1 **FOR: *Biological Control***

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14 Martínez-Ramírez *et al.*: influence of host species on fitness of *D. longicaudata*

21 Nutrient uptake and allocation capacity during immature development determine
22 reproductive capacity in *Diachasmimorpha longicaudata* (Hymenoptera:
23 Braconidae: Opiinae), a parasitoid of tephritid flies.

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Abstract

Fitness in parasitoids is generally influenced by host quality. We measured the effects of three tephritid host species that varied in size, the fruits they developed in, and host nutritional content on some measures of fitness such as egg load, individual egg size, body size, and nutrient composition in the braconid *Diachasmimorpha longicaudata*, a widely used fruit fly biological control agent. The host species were: *Anastrepha ludens* (large host developing in both grapefruit and an artificial diet), *A. obliqua* (small host) and *A. serpentina* (small host). Female parasitoid body size was influenced by host size and the number of eggs per female varied significantly with parasitoid age (0 – 24 h. Female parasitoids that emerged from *A. obliqua* and those that emerged from *A. ludens* reared in grapefruit had the largest eggs, whereas those that emerged from *A. ludens* reared in artificial diet and from *A. serpentina* had the smallest eggs. While nutrient concentrations differed among fly species, there was little to suggest that resources were more accessible in certain hosts. The largest host did not necessarily exhibit the highest nutrient concentration. Larger hosts represented larger macronutrient pools and the nutrient content of the host generally corresponded to the relative abundance of the same nutrients in the parasitoids that developed within them. Nevertheless, the quality of the various hosts tested had little effect on egg load, egg size and adult nutrient composition of the oligophagous parasitoid, *D. longicaudata*. This renders *D. longicaudata* a valuable candidate parasitoid for the biological control of multiple species of *Anastrepha*.

69 **Keywords:** *Anastrepha*, Braconidae, *Diachasmimorpha longicaudata*, Diptera:
70 Tephritidae, host quality, parasitoid, egg load.

Introduction

Since parasitoids feed on a single host during their development, their fitness is strongly influenced by host traits such as the quantity and quality of the host's nutrient resources (Godfray, 1994; Jervis and Kidd, 1996; Roitberg et al., 2001; Van Driesche and Bellows, 1996, Van Driesche et al., 2007). In turn, nutrient quantity and quality are affected by factors such as host diet and size, and in the case of endoparasitoids, they also depend on the physiological alterations inherent to host development (Vinson et al., 2001).

Opiinae parasitoids of Diptera larvae are koinobiont endoparasitoids that attack hosts that are mobile and continue to grow (Askew and Shaw, 1986; Quicke 2014). The braconid *Diachasmimorpha longicaudata* (Ashmead) is a solitary, synovigenic, larval-prepupal, endoparasitoid, originally from the Indo-Australian region where it attacks *Bactrocera* spp. (Diptera: Tephritidae) (Greany et al., 1976), although it can attack a number of tephritid genera in several subfamilies (Ovruski et al., 2000; Wharton and Marsh, 1978). Under laboratory conditions, *D. longicaudata* females can live 34 ± 7.6 days and have an average fecundity of 213.4 ± 3.9 days (González et al., 2007). It is the parasitoid most used to control tephritid fruit flies worldwide (Sivinski, 1996) and it has been introduced into several countries in the New World to control fruit fly pests in the genus *Anastrepha* (Ovruski et al., 2000). *Diachasmimorpha longicaudata* has been mass reared and released to suppress populations of *Ceratitis capitata* (Wiedemann) (Montoya et al., 2005) and *Anastrepha* spp. which infest several species of citrus, guava (*Psidium guajava* L.) and mango *Mangifera indica* L.) (Montoya et al., 2000,

2007) in Mexico, where it is also the most commonly prevalent exotic parasitoid of *Anastrepha* fruit flies in perturbed natural areas (Aluja et al., 1990, 2014; López et al., 1999).

Koinobiont parasitoids can regulate host nutrient availability to manipulate host physiology (Askew and Shaw, 1986; de Eguileor et al., 2001). They commonly begin the uptake of host nutrients since embryonic development due to the fact that they usually produce hydropic eggs (i.e., eggs with reduced yolk content) that do not contain enough nutrients to sustain the morphogenetic process of the embryo (Le Ralec, 1995; Jervis et al., 2008).

