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Intra and interspecific competition between *Diachasmimorpha longicaudata* (Hymenoptera Braconidae) and *Aganaspis pelleranoi* (Hymenoptera Figitidae)

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Abstract

Braconidae and Figitidae parasitoids are important agents of pest population regulation in natural and agricultural systems, with species used in applicate biological control programs of fruit flies (Diptera Tephritidae). However, many aspects of the interactions of parasitoids with their heterospecific and conspecific are poorly understood. Thus, the interspecific competition between the parasitoids *Diachasmimorpha longicaudata* (Ashmead) (DL) (Hymenoptera Braconidae) and *Aganaspis pelleranoi* (Brethes) (AP) (Hymenoptera Figitidae), was studied using *Anastrepha fraterculus* (Wiedemann) (Diptera Tephritidae) as host. Host larvae were offered to only one parasitoid on a single occasion or on two occasions, or even to two parasitoid species, alternating the offering sequence. Thus, six exposure regimes were completed: AP (host exposed for 4 hours); DL (host exposed for 40 minutes); AP-AP (host exposed to AP for 4 hours and then to a conspecific for an additional further 4 hours); DL-DL (host exposed to DL for 40 minutes and then to a conspecific for an additional 40 minutes); AP-DL (host exposed to AP for 4 hours and then to DL for 40 minutes); and DL-AP (host exposed to DL for 40 minutes and then exposed to AP for 4 hours). The mean number of parasitized pupae, emerged parasitoids, oviposition scars per host (larvae) and sex ratio of parasitoids were compared between the different exposure regimes. The mean of parasitized pupae and emerged parasitoids was higher in the DL-DL and DL-AP treatments. The mean number of oviposition scars per host was correlated positively with the mean number of parasitoid offspring and the emerged females in treatments AP, DL, AP-AP, DL-DL for both species, and DL-AP only to *D. longicaudata*. When the hosts were exposed only once to the parasitoids, the sex ratio was male biased (AP and DL treatments); but when exposed twice, the treatments spawned offspring female biased, except for *D. longicaudata* at AP-DL treatment. Irrespective of the parasitism order, *D. longicaudata* suppress the emergence of *A. pelleranoi*.

Key words: *Anastrepha fraterculus*, exotic parasitoid, fruit flies parasitoids, Neotropical parasitoid.

Introduction

Parasitoids play a key role in many ecosystems in terms of biodiversity, ecological impact, and economic importance (Hawkins *et al.*, 1999). In these ecosystems, parasitoids may experience complex interactions with predators, entomopathogens, other parasitoids, and hyperparasites (Boivin and Brodeur, 2006). The competition between individuals of the same species is known to be intraspecific competition and may occur when several individuals of the same species exploit the same resources, sometimes at the same time (Couchoux and van Nouhuys, 2014). In interspecific competition the interaction between parasitoid species occurs among those that have developed ecologically similar strategies (Boivin and Brodeur, 2006). Interspecific competitive effects may occur between adult (extrinsic competition) and immatures parasitoids (intrinsic competition) (de Moraes *et al*., 1999; Wang *et al*., 2008).

Females from many parasitoid families can discriminate between parasitized and non-parasitized hosts (Brodeur and Boivin, 2004; Ruschioni *et al*., 2015). In natural systems, several species may attack the same host, producing multiparasitism and competition between immature stages (Cusumano *et al*., 2011; 2012). When the species of parasitoid attacks a single host, self-superparasitism and superparasitism can also occur (Sirot *et al*., 1997; Montoya *et al*., 2000b; 2003; 2012; González *et al*., 2009). It can also lead to a higher sex ratio of females, without affecting the demographic parameters of the offspring, including longevity and fecundity (van Baaren *et al*., 1999; González *et al*., 2007; Montoya *et al*., 2011; 2013).

Competitive effects may alter the reproductive success of the species, thereby affecting host mortality (Mills, 1994; Follett *et al*., 2000) and establishment of the parasitoid species in the environment. In addition, understanding how interspecific competition in parasitoids can affect pest suppression may improve biological control. Some authors argue that the more species introduced, the greater the reduction of pest density (Stiling and Cornelissen, 2005; Miranda *et al*., 2015), while others suggest that the release of multiple species may impair biological control (Murdoch *et al*., 1998; Denoth *et al*., 2002).

Parasitoids of the Braconidae and Figitidae families are important biological control agents, used for the suppression of fruit flies (Diptera Tephritidae). *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera Braconidae) is widely used as a biological control agent in the world and it is native to the Indo-Australian region, where it parasitizes at least 14 species of *Bactrocera* Macquart (Montoya *et al*., 2000a; Devescovi *et al*., 2017). The species can parasitize *Ceratitis capitata* (Wiedemann) (Mediterranean fly) (Diptera Tephritidae) and several species

of *Anastrepha* Schiner (Carvalho and Nascimento, 2002). *D. longicaudata* has a parasitism efficiency exceeding 50%, and is one of the few species that parasitize hosts larvae in fallen fruit, but it mainly attacks larva inside fruit that is still on the plant, also has the ability to parasitize its host in native and exotic fruits (Sivinski *et al*., 1996; Montoya *et al*., 2000a; García-Medel *et al*., 2007).

