

# Parasitism of *Diachasmimorpha longicaudata* (Ashmead) on two fruit fly species

## *Parasitismo de Diachasmimorpha longicaudata* (Ashmead) em duas espécies de moscas-das-frutas

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**ABSTRACT:** The aim of the present study was to investigate the parasitism of two fruit fly species by different *Diachasmimorpha longicaudata* strains. Females of two parasitoid strains were reared on *Ceratitis capitata* or *Anastrepha fraterculus* that were conditioned in adapted containers. Individual parasitoids were offered 20 larvae for 1 h, using no-choice (20 *C. capitata* or 20 *A. fraterculus*) or free-choice (10 of each species) assays. Parasitism was verified by counting scars, and the pupae were classified as non-parasitized (no scars), parasitized (one scar), or superparasitized (multiple scars). Parasitism intensity was compared by evaluating the proportions of non-parasitized, parasitized, and superparasitized larvae. Parasitism intensity and scar frequency were analyzed as functions of host species and parasitoid strain, using chi-square tests. In the non-choice tests, females of both parasitoid strains parasitized the larvae, regardless of host species. However, when the parasitoids had a choice, they preferred to superparasitize *A. fraterculus* larvae, which are larger than *C. capitata* larvae. In addition, the female parasitoids parasitized most of the host larvae, regardless of parasitoid strain or host species. However, the frequency of non-parasitized pupae was higher when the offered host species was different from the one in which the parasitoid was reared, and the frequency of superparasitized pupae was higher when the offered host species was the same as the one in which the parasitoid was reared.

**KEYWORDS:** Superparasitism; host preference; parasitoid.

**RESUMO:** O objetivo deste trabalho foi avaliar o parasitismo de larvas de Tephritidae por duas linhagens de *Diachasmimorpha longicaudata* em duas espécies de mosca-das-frutas. Foram utilizadas fêmeas do parasitoide criadas sobre *Ceratitis capitata* ou *Anastrepha fraterculus* acondicionadas em gaiolas adaptadas. Para cada fêmea foram oferecidas 20 larvas durante 1 hora, em ensaio com chance de escolha (20 *C. capitata* ou 20 *A. fraterculus*) e sem chance de escolha (10 larvas de cada espécie). Após o parasitismo, o número de cicatrizes foi verificado e os pupários classificados como: não parasitados (ausência de cicatrizes), parasitados (presença de uma cicatriz) e superparasitados (duas ou mais cicatrizes). A intensidade de parasitismo foi avaliada por meio da proporção de larvas não parasitadas, parasitadas ou superparasitadas. As variáveis intensidade de parasitismo e frequência de cicatrizes em virtude do hospedeiro e da linhagem do parasitoide foram analisadas quanto à distribuição de frequência utilizando-se o teste qui-quadrado. No teste sem escolha, independentemente da espécie hospedeira, as fêmeas das duas linhagens parasitaram as larvas oferecidas e, quando tiveram escolha, preferiram superparasitar larvas de *A. fraterculus*, que são maiores que as de *C. capitata*. A frequência de pupários sem cicatriz foi maior quando o hospedeiro oferecido foi diferente daquele em que a fêmea foi criada. Independentemente da linhagem do parasitoide, as fêmeas parasitam a maioria das larvas hospedeiras das duas espécies, mas discriminam o hospedeiro, preferindo superparasitar a espécie na qual foi criada o hospedeiro. *A. fraterculus* foi o preferido para postura de fêmeas.

**PALAVRAS-CHAVE:** superparasitismo; preferência hospedeira; parasitoide.

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## INTRODUCTION

*Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) is a larval-pupal endoparasitoid of fruit flies (Diptera: Tephritidae) in tropical and subtropical regions. The parasitoid can be reared on both *Anastrepha* spp. and *Ceratitis capitata* (Wiedemann), which can be advantageous when using the species to simultaneously control multiple tephritid species (OVRUSKI, 2003; VAN NIEUWENHOVE et al., 2012).