In some cases a resource can only be acquired during the larval stage and used during the adult stage; this type of stored nutrients are referred to as capital reserves (Strand and Casas, 2007). Adult hymenopteran parasitoids are an example of the latter, as they are incapable of synthesizing lipids (Giron and Casas, 2003; Rivero et al., 2001), although some species obtain lipids via host feeding (Jervis and Kidd 1986; Jervis et al., 2008). Although they ingest protein and carbohydrates (e.g., when they feed on nectar and honeydew), little or none of these nutrients are converted to lipids, and thus adult parasitoids depend entirely on reserves acquired as larvae (i.e., the capital reserves) (Giron and Casas, 2003; Visser and Ellers, 2008). Such reserves are later used for egg production (Rivero et al., 2001) and, in a lesser proportion, for maintenance functions (Strand and Casas, 2007; Jervis et al., 2008).

As the host provides the necessary nutrients for parasitoid development, it also has an influence on adult parasitoid size (Godfray, 1994; Jervis and Kidd, 1996). In turn, body size is often correlated with the amount of reproductive tissue,

which can determine egg number (egg load) and size (Jervis et al., 2008), as well as the capacity to carry reproductive payloads (Godfray, 1994). There are exceptions to such generalizations, in some species of mass-reared *Trichogramma* spp. there is no relationship between body size and fecundity, longevity, and locomotor activity (Pavlik, 1993). Independent of fecundity, there may be additional advantages to large body size. Larger individuals may be able to move over greater distances in the search for hosts (Sivinski and Dodson, 1992) or better able to reach hosts within infested substrates with their longer ovipositors (Sivinski et al., 2001).

We addressed the nature of host quality in the nutritional ecology of the koinobiont tephritid parasitoid *D. longicaudata* from both morphological and physiological perspectives. That is, does host body size determine parasitoid size, egg load and egg size or are there other independent, perhaps species specific, nutritional factors outside of sheer quantity that influence parasitoid fitness? While host size represents a realistic measure of quality (Godfray, 1994; Jervis and Kidd, 1996), hosts may also differ in their defensive chemistry and nutritional value, regardless of their size (Häckerman et al., 2007). The importance of host quantity (size) and quality (lipid, protein, glycogen and carbohydrate contents) to *D. longicaudata*'s fitness was experimentally examined by providing parasitoids with hosts from variously sized species that developed in different host fruit and may present varying nutritional profiles. To assess host quality we used: 1) *Anastrepha ludens* (Loew) a polyphagous species considered an important pest of mango (*Mangifera indica* L.) and several species of citrus (Aluja et al., 1996, 2009; Birke et al., 2006), whose distribution ranges from the southern United States to Central

America (White and Elson-Harris, 1992); 2) *Anastrepha obliqua* (Macquart), the West Indian fruit fly, another polyphagous species that shows a preference for plant species in the family Anacardiaceae, whose distribution is from Mexico to Argentina (White & Elson-Harris, 1992) and is considered an important pest of mango (Aluja et al., 1996); and 3) *Anastrepha serpentina* (Wiedemann) considered a pest of “sapodilla” *Manilkara zapota* (L.) van Royen, “zapote mamey” *Pouteria sapota* (Jacq.) H. Moore and Stearn, as well as other less common commercially used species of Sapotaceae [e.g., *Calocarpum mammosum* (L.) P. Royen, *Chrysophyllum cainito* (L.) and *Pouteria campechiana* (Kunth) Baehni (Robacker et al., 2009)]. We determined the nutrient composition of both, the host species and the parasitoids they nourished to see if the amounts and proportions of host nutrients were reflected in their parasitoids. The value of body size and the outcomes of additional nutritional inputs, both in hosts and parasitoids, are an issue of economic importance because they can influence the costs of mass-rearing and the efficacy of biological control programs based on augmentative releases of parasitoids for the management of fruit fly species, several of which represent the most devastating agricultural pests of the New World (Aluja, 1994; Reyes et al., 2000).