Aganaspis pelleranoi (Brethes) (Hymenoptera Figitidae) has a natural abundance superior to other species of Neotropical parasitoids, and it is considered a promising species in biological control programs against fruit flies (Núñez-Campero *et al*., 2014; Gonçalves *et al*., 2016; Schliserman *et al*., 2016). It has the ability to parasitize fruit fly larvae in native and exotic fruits, increasing its chances of success in parasitism, being more competitive than other species of native parasitoids (Guimarães and Zucchi, 2004; Schliserman *et al*., 2016). It also parasitizes larvae of *C. capitata*, several species of the genus *Anastrepha* and individuals of Lonchaeidae (Wharton *et al*., 1998; Guimarães *et al*., 1999; Ovruski *et al*., 2000; 2004; Rohr *et al*., 2019a). Females forage and attack their hosts inside fallen or cracked fruits, because it has a proportionally smaller ovipositor than other fruit fly parasitoids (Sivinski *et al.*, 2000).

A. pelleranoi and *D. longicaudata* are both solitary endoparasitoids, koinobionts, and the adults emerge from the puparia (Wharton and Gilstrap, 1983). Females of both species are synovigenic and can recognize whether the hosts have been parasitized or not (Montoya *et al*., 2000b; Golçalves *el al*., 2013; Diáz-Fleicher *et al.*, 2015; Ruschioni *et al*., 2015). The two species mainly parasitize third-instar fruit fly larvae (Ovruski, 1994a; Sime *et al*., 2006; Gonçalves *et al*., 2013), although *D. longicaudata* can parasitize other instars (Alvarenga *et al*., 2005; Sime *et al*., 2006; Montoya *et al*., 2012; Rohr *et al*., 2019b). Also, both parasitoids are capable of parasitizing *Anastrepha fraterculus* (Wiedemann) (South American fruit fly) (Diptera Tephritidae), responsible for significant economic losses in commercial orchards in South America (Van Nieuwenhove *et al*., 2016; Araujo *et al*., 2019).

The interactions between *D. longicaudata* and other parasitoids have already been studied, such as the competitiveness of first instar larvae of this species, which are capable of succeeding in physical competitions with other parasitoid larvae such as the braconids *Fopius ceratitivorus* Wharton, *Fopius arisanus* (Sonan) and *Fopius persulcatus* (Silvestri), with up to 64% suppression of competing species (Palacio *et al*., 1991; Wang *et al*., 2008). They can also suppress its congener *Diachasmimorpha tryoni* (Cameron), in addition to other braconid species such as *Opius hirtus* Fischer, *Doryctobracon areolatus* (Szepligeti), *Doryctobracon crawfordi* (Viereck) and *Utetes anastrephae* (Viereck), due to morphological adaptations of larva or the size of the ovipositor (Miranda *et al*., 2013; Murillo *et al*., 2016; Montoya *et al*., 2017). In competition with *Coptera haywardi* (Oglobin) (Hymenoptera Diapriidae), when parasitism occurs with little difference in time, *D. longicaudata* has an advantage over the immature ones of the other species, but after a few days, hyperparasitism by *C. haywardi* may occur (Montoya *et al*., 2018).

In Florida, *D. longicaudata* was one of the species used to control *Anastrepha suspensa* (Loew) (Caribbean fruit flies) (Diptera Tephritidae), in which process, this parasitoid suppressed the already established population of *D. areolatus*. This has led to the latter parasitoid species moving from the region where it was already established, looking for a new environment where it could adapt (Eitam *et al*., 2004). In Mexico however *D. longicaudata* and *D. areolatus* coexist with niche separation (López *et al*., 1999; Sivinski *et al*., 2000).

Studies evaluating the competition between *D. longicaudata* and *A. pelleranoi* parasitizing larvae of *A. fraterculus*, have not been recorded. Knowing that *A. pelleranoi* and *D. longicaudata* can parasitize the same larval instar and that the female fruit fly parasitoids recognize whether or not the host is parasitized, it is possible that the exotic parasitoid can suppress native populations of *A. pelleranoi*, or displace them. So, this work aimed to evaluate the interspecific competition between *D. longicaudata* and *A. pelleranoi*, in *A. fraterculus* larvae.

Materials and methods

Study site

The study was conducted in the Laboratório de Biologia, Ecologia e Controle Biológico (Bioecolab), at the Universidade Federal do Rio Grande do Sul, under controlled conditions (26 ± 1 °C, 60 ± 10% RH, photoperiod of 14:10 L:D).