The measurable biological parameters of *D. longicaudata* parasitism have been evaluated previously in *Anastrepha suspensa* Loew (LAWRENCE et al., 1976), *Anastrepha ludens* Loew (MONTTOYA et al., 2003), and *C. capitata* (OVRUSKI et al., 2003). However, few studies have investigated the development of *D. longicaudata* in different hosts or the parasitism behavior of *D. longicaudata* when offered different host species simultaneously. The few studies that have been conducted with these objectives have reported that *D. longicaudata* superparasitizes both *A. fraterculus* and *C. capitata* (ALTAFINI et al., 2013; MEIRELLES et al., 2013).

Host discrimination ability, *i.e.*, the ability to recognize previously parasitized hosts, has been reported for several parasitoids and can result in subsequent rejection or superparasitization (VAN ALPHEN; VISSER, 1990). At one time, superparasitism was considered to result from parasitoid error, since there were no apparent benefits to laying eggs in previously parasitized hosts. However, it is now recognized that superparasitism can promote natural selection by fostering intraspecific competition (BAKKER et al., 1985). GONZÁLEZ et al. (2007) even suggested that superparasitism could have positive effects on *D. longicaudata* populations by promoting the selection of better flying ability and greater female production, thereby increasing populations' reproductive success. Indeed, even though female *D. longicaudata* exhibit host discrimination ability, the females prefer to superparasitize previously infected hosts, a behavior whose negative effects on offspring demography have yet to be reported (MONTTOYA et al., 2003; MONTTOYA et al., 2012).

Despite the acceptable performance of *D. longicaudata* in controlling fruit flies, few studies have evaluated the parasitism potential of the species' many strains. Because female parasitoids are expected to target the same host species in which they developed, understanding parasitoid-host interactions and parasitism behavior will facilitate the management of fruit fly populations in the field. Therefore, the aim of the present study was to evaluate the parasitism of two fruit fly species by two strains of *D. longicaudata*.

## MATERIALS AND METHODS

Insects (fruit flies and parasitoids) were obtained from colonies maintained at the Biological Control Laboratory of the State

University of Montes Claros (Minas Gerais, Brazil) under controlled conditions (26±2°C, 65±10% RH, and 12 h photoperiod).

Third instar *A. fraterculus* and *C. capitata* larvae were used to rear two parasitoid strains (AS and CS, respectively) for 10 generations prior to the trials, and eight-day-old females were used in the experiments for both strains. For the tests, the female parasitoids were kept in transparent 500-mL plastic containers (11 cm diameter, 8 cm height). Circular openings were cut in the container lids and covered with voile (a fine-meshed fabric), in order to simultaneously allow air flow and confine the insects. The containers were also covered laterally with paperboard, so that the females could not see one another, which could have affected their behavior. Two female parasitoids were kept in each container and were fed an artificial diet of honey and water, following the methodology of CARVALHO et al. (1998).

To facilitate mating and oviposition by the females, two newly emerged pairs were individualized in the experimental containers for 5 d. Third-instar host larvae (*A. fraterculus* and/or *C. capitata*) were then enclosed in a structure of voile (*i.e.*, parasitism exposure unit, PEU) and offered daily to the parasitoids for 3 d. The presence of the male as well as the process of experience with the PEUs were terminated on the day before the start of the assay.

Two assays were performed, with either no choice of host species or with a free choice of host species. In the no-choice assays, parasitoids (CS or AS) were offered either 20 *C. capitata* larvae or 20 *A. fraterculus* larvae, whereas in the free-choice assays, parasitoids (CS or AS) were offered PEUs containing both host species (10 *C. capitata* larvae and 10 *A. fraterculus* larvae).

For all the assays, the PEUs were removed after 1 h, and the parasitoid-exposed larvae were transferred to containers containing moist vermiculite, where they could pupate. Oviposition scars on the pupae were counted under a stereomicroscope, and the pupae were classified as non-parasitized (no scars), parasitized (one scar), or superparasitized (multiple scars). In order to allow the emergence of flies and parasitoids, the pupae were then transferred to containers with moist vermiculite and covered with voile.