Materials and methods

Parasitoids and fruit fly hosts

For this experiment, *D. longicaudata* individuals were obtained from colonies maintained in the laboratories of the Red de Manejo Biorracional de Plagas y Vectores (RMBPV) of the Instituto de Ecología, A.C (INECOL), in Xalapa, Veracruz, Mexico and reared on *A. ludens* larvae of seven to nine days of age. At adult emergence, female and male parasitoids were placed together in Plexiglass cages (25 × 25 × 25 cm), fed with diluted honey (70% honey, 30% water) (Miel Carlota®; Herdez S.A. de C.V., Cuernavaca, Morelos, Mexico) and maintained at 27 ± 1°C with a relative humidity of 70 ± 5% and a photoperiod of 12:12 h (L:D).

For the experiment, third-instar *A. ludens* (large = 29.31 ± 0.55 mg), *A. obliqua* (small = 21.6 ± 0.54 mg) and *A. serpentina* (small = 19.74 ± 0.54 mg) larvae were used as hosts. Larvae were obtained under laboratory conditions from fruit infested by exposure to a semi-domesticated laboratory colony of fruit flies. Tephritids used for fruit infestation were collected in the field at several localities in the central region of the state of Veracruz, Mexico. We used different host fruit species for each fly: *Citrus paradisi* Macfayden (pink grapefruit) in the case of *A. ludens*, *M. indica* (mango, cultivar Tommy Atkins) for *A. obliqua*, and *M. zapota* (sapodilla) in the case of *A. serpentina* (details of fruit nutrient composition in Table 1). Fruit was purchased at local markets in order to assure that they were free of fruit fly infestation and hand-washed with water and a neutral detergent prior to infestation to eliminate possible pesticide residues. Following exposure to flies, fruits were maintained at 26 ± 1°C under a LD 12:12 h photoperiod (lights on at 07.00 h) and 70 ± 10% relative humidity to guarantee an adequate development of fly larvae. In the case of *A. ludens*, additional larvae from a laboratory colony were

used as a control and were reared with an artificial diet consisting of yeast, sugar, corncob powder, citric acid, sodium benzoate, wheat germ and vitamins (Table 1).

Host exposure to parasitoids

Parasitoids

Larvae of each fly species were exposed to *D. longicaudata* females to obtain adult female parasitoids from each host species. Groups of 25 third-instar larvae of each fruit fly species were rinsed with water to remove odors and were placed in “sandwich-type” oviposition units, consisting of a Petri dish (4.0 cm diameter) base covered with two pieces of organdie cloth (15 × 15 cm) with larvae placed in the middle of the cloth pieces. To render oviposition devices attractive to parasitoids, a thin slice of guava peel (*P. guajava*) was placed on the top of the unit (Aluja et al., 2009). The oviposition unit was then exposed to five, four to 10-day old female parasitoids with no previous oviposition experience (Montoya et al., 2000). Exposures were conducted in plastic containers 14.5 cm in height by 12.5 cm in diameter for a period of 25 to 40 min.

Fly larval weight

A total of 24 third instar *A. ludens* reared on artificial diet, 46 *A. ludens* reared in grapefruit, 46 *A. obliqua* reared in mango, and 45 *A. serpentina* reared in sapodilla were used to record differences in host weight.

Adult emergence, dissection and oocyte counts

Following parasitism, host larvae were placed in labeled plastic containers with Vermiculite for pupation. Vermiculite was moistened with 2.0% sodium benzoate solution every two days to suppress fungal growth. Ten days after pupation, pupae were individually placed in Petri dishes 4.3 cm in diameter by 1.6 cm in height with vermiculite, and parasitoid emergence was monitored every 30 min, from day 14 to day 30 post parasitism. Unfed female *D. longicaudata* were dissected under a stereoscopic microscope at 0, 6 or 24 h after emergence to extract the ovaries. Ovaries were stored together with the thorax in individual glass containers in a phosphate buffer containing formaldehyde at 5°C. Egg counts and size were measured in 15 randomly selected females from each host species treatment. Ovaries were placed in saline solution and were dissected to release the eggs. Eggs were counted and their area (μm^2) was estimated using the NIS-Elements 3.0 (Nikon) software. The sizes of the dissected adults were estimated based on the measurement of the length of the left hind tibia (Godfray, 1994; Rivero and West, 2002) using the same software.