Host rearing

Adults of *A. fraterculus* from the rearing of Bioecolab (over 100 generations) were kept in wooden cages (45 × 30 × 30 cm), covered with voile fabric, receiving distilled water and a solid diet given on an ad libitum basis, which consisted of granulated sugar, hydrolysed protein, soybean extract (3:1:1), and vitamin complex (Lavitan - A-Z®), in the ratio of two macerated tablets (1.26 g) per 250 g of diet (adapted from Jaldo *et al*., 2001). The oviposition substrate was a blue tissue bag covered with silicone (30 × 30 cm), having at one end a bottleneck with a cap, through which water was placed (Meirelles *et al*., 2016). The substrate was rested on the upper part of the cage, to obtain the eggs. The eggs were collected daily and placed in polystyrene trays (23.5 × 18 × 1 cm), with an artificial diet based on organic carrot, beer yeast, corn flour, sugar, and distilled water (modified from Terán, 1977). After seven days, these were placed inside larger plastic trays (51 × 30 × 9.5 cm), with sterile sand and covered with voile, where they stayed for approximately seven days to allow pupation. Subsequently, the sand was sifted, and the collected pupae were placed in plastic pots (6.6 × 6.6 × 6 cm) until emergence, under controlled conditions (26 ± 1 °C, 60 ± 10% RH, photoperiod of 14:10 L:D).

Parasitoids rearing

To rear *A. pelleranoi*, araçá fruit - *Psidium cattleianum* Sabine (Myrtaceae) - infested with *A. fraterculus* was collected from orchards of native fruit species at the Fundação Estadual de Pesquisa Agropecuária, in Taquari, RS,

Brazil. In the laboratory, the fruits were placed in plastic trays (51 × 30 × 9.5 cm) on a layer of sterilized sand and covered with voile fabric. The sand was sieved after 15 days, and the pupae obtained were conditioned in plastic pots (6.6 × 6.6 × 6 cm) until the emergence of parasitoids. The rearing of *D. longicaudata* in our laboratory began with parasitized pupae of *A. fraterculus* obtained from the experimental establishment of Embrapa Clima Temperado, in Pelotas, RS, Brazil. The pupae obtained were stored in a plastic pot and kept under controlled conditions (26 ± 1 °C, 60 ± 10% RH, 14 photophase hours) until the emergence of the flies or parasitoids.

The adults of both species were placed in wooden cages (19.5 × 16.5 × 25.5 cm), covered with voile fabric, and received water by capillarity and honey dissolved in water (7:3), offered in Petri dishes (5 × 5 × 1.5 cm) with cotton. Larvae of third-instar *A. fraterculus* (Ovruski, 1994b; Sime *et al*., 2006) were offered to the parasitoids. The larvae were placed in parasitism units, which consisted of a circular plastic plate (0.3 cm tall, 4 cm in diameter), encased in white voile fabric, and fastened with an elastic band. After the exposure for 4 hours to *A. pelleranoi* (based on Gonçalves *et al*., 2016) or 40 minutes to *D. longicaudata* (based on Suárez *et al*., 2012), the larvae were returned to the diet in polystyrene trays (15.5 × 15.5 × 1 cm) which was placed in plastic trays (41 × 28 × 7 cm) on a layer of sterilized sand and covered with voile fabric. After five days, the sand was sifted and the puparia were packaged in the same manner as for fly rearing, waiting for the emergence of the parasitoids that were reintroduced to the breeding in new cages.

Bioassay

The bioassay was performed in arenas constituted of plastic bottles (7 cm tall, 10.5 cm in diameter), with an upper opening (6 cm in diameter) covered with voile fabric for ventilation. Within each arena, we placed an 8-days-old female of *A. pelleranoi* (10^{th} generation of laboratory) or *D. longicaudata* (10^{th} generation of laboratory), already mated and with an oviposition experience. Females received water and food as described above, and were used in the bioassay 24 hours after the experience with the host. For each female, 10 larvae washed with water were offered in parasitism units, the units consisted of a circular plastic plate (0.2 cm tall, 2.7 cm in diameter), formed by a small layer of silicone and enveloped with white voile, fastened with an elastic band. To dispose of the parasitism units within the cages, plastic vials (0.9 × 5 × 1.2 cm) were used as carriers.

To assess the effects of competition among species, different exposure regimes of *A. fraterculus* larvae were established: AP - host exposed for 4 hours to *A. pelleranoi*; DL - host exposed for 40 minutes to *D. longicaudata*; AP-AP - host exposed to *A. pelleranoi* for 4 hours and then to a co-specific for a further 4 hours; DL-DL - host exposed to *D. longicaudata* for 40 minutes and then to a co-specific for a further 40 minutes; AP-DL - host exposed to *A. pelleranoi* for 4 hours and then to *D. longicaudata* for 40 minutes; and DL-AP - host exposed to *D. longicaudata* for 40 minutes and then exposed to *A. pelleranoi* for 4 hours. The experiment was conducted with 40 replicates for each treatment. The different exposure times for each species of parasitoid were determined from tests done before the bioassay began, and based on the works of Suárez *et al.* (2012) and Gonçalves *et al.* (2016), which show that these times are the best to obtain the highest rates of parasitism, lower mortality rate and superparasitism for each species.

To evaluate the mortality of larvae without action of the parasitoids (control), 10 larvae of *A. fraterculus*, totalizing 40 replicates for each exposure time, were placed in parasitism units and positioned in the cages for the same periods of time as described above (40 minutes, 80 minutes, 4 hours, 4:40 hours and 8 hours), but without the presence of parasitoids, and were then placed in the same manner as described above.