A completely randomized design was used for both experiments, with four treatments (two strains × two hosts) and 25 replicates per treatment. The frequency distributions of parasitism intensity (non-parasitized, parasitized, and superparasitized pupae frequency) and scar frequency were analyzed using  $\chi^2$  tests in SAS (SAS INSTITUTE, 2003).

## RESULTS

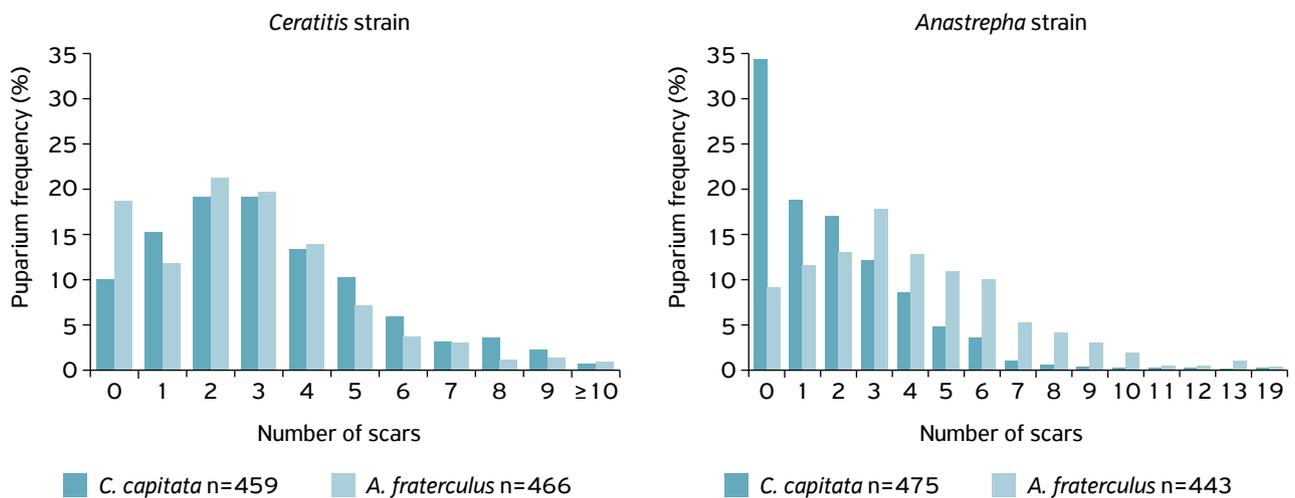
The distribution of parasitism intensity in the no-choice assays was significantly influenced by host species ( $\chi^2=261.16$ ,  $p<0.001$ ; Fig. 1). When CS females were used, the frequency of non-parasitized *A. fraterculus* larvae was greater than that of non-parasitized *C. capitata* larvae, whereas the frequency of parasitized (one scar)

*C. capitata* larvae was greater than that of parasitized *A. fraterculus* ones. Furthermore, among the superparasitized larvae, a relatively greater proportion of *A. fraterculus* pupae had two to four scars, whereas a relatively greater proportion of *C. capitata* pupae had five or more scars, and both host species received the same maximum number of scars (Fig. 1). Meanwhile, when AS females were used, most of the *C. capitata* larvae were non-parasitized, compared to <10% of the *A. fraterculus* larvae ( $\chi^2=261.16$ ,  $p<0.001$ ), and most of the scarred *C. capitata* pupae had two scars, whereas most of the scarred *A. fraterculus* pupae had three or more scars. Furthermore, the *A. fraterculus* pupae had a higher maximum number of scars ( $n=19$ ; Fig. 2).