Analyses of proteins, lipids, carbohydrates and glycogen

Parasitoid specimens derived from each host species were stored at -80°C until used for protein, lipid, carbohydrate and glycogen content analyses. Protein content was determined using the Bradford reagent (standard: bovine serum albumin), lipids with vanillin in phosphoric acid (standard: triolein), and glycogen (standard: glycogen) and carbohydrates (standard: glucose) were determined using the Anthrone reagent. All nutrients were determined using an ELISA spectrophotometer (details are described in Warburg and Yuval, 1996; 1997; Yuval

et al., 1998; Kaspi et al., 2002; Nestel et al., 2003; Nestel et al., 2004). These analyses were also conducted for third instar larvae of each host species.

Statistical analyses

The effects of host species and parasitoid age on egg load and egg size were evaluated by factorial ANOVAs. To test if parasitoid size and its interaction with host had a significant effect on egg load or egg size, a GLM homogeneity of slopes analysis was run. An egg load*egg size correlation was run to ascertain if there was an association. To test for differences in nutrient content among host species, as well as to determine if host species had an effect on female parasitoid size, egg load at 0, 6 and 24 h, and egg size, separate one-way ANOVAs. In all cases, means were compared by post-hoc Tukey tests. Egg load values were $\sqrt{x+1}$ transformed and egg size values were squared to achieve normality and homoscedasticity. Nutrient content was analyzed in two ways: 1) considering the nutrient concentration per larvae and 2) considering the absolute amount per larvae among different species. In the case of parasitoids, nutrient concentration and absolute amount per larvae, were first analyzed with an one-way MANOVA including all the nutrients and then each nutrient was analyzed individually. For nutrient concentration, one-way ANOVAs were performed to compare protein, lipid, glycogen and carbohydrate concentration among fly larvae from different host species and for lipid, carbohydrate and glycogen from parasitoids emerging from different host species. Nutrient concentration values were $\sqrt{x+1}$ transformed to meet parametric assumptions. For proteins, a Kruskal Wallis test was performed as normality and homoscedasticity could not be achieved via transformations. To

compare absolute amounts of nutrients among different species, one way ANOVAs were performed to analyze fly larvae and parasitoid lipid, carbohydrate, glycogen and protein content. Absolute nutrient values were $\sqrt{x+1}$ and x^2 (parasitoid proteins) transformed to meet parametric assumptions. Means were compared by post-hoc Tukey tests for parametric tests and with Dunn tests when non-parametric analyses were performed. Analyses were run in R (version 2.12.2) (R Development Core Team, 2011) and the Statistica 7.0 program (StatSoft Inc., OK, USA).

Results

Hosts

There were significant differences in larval weight among species (ANOVA: $F_{3, 156} = 58.98$, $P < 0.001$). *Anastrepha ludens* larvae fed with grapefruit (29.31 ± 0.55 mg) had similar weights to *A. ludens* fed with artificial diet (27.89 ± 0.77 mg), and both were significantly heavier than *A. obliqua* (21.6 ± 0.54 mg) and *A. serpentina* (19.74 ± 0.54 mg) (Fig. 1).

Host species differed significantly in protein, lipid, and carbohydrate concentrations (ANOVAs; proteins: $F_{3,102} = 5.88$, $P < 0.001$; lipids: $F_{3,50} = 6.48$, $P < 0.001$; carbohydrates: $F_{3,50} = 3.67$, $P = 0.05$) but this was not the case with glycogen concentration ($F_{3, 50} = 0.93$, $P = 0.43$) (Fig. 2A). (Figs. 2B, C and D.

Mean absolute amount of proteins, lipids, and carbohydrates differed significantly among species (ANOVA; proteins: $F_{3, 102} = 3.40$, $P < 0.05$; lipids: $F_{3, 50} = 22.78$, $P < 0.0001$; carbohydrates: $F_{3, 50} = 9.04$, $P < 0.0001$. There was no difference in the absolute weight of glycogen in the host larvae ($F_{3, 50} = 2.68$, $P = 0.051$) (Fig. 2).