After each exposure, in all treatments, the larvae were observed individually with the aid of a stereomicroscope (Wild Heerbrugg - Wild M5A), to count the number of oviposition scars per host (larvae) and also checked for dead larvae. For treatments with more than one offering, after examined the larvae were placed in the parasitism units again, exposed to the second moment of parasitism, and examined a second time for scoring. After the larvae were returned to the diet in plastic units (4.4 cm tall, 0.9 cm in diameter) and packed in plastic containers (7 × 6.8 × 5.5 cm) on a layer of sand. After, five days, the sand was sifted, and the puparia were packed in plastic pots (6.6 × 6.6 × 6 cm) until the emergence of flies or parasitoids.

For all treatments, the puparia in which emergence did not occur were dissected to check for the presence of parasitoids or flies. The numbers of emerged flies, parasitized puparia (emerged parasitoids + puparia dissected with parasitoids), emerged parasitoids, species of the emerged parasitoids, oviposition scars per host (larvae), parasitism rate (number of emerged parasitoids/number of puparia formed × 100), larvae with scar (number of larvae that have at least one scar) and sex ratio (number of females/number of females + number of males) of the parasitoids were recorded.

Data analysis

Generalized linear models (GLMs) of the quasi-Poisson family were used for data of emerged flies, parasitized puparia, emerged parasitoids, species of the emerged parasitoids, and larvae with scar. For parasitism rate and sex ratio GLMs of the quasi-binomial family were performed. The quality of the fit of the models was assessed through half-normal probability charts with a simulation envelope (package hnp) (Moral *et al.*, 2017). Post-hoc tests were done for pairwise comparisons of least-square means using the compact letter display (CLD) function and Tukey HSD adjust ($\alpha = 0.05\%$) (packages emmeans and multcompView) (Piepho, 2004). Oviposition scars per host (larvae) data were transformed ($\sqrt{x} + 0.5$), assessed for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test), and subjected to ANOVA, followed by the Tukey HSD test ($\alpha = 0.05\%$) (package agricolae). These analyses were conducted in the statistical software "RStudio" version 1.3.959 (RStudio Team, 2020).

The relationship between the number of parasitoids and emerged females, and the number of oviposition scars per

host (larvae) was tested using the Spearman Correlation Coefficient ($\alpha = 0.05$), followed by a polynomial regression performed using the BioEstat 5.0 software (Ayres *et al*., 2007).

Results and discussion

The number of flies that emerged in the controls was significantly higher than in all treatments (table 1), indicating that exposure conditions and different time intervals (40 minutes, 80 minutes, 4 hours, 4:40 hours and 8 hours) may not have affect the emergence of flies. Certainly, mortality was expected to be higher in treatments, but it could increase by manipulating the larvae and exposure outside the diet, even without the presence of parasitoids. Thus, as in the control mortality was close to records of natural mortality of the larvae in laboratory which ranged from 7% to 19.5% (Bressan-Nascimento, 2001; Jaldo *et al*., 2007), we consider that manipulation did not affect the survival of fly larvae.

The mean number of parasitized pupae was higher in the regimens in which the larvae were twice offered to *D. longicaudata* (DL-DL); first to *A. pelleranoi* and then to *D. longicaudata* (AP-DL) and, first to *D. longicaudata* and then to *A. pelleranoi* (DL-AP) (table 1). Conversely, the lowest mean number of parasitized pupae was recorded in the treatments in which the larvae were only offered to *A. pelleranoi*, either in one or two exposures (AP or AP-AP), the latter does not differ from a single exposure for *D. longicaudata* (table 1). Gonçalves *et al*. (2016), using third-instar larvae of *A. fraterculus*, with a single exposure to *A. pelleranoi* found mean values similar to those of our study (3.8 ± 0.13).

Reflecting the number of parasitized pupae, the highest parasitism rate was for *D. longicaudata* when the larvae were exposed twice to the parasitoid in the treatments DL-DL and DL-AP, and the AP-DL treatment did not differ from the parasitism rate in the only exposure to DL (table 1). Ovruski *et al*. (2011) recorded 43.2% of parasitism of *D. longicaudata* on host *A. fraterculus*, and assert that this index indicates potential for the use of the species in fly control. However, in their experiment, the authors left a proportion of 30 larvae per female, exposed to parasitism for 24 hours, while in our experiment a female was exposed to 10 larvae for only 40 minutes, showing that the great potential for parasitism of this species.

The high parasitism rate of *D. longicaudata* reported in our study for *A. fraterculus* individually or in interspecific competition was also reported by Bautista and Harris (1997) in larvae of another fruit fly species, *Bactrocera dorsalis* (Hendel), with the highest offspring percentage (99%) reported when *D. longicaudata* was the first parasitoid to have access to the host, followed by the braconid *Psyttalia incisi* (Silvestri). Similarly, *D. longi-*

Table 1. Mean number (± SE) of emerged flies, parasitized pupae, emerged parasitoids by species and total, oviposition scars per host (larvae), larvae with scar, parasitism rate, and sex ratio of *Aganaspis pelleranoi* (AP) and *Diachasmimorpha longicaudata* (DL) parasitizing larvae of the host *Anastrepha fraterculus* in different intra- and interspecific competition arrangements (N = 10 larvae/unit).

