fed with an artificial diet, mango and grapefruit, or wild *A. ludens* fed with white sapote. Different letters indicate significant differences among host food substrate treatments ($P < 0.05$).

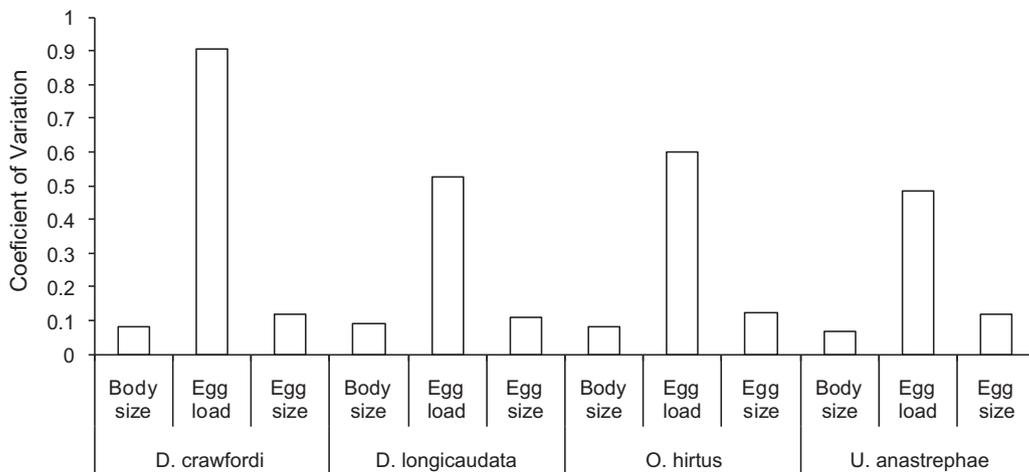


Fig. 5. Coefficient of variation of egg load, egg size and tibia length (body size) parameters among host treatments in female *D. crawfordi*, *D. longicaudata*, *O. hirtus* and *U. anastrephae* parasitoids reared in *A. ludens* larvae.

4. Discussion

Phenotypic plasticity is the capacity of a genotype to express different phenotypes according to the environment in which it develops. The environment can be represented as the physical surroundings and/or the internal conditions that may be affecting gene expression (Thompson, 1991; Stearns, 1989; West-Eberhard, 1989; Whitman and Agrawal, 2009). In parasitoids, the size and quality of the host can be unpredictable and the capacity to allocate proteins and lipids to various physiological and growth needs is thought to be a means of optimizing reproductive investments. For example, Ozkan (2007) found that egg load was higher in *Venturia canescens* (Hymenoptera: Ichneumonidae) females reared from third instars of its host *Ephestia kuehniella* (Lepidoptera: Pyralidae) compared to those reared from fifth instars. In another study, Jenner and Kuhlmann (2006) showed that *Campoplex dubitator* (Hymenoptera: Ichneumonidae) adjusted body size and fecundity depending on the age of its host *Enarmonia formosa* (Lepidoptera: Tortricidae); younger hosts produced later emerging but larger and more fecund female parasitoids, and older hosts produced less fecund, smaller females that emerged earlier. Similarly, Harvey et al. (1994) found that larvae of the parasitoid *V. canescens* adjust developmental time according to the larval stage of their host *Plodia interpunctella* (Lepidoptera: Pyralidae) shortening such time in late instar larvae. Another example of how parasitoids adjust some developmental traits under different environments, is represented by *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae), that varies developmental time according to the plant genotype its host has developed in (Sarfraz et al., 2008).

Here we uncovered various forms of plasticity through conditional responses by opiine fruit fly parasitoids to different host-nutritional histories: These were: (1) differences within species due to host food-substrate, (2) differences across species in the magnitude of their responses to the host substrate, and (3) differences in the apparent capacity of fitness components (traits) for change. In many cases these responses were interrelated.

4.1. Plasticity in reproduction components

Egg load, either initial or after 24 h, was relatively insensitive to host substrate. Only *D. longicaudata* had significant differences in initial egg load on different hosts and only for *U. anastrephae* changes were observed after 1 day of age. Intraspecific variance in egg load was highly variable, but host larval diet explained little of this variance. In contrast, egg size was affected by host substrate in all four species. Egg size may adaptively respond to changes in resource availability if it is a relatively unimportant trait and there is weaker selection to maintain a standard investment per egg. Koinobionts in general produce yolk-poor (hydropic) eggs (Jervis and Kidd, 1986), perhaps because hatchlings are immediately afloat in a nutrient suspension (Gauld and Bolton, 1988; Jervis et al., 2001; Strand, 2000). Although the significance of species' differences in egg size is unknown, larvae from opiine species with smaller eggs can successfully develop in the same host as those derived from larger eggs (Sivinski et al., 2000). A reduction in the number of eggs, and hence oviposition opportunities, may represent a greater cost to total female reproduction than under provisioning individual progeny in the egg stage. Adult body size also showed a consistent significant response to host diet. However, the combination of egg number and size relative to body size (reproductive investment) was, like egg load, insensitive to host diet in all species other than *D. longicaudata*. To extend the argument above, total reproductive investment is likely to represent a critical component of reproduction, and selection will act to stabilize a minimum investment in the face of nutritional shortfalls.