Parasitoids

The body size (tibia length) of female parasitoids was significantly influenced by the host species ($F_{3, 243} = 41.89$, $P < 0.001$). Females that emerged from grapefruit or diet-reared *A. ludens* were significantly larger (1.83 ± 0.017 mm and 1.86 ± 0.019 mm respectively; mean $\pm$ SE) than females emerged from *A. obliqua* (1.68 ± 0.01 mm) and *A. serpentina* (1.60 ± 0.02 μ m).

Host species influenced the nutrient concentration per host larvae (one-way MANOVA; *Wilks Lambda* = 0.639, $F_{12,212} = 3.25$; $P = 0.001$). When nutrients were individually analyzed, protein, lipid, and glycogen concentration in female parasitoids differed significantly among host species (Kruskal Wallis; protein: $H_{3, 97} = 12.34$, $P < 0.05$; ANOVA's: lipid: $F_{3, 84} = 4.10$, $P < 0.001$; glycogen: $F_{3, 85} = 4.25$, $P = 0.01$; Figs. 3A, B and D), whereas carbohydrate concentration did not ($F_{3, 86} = 1.02$, $P = 0.388$; Fig. 3B).

Mean absolute weights of nutrients in parasitoids were significantly influenced by host species (one-way MANOVA: *Wilks Lambda* = 0.59, $F_{12,215} = 3.98$, $P < 0.0001$). When nutrients that *D. longicaudata* obtained from its hosts were analyzed individually, they differed significantly (ANOVA's: proteins: $F_{3, 97} = 5.23$, $P < 0.05$; lipids: $F_{3, 86} = 6.68$, $P < 0.001$; carbohydrates: $F_{3, 86} = 4.39$, $P < 0.05$; [Fig. 3]). Glycogen was the exception ($F_{3, 85} = 2.53$, $P = 0.06$). In general, absolute weights were highest in parasitoids derived from the larger hosts (*A. ludens*) and lower for small hosts (*A. obliqua* and *A. serpentina*).

Egg load increased significantly with female age (factorial ANOVA: Age; $F_{2, 236} = 94.11$, $P < 0.0001$), but it was not affected by host species; as illustrated in Figure 4, parasitoids stemming from different host species had similar egg loads (factorial ANOVA: Host: $F_{3,236} = 2.52$, $P = 0.058$) and the interaction host*age (factorial ANOVA: Host: $F_{3,6 236} = 0.93$, $P = 0.48$). Parasitoid size and the interaction host*parasitoid size influenced egg load (Homogeneity of slopes analysis: Parasitoid size $F_{1,238} = 25.67$, $P < 0.0001$; Host*parasitoid size $F_{3,238} = 3.86$, $P < 0.05$).

Egg size was significantly influenced by the host species, parasitoid age and the interaction host*age (factorial ANOVA: Host: $F_{3,230} = 28.81$, $P < 0.0001$; Age: $F_{2,230} = 5.90$, $P < 0.005$; Host*age: $F_{6,230} = 5.80$, $P < 0.0001$, Fig. 5). We also found a significant effect of parasitoid size on egg size (Homogeneity of slopes analysis Parasitoid size: $F_{1,232} = 27.06$, $P < 0.0001$), but not in the case of the interaction between parasitoid size and host (Homogeneity of slopes analysis Host*parasitoid size: $F_{3,232} = 0.93$, $P = 0.42$, Fig. 5). With respect to the relationship between egg load and egg size, we found a positive albeit weak correlation ($r=0.26$; $P < 0.0001$).

Female parasitoids that emerged from *A. obliqua* and those that emerged from *A. ludens* reared in grapefruit had the largest eggs ($40135.16 \pm 418 \mu^2$, and $39598.35 \pm 370 \mu^2$ respectively), whereas those that emerged from *A. ludens* reared in artificial diet with 0 and 6 h of age ($35947.33 \pm 725 \mu^2$) and from *A. serpentina* had the smallest eggs ($35599.61 \pm 482 \mu^2$).

Discussion

Parasitoid fitness is known to be closely associated with the quality of their host (Henry et al., 2005; Vinson, 2010). In this study, we tested the effect of host species on fitness parameters such as nutrient content, egg load, egg size, and adult size of the oligophagous parasitoid *D. longicaudata*. We found that, despite host differences in size and nutritional content, the fitness of *D. longicaudata* was unaffected and no decline in reproductive potential was observed, which is consistent with results obtained for this species in previous studies (Cicero et al., 2011, 2012; López et al., 2009).