Variables	Treatments					
	AP	DL	AP-AP	DL-DL	AP-DL	DL-AP
Emerged flies (control)[(a)]	9.1 ± 0.20 A	9.1 ± 0.16 A	8.4 ± 0.26 A	9.2 ± 0.26 A	8.6 ± 0.34 A	9.5 ± 0.26 A
Emerged flies (treatment)[(a)]	3.9 ± 0.60 aB	2.2 ± 0.60 bcB	3.6 ± 0.52 abB	0.6 ± 0.24 dB	1.2 ± 0.40 cdB	2.6 ± 0.53 abB
Parasitized pupae [(1)(a)]	4.0 ± 0.48 c	5.3 ± 0.45 ab	4.1 ± 0.49 bc	6.4 ± 0.38 a	5.3 ± 0.42 a	6.5 ± 0.61 a
Emerged *A. pelleranoi*[(a)]	4.0 ± 0.48 a	-	4.1 ± 0.49 a	-	1.1 ± 0.29 b	0.8 ± 0.23 b
Emerged *D. longicaudata*[(a)]	-	5.0 ± 0.46 ab	-	6.4 ± 0.38 a	4.2 ± 0.48 b	5.7 ± 0.65 a
Total emerged parasitoids[(a)]	4.0 ± 0.48 b	5.0 ± 0.46 ab	4.1 ± 0.49 b	6.4 ± 0.38 a	5.3 ± 0.42 ab	6.5 ± 0.61 a
Oviposition scars[(b)]	2.3 ± 0.30 c	2.7 ± 0.26 bc	2.6 ± 0.30 bc	5.3 ± 0.35 a	3.7 ± 0.38 b	3.6 ± 0.35 b
Larvae with scar[(b)]	6.6 ± 0.58 b	7.5 ± 0.53 ab	6.8 ± 0.41 b	9.2 ± 0.28 a	8.7 ± 0.735 a	7.7 ± 0.44 ab
Parasitism rate (%)[(2)(a)]	40.3 ± 4.79 c	52.5 ± 4.59 bc	43.4 ± 4.73 c	70.0 ± 3.51 a	59.0 ± 3.75 ab	68.9 ± 5.46 a
Sex ratio[(a)]	0.45 ± 0.04 ab	0.48 ± 0.04 ab	0.62 ± 0.05 a	0.51 ± 0.04 ab	0.66 ± 0.09 ab (AP) 0.41 ± 0.06 b (DL)	0.49 ± 0.11 ab (DL) 0.50 ± 0.04 ab (AP)

Lowercase letters compare treatments on the same line. Upper case letters compare controls and treatments. [(a)] Means followed by the same letter do not differ from each other by contrasts of the generalized linear model (Tukey's adjustment; $P < 0.05$). [(b)] ANOVA, followed by Tukey HSD ($P < 0.05$). [(1)] Parasitized pupae = parasitoids emerged from the puparia + puparia dissected with parasitoids. [(2)] Parasitism rate (%) = (number of total emerged parasitoids/number of puparia formed) × 100.

caudata, when competing with its congener *D. tryoni*, also won the competition, generating more offspring (57% when *D. longicaudata* was the first to parasite; and 58% when this was the second) (Ramadan *et al*., 1994). This occurred independently of the order in which *B. dorsalis* larvae were offered, a fact that may be associated with physiological suppression and may result from the release of substances at the time of oviposition, by the egg or larva, inhibiting the development of competitors (Mackauer, 1986; Silvers and Nappi, 1986; Vinson and Hegazi, 1998). The prevalence of *D. longicaudata* in competitions may not be host-dependent, similar results was observed in the competition between *D. longicaudata* and *D. areolatus* on larvae of *A. suspensa* (Paranhos *et al*., 2013) and with *D. crawfordi* on other host species, like *Anastrepha ludens* (Loew), *Anastrepha obliqua* Macquart, *Anastrepha serpentina* (Wiedemann) and *C. capitata* (Miranda *et al.*, 2015).

In our work we observed that the order in which the parasitoids, *D. longicaudata* and *A. pelleranoi*, had access to the host did not influence the parasitism rate, and *D. longicaudata* always had an advantage over the native parasitoid (table 1). However, the emergence of *D. longicaudata* was significantly lower in the AP-DL treatment, compared to the DL-AP. Other studies report irrespective of the host species, that the order in which the parasitoid had access to the host may alter viability. Wang *et al*. (2008), observed that the greatest number of offspring occurred when *D. longicaudata* was the second parasitoid to have contact with the host *C. capitata* being its precursor the egg parasitoid *F. ceratitivorus*. In contrast, when competing with *U. anastrephae*, if the *A. suspensa* larvae were first offered to *D. longicaudata*, the latter wins the competition, but if the order of oviposition is reversed, *U. anastrephae* generated more offspring (Paranhos *et al*., 2013). On the other hand, the emergence of *A. pelleranoi* offspring was affected when in the presence of the competitor (AP-DL and DL-AP), being lower than in in single exposure (AP) or double exposure (AP-AP).