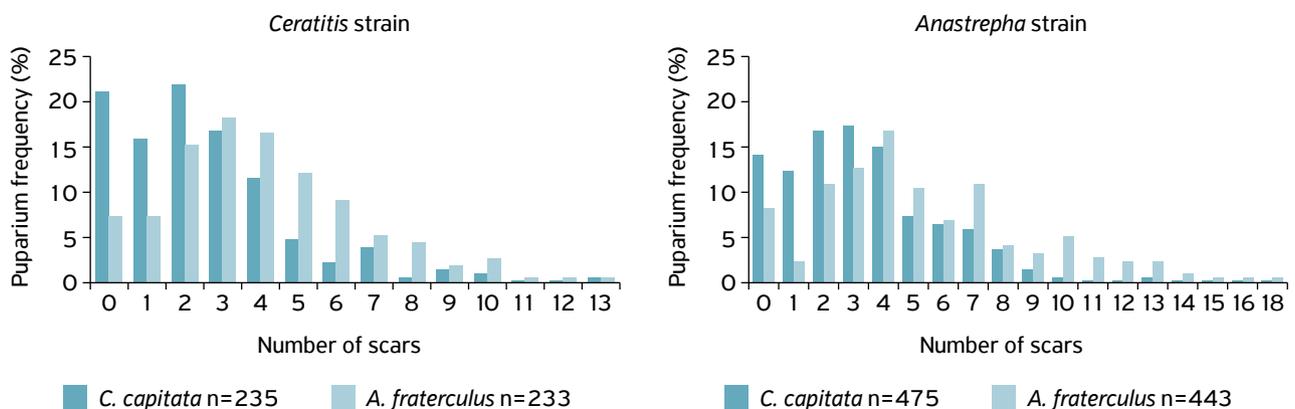
The distribution of parasitism intensity was also significantly influenced by host species in the free-choice assays ( $\chi^2=159.92$ ,  $p<0.001$ ; Fig. 2). The CS females preferred to parasitize *A. fraterculus* larvae, leaving most *C. capitata* larvae non-parasitized; only 7% of the *A. fraterculus* larvae were non-parasitized. In addition, *C. capitata* was over-represented among the parasitized (one scar) larvae, whereas *A. fraterculus* was over-represented among the superparasitized (three or more scars) larvae (Fig. 2).

Similarly, the AS females also preferred to parasitize *A. fraterculus* larvae, most of which were superparasitized (two or more scars), and left most *C. capitata* larvae non-parasitized, and *C. capitata* was again over-represented among the parasitized (one scar) larvae, whereas *A. fraterculus* was over-represented among the superparasitized larvae. Furthermore, the *A. fraterculus* pupae had a higher maximum number of scars ( $n=18$ ; Fig. 2).

Finally, even though both host species were consistently parasitized, assay type (no-choice and free-choice) and parasitoid strain each affected the distribution of parasitism intensity (Table 1). For example, superparasitism was more frequent in the no-choice assays, regardless of host species or parasitoid strain ( $\chi^2=170.34$ ,  $p<0.001$ ). In the assays with CS females, ~19% of the *A. fraterculus* larvae were non-parasitized, whereas only ~10% of the *C. capitata* larvae were non-parasitized. In the assays with AS females, 79% of the *A. fraterculus* larvae were superparasitized, whereas >34% of the *C. capitata* larvae were non-parasitized. In the free-choice assay, both parasitoid strains preferred to superparasitize *A. fraterculus* larvae and parasitize (one scar) *C. capitata* larvae ( $\chi^2=59.12$ ,  $p<0.001$ ; Table 1).



**Figure 1.** Distribution of parasitism intensity among the pupae of *Anastrepha fraterculus* and *Ceratitis capitata* parasitized by *Diachasmimorpha longicaudata* in no-choice assays. CS: *Ceratitis* strain; AS: *Anastrepha* strain.



**Figure 2.** Distribution of parasitism intensity among the pupae of *Anastrepha fraterculus* and *Ceratitis capitata* parasitized by *Diachasmimorpha longicaudata* in free-choice assays. CS: *Ceratitis* strain; AS: *Anastrepha* strain.