As other studies showing a relationship between host and parasitoid size (Godfray, 1994). The size of *D. longicaudata* was significantly linked to host size. Moreover, smaller larvae supplied similar amounts of nutrients than larger larvae due to their higher nutrient concentration (e.g., *A. serpentina* and *A. obliqua* larvae had higher concentrations of protein and similar concentrations of glycogen than *A. ludens* from both diets [Fig. 2]), which allowed the parasitoid larvae to store adequate amounts of nutrients and produce similar egg loads (Fig. 4). This is consistent with Strand and Casas (2007), who stated that female parasitoids depend on capital reserves from their host for egg production. A different pattern was found for *D. longicaudata* egg size, which differed significantly among host species, particularly in newly emerged parasitoids and those reaching six hours of age. The relationship between adult parasitoid size and egg size was more apparent in *A. obliqua* (= small host) and *A. serpentina* (= small host) than *A. ludens* (= large host) reared in grapefruit. We do not know if in the case of *D. longicaudata* egg size could be associated with sex ratio or fertility. At present,

there is no evidence of a fitness advantage accruing to koinobiont parasitoid offspring that develop from larger eggs (Lalonde, 2005), which usually are hydropic (i.e., eggs with low yolk content) (Jervis et al., 2008; Quicke, 2014).

Within the present range of measurements, it was better to have as many eggs as possible, but, after reaching a certain volume, increased egg size served no further purpose. If true, parasitoids attacking *A. serpentina* were confronted with the dilemma of how to best balance egg number and the nutrients they contained. Given the fact that adult parasitoids depend on their capital reserves of lipids obtained during the larval stage for egg production (Strand and Casas, 2007), we assumed that *D. longicaudata* from *A. serpentina* exhibited a tradeoff between egg quality (i.e., egg size) and quantity (i.e., egg load). The habitats of endoparasitic larvae mitigate this challenge and might have tipped the balance in favor of investment in egg load. Hatchlings often eclose already immersed in nutrients and are confronted with dangers where size does not necessarily lend itself to defense the host's immune system and the larvae of other con- and heterospecific parasitoids (Godfray, 1994). Perhaps because of this, endoparasitoid eggs tend to be relatively small and contain little yolk (Strand, 2000; Quicke, 2014).

While it is often found and commonly assumed that a larger host represents a better host (López et al., 2009; Otto and Mackauer, 1998; Wang et al., 2008), host size does not always reflect host quality (Häckermann et al., 2007). Recently, the primacy of host size has been undermined by findings that stress the ability of parasitoid larvae to assimilate nutritional resources in a particular within-host environment rather than their abundance (Sampaio et al., 2008). In our case, while

nutrient concentrations differed among fly species, there was little to suggest that resources were more accessible in certain hosts. Larger hosts represented larger macronutrient pools and the nutrient content of the host generally corresponded to the relative abundance of the same nutrients in the parasitoids that developed within them. Here, we measured the main macronutrients (Table 1, Fig. 2), but it is possible that specific micronutrients accrued from the host larvae developed in could have influenced egg quality. In our study, *A. obliqua* larvae were reared in mango fruit, one of the preferred hosts of several species of *Bactrocera* spp. flies, the original *D. longicaudata* hosts (Wharton and Marsh 1978). It is likely that this host provided female parasitoids with specific nutrients allowing them to consistently produce bigger eggs since the moment of emergence and shortly thereafter (six hours; Fig. 5). Cicero et al. (2011) found that *Opius hirtus* and *D. longicaudata* females reared on *A. ludens* larvae fed with mango produce higher egg loads than those fed on an artificial diet. In other parasitoid species an indirect effect of host plant species on parasitoid resource allocation has been shown (Stoepler et al., 2011). In our study, parasitoid females reared in *A. ludens* (= big host) fed with grapefruit and *A. obliqua* (= small host) exhibited bigger egg sizes (Fig. 5) and similar egg loads (Fig. 4), despite differences in host size. This pattern underscores the plasticity/capacity of *D. longicaudata* to adapt to varying environmental conditions.