Another factor that may influence competition is the specificity of the parasitoid in relation to the larval instar of the host. The competition between *D. longicaudata* and *P. incisi* was tested by offering the third instar larvae to both (Bautista and Harris, 1997). However, it is known that *P. incisi* prefer first instar larvae (Yang *et al*., 2018), so this must have affected the performance of the parasitoid in relation to *D. longicaudata*. Similarly, when testing the competition between *D. longicaudata* and *D. areolatus* offering third instar larvae to both parasitoids (Paranhos *et al*., 2013), but is known for *D. areolatus* the preference to parasitize young larvae, of first and second instar (Murillo *et al*., 2015). We offered the third larval instar, which is the preference of both parasitoid species, irrespective of the host species, which may be *C. capitata*, *Bactrocera oleae* (Rossi) or *Anastrepha* spp. (Ovruski, 1994b; Sime *et al*., 2006). Therefore, this factor should not have influenced the parasitoid response in our study.

The competitiveness of *D. longicaudata* could also be associated with the morphological characteristics of its early larval stages, whose mandible and cephalic capsule are well developed, which may make it more competitive (Paladino *et al*., 2010; Murillo *et al*., 2016), although this was not evaluated in this study. *D. longicaudata* has well-developed mandibles in its first instar (Murillo *et al*., 2016), and this may have provided an advantage when the host larvae were offered to it first and subsequently to *A. pelleranoi*, as described for other studies (Palacio *et al*., 1991; Wang *et al*., 2008). Furthermore, eggs of *D. longicaudata* hatch at around 24 to 48 hours after parasitism (Paladino *et al*., 2010), whereas those of *A. pelleranoi*, hatch at between 78 and 80 hours (Ovruski, 1994b), also promoting an advantage in the initial development time of the exotic species.

An interesting aspect regarding *A. pelleranoi* was the lack of increased parasitism when the larvae were exposed twice to the females of this species being 41.6% (AP) and 43.5% (AP-AP), with no significant difference ($P > 0.05$). This could be associated with the recognition of the conspecific parasitoid by hosts previously parasitized, which can interfere with the occurrence of superparasitism (Montoya *et al*., 2000b; 2003). In addition, the exposure time of four hours was considered the most suitable for a larger generation of offspring for this species, parasitizing *A. fraterculus* since an exposure of larvae for longer periods, such as 8 hours, can reduce the production of the offspring (Gonçalves *et al*., 2016). When we offered the same larvae again for a second period, the total exposure time reached 8 hours, and in this case, there was no increase in parasitism. Additionally, the mean number of *A. pelleranoi* emerged was lower when larvae were also offered to *D. longicaudata*, irrespective of order (AP-DL or DL-AP) (table 1).

Studies that evaluated the competition between *A. pelleranoi* and *D. longicaudata* were not found in the literature, although there are reports that, when released to the field, *D. longicaudata* can alter the abundance of *A. pelleranoi* and other native parasitoids (Montoya *et al*., 2017). Therefore, Paranhos *et al*. (2013) warned that the establishment or augmentation of *D. longicaudata* could result in the elimination of native parasitoids. On the other hand, other studies evaluating the release of *D. longicaudata* in field (Carvalho, 2005; dos Santos *et al*., 2016, Meirelles *et al*., 2016) observed no loss of biodiversity of native species present in the orchards. Carvalho (2005) found alterations in the frequency of species, which may have occurred due to the existence of interspecific competition in the exploitation of oviposition sites.

The mean number of oviposition scars per host (larvae) (table 1) was higher in the DL-DL treatment than in all others, which did not differ, except for AP compared to the AP-DL and DL-AP, again indicating the highest aggressiveness of *D. longicaudata* in relation to the hosts. The increase in oviposition scars may be related to mechanisms of recognition of previously parasitized hosts through proof punctures, which has been described for other parasitoids of Tephritidae, such as *Psyttalia concolor* (Szepligeti) (Hymenoptera Braconidae) on *C. capitata* (Canale and Loni, 2006; Benelli *et al*., 2013). According to the authors, females have sensory structures on the ovipositor that may be related to the decision to oviposit in larvae that already have eggs of other parasitoids.

The mean number of oviposition scars per host (larvae) was positively correlated with the mean number of offspring generated as well as the mean number of emerged females in the treatments in which only one parasitoid had contact with the hosts or in the intraspecific interaction (figure 1 a-d). In cases with interspecific interaction, this correlation was not observed for *A. pelleranoi* offspring, (figure 1 e, h), probably because just a few parasitoids of this species have emerged. For the offspring of *D. longicaudata*, when the hosts were first offered to *A. pelleranoi* (AP-DL) (figure 1 f), the increase in scars also did not correlate with emerged individuals, which is the only indication of possible interference from the pioneer species. In all cases that had a positive correlation for the average number of offspring generated, this also applied to the number of emerged females, corroborating Montoya *et al*. (2011; 2012), who found a positive correlation between the number of scars and the number of females generated. Information on superparasitism in *A. pelleranoi* is not available in the literature, therefore, our study raises some possibilities about superparasitism and multiparasitism in the offspring of this species.