## DISCUSSION

The distribution of parasitism intensity among the no-choice assays suggests that female parasitoids prefer not to parasitize unknown host species. For example, regardless of parasitoid strain, the frequency of non-parasitized pupae was higher when the offered host species was different from the one in which the parasitoid was reared (Fig. 1). However, *C. capitata* larvae were preferred by the females to laying a few eggs, up to two scars. Similarly, MEIRELLES et al. (2013) dissected parasitized larvae and observed  $\leq 3$  parasitoid eggs within *C. capitata* larvae but  $\leq 6$  parasitoid eggs within *A. fraterculus* larvae. In contrast, CS females preferred to superparasitize their original host. Meanwhile, among the free-choice assays (Fig. 2), *A. fraterculus* larvae were more intensely superparasitized than *C. capitata* ones. Our results resemble those of OVRUSKI et al. (2011), who also reported that *D. longicaudata* females parasitize both *A. fraterculus* and *C. capitata*. However, when female parasitoids had no choice, the parasitism was similar, and when given a choice, parasitoids preferred to parasitize *A. fraterculus* larvae. ALTAFINI et al. (2013) also reported this behavior and mentioned that, although *D. longicaudata* superparasitized both hosts, *A. fraterculus* larvae received a greater number of scars.

The results of the present study suggest that individual *D. longicaudata* females have preferred host species and that, when given a choice, select host larvae accordingly. SAMPAIO et al. (2001) reported that the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) preferred to parasitize *Aphis gossypii* Glover (Hemiptera: Aphididae), even though it was previously reared on *Myzus persicae* Sulzer (Hemiptera: Aphididae). In the present study, the distribution of parasitism intensity indicated that female *D. longicaudata*, in the absence of choice, were more likely to superparasitize their original

hosts (Table 1). This suggests that host acceptance or preference by parasitoids can be influenced by larval environment. In the no-choice assays, *D. longicaudata* parasitized its original host, and in the free-choice assays, the parasitoid preferred to parasitize the larger host, *A. fraterculus*, as reported previously (MESSING et al., 1993; CANCELO et al., 2002; LOPEZ et al., 2009). This preference for larger hosts may indicate an adaptation for producing larger offspring and, consequently, more efficient in the search for hosts, since the size of the host can directly influence the size of the parasitoid (MEIRELLES et al., 2013) and this may interfere with the search efficiency of its host (LAWRENCE et al., 1976; GODFRAY, 1994). Larger individuals may exhibit greater fertility and fecundity (JERVIS, 2005).

According to the results of the present study, it is clear that female *D. longicaudata* parasitize most available host larvae, regardless of parasitoid strain or host species, but discriminate among hosts, in regard to superparasitism. Therefore, elucidating the effects that rearing *D. longicaudata* on different hosts will have on production efficiency, production cost, and efficiency of host suppression are important for deciding which host to use in the breeding of *D. longicaudata* for biological control programs.

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**Table 1.** Percent distribution of parasitism intensity among the pupae of *Anastrepha fraterculus* and *Ceratitidis capitata* parasitized by *Diachasmimorpha longicaudata*.

Host/Parasitoid (Assay type)	Non-parasitized (n)	Parasitized (n)	Superparasitized (n)	Total (n)
C/CS (NC) <sup>1</sup>	9.81 (45)	15.03 (69)	75.16 (345)	459
A/CS (NC)	18.45 (86)	11.59 (54)	69.96 (326)	466
C/AS (NC)	34.31 (163)	18.74 (89)	46.95 (223)	475
A/AS (NC)	9.03 (40)	11.51 (51)	79.46 (352)	443
<b>Total (n)</b>	<b>334</b>	<b>263</b>	<b>1,246</b>	<b>1,843</b>
C/CS (WC) <sup>1</sup>	20.85 (49)	15.74 (37)	63.40 (149)	235
A/CS (WC)	7.30 (17)	7.30 (17)	85.41 (199)	233
C/AS (WC)	13.96 (31)	12.16 (27)	73.87 (164)	222
A/AS (WC)	8.11 (18)	2.25 (5)	89.64 (199)	222
<b>Total (n)</b>	<b>115</b>	<b>86</b>	<b>711</b>	<b>912</b>

A: *Anastrepha fraterculus*; C: *Ceratitidis capitata* CS: *Ceratitidis capitata* parasitoid strain; AS: *Anastrepha fraterculus* parasitoid strain; NC: no choice assay; WC: with choice assay; n: frequency.

<sup>1</sup>Distribution significantly different than expected by chance ( $p < 0.01$ , chi-square test).

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