Phenotypic plasticity involves a differential response to environmental variation by individuals possessing similar genotypes (Stearns, 1989). In this context, *D. longicaudata* displayed phenotypic plasticity in adult body size, egg load, and egg-size in response to host size and nutrient concentration. Our

findings suggest that there was selection to maximize the number of eggs produced, which likely translates into egg-laying opportunities and perhaps less in investments in egg size. Such flexibility might be particularly adaptive in an oligophagous species like *D. longicaudata*. When Cicero et al. (2011) compared the effects of different *A. ludens* diets on the fitness of several parasitoid species, they found that the more specialized parasitoids were less plastic in some developmental responses when compared to *D. longicaudata*. These authors also found that the reproductive investment index ($RII = \text{mean egg size} * \text{egg load} / \text{hind tibia length}$) did not change with host diet in the more specialist parasitoids, while *D. longicaudata* was the only species that changed and adjusted RII index to host diet/quality. Similar to Cicero et al. (2011), in this study we demonstrated that *D. longicaudata*, can cope with differences in host quality (i.e., concentration of protein, lipids, glycogen and carbohydrates present in larvae) without experiencing a significant loss in key reproductive traits.

Our results have broad implications for fruit fly biological control as *D. longicauda* performed equally well independent of fly species despite the fact that not all host species were of the same quality (Fig. 2). This means that augmentative biological control programs using this species are likely to be equally successful against various species within *Anastrepha* if parasitoid females are released in the right place and at the right time.

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Figure Legends

Figure 1. Host size (= weight) and parasitoid size (= tibia length) by host species.

Figure 2. (A) Concentration and absolute content (mean $\pm$ SE) of glycogen (B) proteins, (C) carbohydrates, and (D) lipids of *A. ludens* larvae fed with an artificial diet (ludens [d]) or grapefruit (ludens [g]), *A. obliqua* fed with mango (obliqua), and *A. serpentina* fed with sapodilla (serpentina). Different letters indicate significant differences among host species ($P < 0.05$).

Figure 3. (A) Concentration and absolute content (mean $\pm$ SE) of proteins, (B) carbohydrates, (C) lipids and (D) glycogen in females of *D. longicaudata* reared on larvae of different host species: *A. ludens* fed with an artificial diet (ludens [d]) or grapefruit (ludens [g]), *A. obliqua* fed with mango (obliqua) and *A. serpentina* fed with sapodilla (serpentina). Different letters indicate significant differences among treatments ($P < 0.05$).

Figure 4. Egg load (mean $\pm$ SE) of *D. longicaudata* females of different ages (0, 6 and 24 h after emergence) reared on three different host species: *A. ludens* reared in an artificial diet (ludens [d]) or grapefruit (ludens [g]), *A. obliqua* reared in mango (obliqua), and *A. serpentina* reared in sapodilla (serpentina). Different letters indicate significant differences among treatments ($P < 0.05$).

Figure 5. Egg area measured in μm^2 (mean $\pm$ SE) for *D. longicaudata* females of three different ages reared on larvae of three host species: *A. ludens* reared in an artificial diet (ludens [d]) or grapefruit (ludens [g]), *A. obliqua* reared in mango (obliqua) and *A. serpentina* reared in sapodilla (serpentina). Different letters indicate significant differences among treatments ($P < 0.05$).

663 Table 1. Nutrient composition of different diets (artificial and natural) used to rear
664 host larvae of *D. longicaudata* (percentage of dry matter)

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	Artificial diet	Grapefruit (Ruby red)	Mango	Sapodilla
Fat	0.3	0.1	0.1	0.1
Carbohydrate	27.3	9.4	16.6	22
Protein	7.2	0.5	0.8	0.4

667

Figure 1

Figure 2

Figure 3

Nutrient content/parasitoid fresh weight in $\mu\text{g}/\text{mg}$ (mean $\pm$ SE)

Figure 4

Figure 5

Graphical abstract

Egg load of the parasitoid *D. longicaudata* reared in different species of *Anastrepha* larvae (*A. ludens* (d)= *A. ludens* reared in artificial diet; *A. ludens* (g) = *A. ludens* reared in grapefruit).

The square on the top of bars represents the mean weight of larvae .

Diachasmimorpha longicaudata, an oligophagous parasitoid, coped with different qualities of hosts without experiencing a significant loss in its reproductive output.