The correlation between oviposition scars per host (larvae) and the emergence of parasitoids and females has also been studied for *D. longicaudata* (Altafini *et al*., 2013) in different hosts. Several studies indicated that moderate superparasitism (2-6 scars per pupa) increases the tendency for females to emerge (González *et al*., 2007; Montoya *et al*., 2011; 2012; 2013), which corroborates our results (table 1), where the averages ranged from of 2.3 to 5.3 scars per larva was found. These authors also commented that this superparasitism does not lead to detrimental effects on the demographic parameter of the offspring, including longevity and fecundity.

To evaluate whether this behaviour occurred naturally in the field, Montoya *et al*. (2013) collected mango fruits - *Mangifera indica* L. (Anacardiaceae) - and evaluated the puparia from these fruits. The authors showed a positive correlation between the fruit size and the infestation levels of *Anastrepha* spp. as well as the number of parasitized pupae and superparasitized by *D. longicaudata*. Superparasitism was also positively correlated with a biased sex ratio for females, demonstrating that superparasitism is present in natural populations of *D. longicaudata*. Notwithstanding, our study found that when in competition with *A. pelleranoi*, this pattern was altered (table 1), as the sex ratio of *D. longicaudata* in the treatments AP-DL and DL-AP was 0.41 and 0.49 respectively (table 1), that is, it was males biased. This fact may indicate that the competition may have occurred between the larvae of the parasitoid that will originate females (Mackauer, 1990), with the larvae of the native species being more competitive. However, this aspect has not been studied in our work.

The mean number of larvae with scar was higher in treatments where larvae were twice exposed to parasitoids, being DL-DL, AP-DL (table 1). This result indicates that parasitoids can recognize parasitized hosts, preferring those that have not yet been parasitized, being able to minimize the waste of time and energy associated with this behaviour (Godfray, 1994; Montoya *et al*., 2000b; 2003; Ruschioni *et al*., 2015). The host's search and acceptance strategies, including the search time in relation to the number of healthy pupae available (Tamò *et al*., 2006), also may have affected these results, as the parasitoids are influenced by chemical and mechanical sensory information that they receive from the hosts (Ayala *et al*., 2014; 2018), although our work did not evaluated this.

The sex ratio (SR) varied between the treatments (table 1). When the larvae were only offered once, both *D. longicaudata* and *A. pelleranoi* had an offspring male biased (table 1). In the treatments with two exposures to the females of the conspecific parasitoid, both species generated more females. For both species, *A. pelleranoi* and *D. longicaudata* are known to generate more females when they parasite larvae in later and larger instars (Eben *et al*., 2000; Ovruski *et al*., 2011; van Nieuwenhove and Ovruski 2011; Gonçalves *et al*., 2013; 2016). This occurs because parasitoids select the best host for their offspring and, upon finding it, they tend to breed offspring with more females (Godfray and Shimada, 1999). In general, larger hosts have more resources and are considered qualitatively superior in terms of efficiency to the parasitoid (Mattiacci and Dicke, 1995; Ovruski *et al*., 2011). The most common component in host quality is its size. Females tend to produce more females in larger hosts and males in smaller ones (Godfray and Shimada, 1999; Harvey *et al*., 2013). However, because all hosts in our study had approximately the same size, this factor may not have influenced the results in the treatments AP, DL, and DL-AP (DL).

Another result of our study (table 1) shows that *A. pelleranoi* had fewer emerged parasitoids than *D. longicaudata* but presented a higher sex ratio in treatments with two exposures (table 1). In an interspecific competition test between *D. tryoni* and *D. longicaudata*, Ramadan *et al.* (1994) observed similar to our study, that although the second species generated more offspring, it did not have the largest number of females. The authors inferred that this may have occurred because immature females of *D. tryoni* may be better competitors than males, or simply because adults oviposit more fertilized eggs (eggs that would give rise to females) in case of competition.

Due to the similarities in the behaviour and preference between *A. pelleranoi* and *D. longicaudata* already described and confirmed in this study, experiments should be conducted on the competition of these species in the field, because although some works state that *D. longicaudata* does not compete with native parasitoids (dos Santos *et al.*, 2016; Meirelles *et al*., 2016), others state the opposite (Paranhos *et al*., 2013; Montoya *et al*., 2017), leaving questions regarding this issue. Our results show that *D. longicaudata* competes and diminishes the emergence of *A. pelleranoi* when both species are exposed to the same larva. Thus, this competition may also occur in the field, especially if the number of available hosts is limited. Moreover, other biotic and abiotic factors should be considered, as they may cause this parasitoid to coexist with *A. pelleranoi*. One of the aspects that may interfere in this field competition is the fact that the ovipositor size of *A. pelleranoi* has a mean size of 0.2 mm (Tormos *et al*., 2013), smaller than that of *D. longicaudata* (5.27 mm)