4.2. Niche breadth, host predictability and plasticity

It was hypothesized that the generalist parasitoid (exploiting several species of hosts) would encounter more reproductive opportunities (available hosts) than specialized species exploiting fewer hosts. As a consequence, reproductive plasticity would be of greater importance to parasitoids with broader host ranges and wider environmental tolerances. The opiines examined represented a considerable range of specialization, some based on differences in ovipositor length. For example, species with longer ovipositors are able to exploit larvae in a greater size and fly species that infest a range of fruits (Sivinski et al., 2000). There were additional components of host range. Both *O. hirtus* and *U. anastrephae* have short ovipositors, although *U. anastrephae* exploits more fruit species than *O. hirtus* (J. Sivinski, unpublished data). The long ovipositor of *D. crawfordi* is a near match for that of *D. longicaudata*, however it is more restricted to cooler environments and has been recovered from a lower diversity of fruits. Given this, we predicted that *D. longicaudata* would exhibit the greatest plasticity in response to host variability and *O. hirtus* the least. This predicted pattern was partially observed. Of the five reproductive components, initial egg load, egg load after 24 h, egg size, body size and reproductive investment, *D. longicaudata* showed a significant response to host origin in four (excepting egg load at 24 h). In contrast, *O. hirtus* and *D. crawfordi* were insensitive to three of the five components (egg and body size excepted). We also predicted that since specialized parasitoids feeding on specific hosts would face lower variance in host quality in nature they would be ill-equipped to conditionally respond to artificially high variances in host quality in the laboratory. As a result, the variances in body size, egg size and egg load would be similar i.e., there would be no mechanism to maintain an optimal reproductive investment at the expense of investment in total body size. This prediction was not upheld, since all four parasitoid species showed similar responses with higher variation in egg load than in body size and egg size.

Overall, *D. longicaudata* seems to be a good candidate for future tests of the environmental matching hypothesis (Dmitriew and Rowe, 2011), where a plastic developmental response to poor nutrition results in an adult phenotype that is better adapted to restricted food conditions.

4.3. Predicted host "quality" and reproductive investments

We predicted that the near-ancestral rutaceous diets of grapefruit and white sapote and the artificial diet (designed to be nutritionally complete), would produce fly larvae of large size and/or high nutritional content. Further, we predicted these characteristics would be reflected in the reproductive traits of parasitoids that developed in large, high quality hosts. In another tri-trophic system diet influenced the development of a pentatomid host and the egg loads of its parasitoid (Senravan and Annadurai, 1991). Host food availability was also among the factors that influenced the ovigeny index measured as the proportion of the potential lifetime complement of eggs that is mature upon female emergence in the parasitoid *V. canescens* (Ozkan, 2007). However, our prediction that rutaceous diets would result in more numerous and larger parasitoid eggs often proved erroneous.

As previously noted, egg size differed significantly with host substrate in all the parasitoid species. Individuals of *D. crawfordi*, *D. longicaudata* and *O. hirtus* that emerged from hosts reared in Rutaceae, white sapote and grapefruit, had larger eggs than those from the alternative substrates. In this case the smallest eggs were observed in females from hosts that had fed on non-Rutaceae diets, mango and artificial diet. However, initial egg load (EL₀) was influenced by host substrate only in the exotic generalist *D. longicaudata*, and in this case both the highest and lowest numbers of eggs

were found in parasitoids derived from the Rutaceae fed hosts, grapefruit and white sapote, respectively. *U. anastrephae*, was insensitive to host diet at EL₀, but after 24 h grapefruit fed hosts yielded parasitoids with the lowest egg loads.

Adult tibia length was plastic for all species and had highly significant responses to host substrate that conformed to the expectation that *A. ludens* larvae developing in Rutaceae should make better hosts. This was true only for larvae fed with white sapote. *D. crawfordi*, *D. longicaudata* and *O. hirtus* that emerged from hosts reared in white sapote and artificial diet were larger than those from the alternative host substrates. These diets yielded larger and more protein-rich *A. ludens* larvae. In all parasitoid species, smaller individuals developed in hosts that fed on the other rutaceous species, grapefruit. In the case of *U. anastrephae*, females were largest when they developed in larvae from mango.

Such mixed results, with poorer outcomes arising on occasions from hosts fed on fruits from the proposed ancestral family, suggests that more than the ability to acquire nutrients in a fruit, it is the evolution of *Anastrepha* host ranges and their ecological consequences that shape resource allocation to reproduction in associated parasitoid species.

4.4. Summary and implications for biological control

The four species of parasitoids responded in different ways to host diet, despite belonging to the same subfamily and attacking hosts in the same genus. The differences in resource allocation strategy to reproduction among the parasitoids studied may be due to the evolution of life histories in environments with differential variation in host availability (Ellers and van Alphen, 1997).

On the other hand, there were important similarities, particularly in the non-significant host diet effects on egg counts over a 24 h period, and the differences between host substrate in egg size and tibia length. However, for all the differences involving changes over time and treatment interactions between and within species, reproductive investment values that estimate the amount of resources allocated to reproduction per unit body size, were remarkably stable. Over a very short period of time RI differences due to host fruit treatments were trivial, at least for the native species. It seems that for these species there exists a fixed reproductive budget, which is independent of host quality. This may reflect “time-limited” rather than “egg-limited” foraging strategies (Ellers et al., 2000). That is, the most expensive component of reproductive success is to locate and handle patchily-distributed and fruit-sequestered hosts. If so, the cost of eggs would be relatively trivial and all of the host substrates were sufficient to produce adequate numbers and sizes of eggs.

The general robustness of RI in these species has implications for biological control, both in the selection of candidate species for introduction in classical biological control programs and when calculating the costs associated with inundative parasitoid releases (Aluja et al., 2009; Cancino et al., 2009; Montoya et al., 2000). It suggests that the hosts on which *A. ludens* populations are sustained are relatively unimportant to the success of parasitoid establishment efforts. Mass-rearing programs may not need to search for additional, perhaps expensive, foods for *A. ludens* since the presently used artificial diet recipe produces hosts of equal value as those that develop in natural fruit.

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