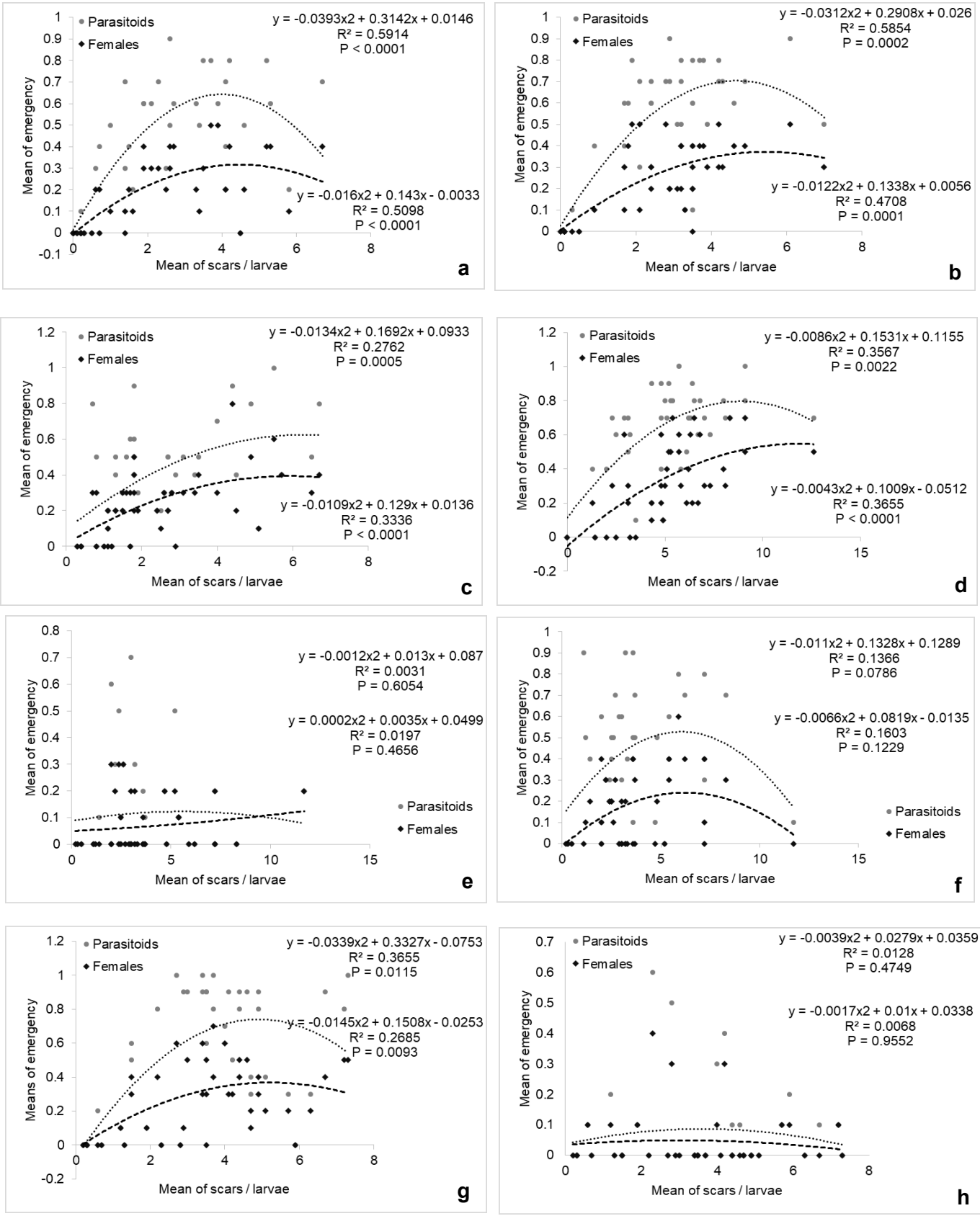

Figure 1. Correlation between oviposition scars caused by *Diachasmimorpha longicaudata* (DL) or *Aganaspis pelleranoi* (AP) on larvae of *Anastrepha fraterculus* and parasitoids and females emerged in the treatments **(a)** AP, **(b)** DL, **(c)** AP-AP, **(d)** DL-DL, **(e)** AP-DL (progeny of *A. pelleranoi*), **(f)** AP-DL (progeny of *D. longicaudata*), **(g)** DL-AP (progeny of *D. longicaudata*), and **(h)** DL-AP (progeny of *A. pelleranoi*). Spearman correlation coefficient ($\alpha = 0.05$).

(Sivinski *et al*., 2001), which limits the search of the first species for hosts in fruits with a thicker mesocarp. *A. pelleranoi* exhibits a specific foraging behaviour by depositing on cracked or fallen fruits (Sivinski *et al*., 2000); however, if host larvae have already been parasitized by *D. longicaudata*, our study shows that the species will not be able to succeed in the competition.

The results presented and discussed in this study indicate that in interspecific interactions with *A. pelleranoi*, *D. longicaudata* is considered the best competitor as it has already been registered with other larval parasitoids (Miranda *et al*., 2015; Murillo *et al*., 2016 ; Montoya *et al*., 2017) able to win in an intrinsic competition with native parasitoids (Paranhos *et al*., 2013). Given that *D. longicaudata* is considered a highly concurrent species, with ease of adaptation to new environments and larvae with competitive characteristics, its introduction into new environments should be carefully evaluated, as it may not only suppress *A. pelleranoi*, but also other species present in the environment.

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