Ecological Entomology

Ecological Entomology (2013), **38**, 485–496 DOI: 10.1111/een.12039

Inter-specific competition and competition-free space in the tephritid parasitoids *Utetes anastrephae* and *Doryctobracon areolatus* (Hymenoptera: Braconidae: Opiinae)

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Abstract. 1. *Utetes anastrephae* (Viereck) and *Doryctobracon areolatus* (Szépligeti) are common, native, Neotropical braconid parasitoids of tephritid fruit flies that are sympatric and often found attacking the same host.

2. The coexistence of the two species may be due in part to the longer ovipositor of *D. areolatus* that permits it to attack larvae in larger fruit than can *U. anastrephae*. This increases its potential host range and provides 'competitor-free space'.

3. The capacity of *U. anastrephae* to persist in smaller fruit, exploitable by *D. areolatus*, suggested that it was a superior competitor in multiparasitised hosts. As predicted *U. anastrephae* had a competitive advantage over *D. areolatus* and this advantage occurred regardless of the order in which the two parasitoids attacked. Although we could not identify the precise mechanisms used for elimination of competitors, a possible cause is suggested by the formidable mandibles of the first-instar *U. anastrephae*.

4. However, *D. areolatus* survival increased significantly if eggs had been deposited 24 h prior to exposure to *U. anastrephae*. Older *D. areolatus* larvae might be more competitive after a period of development.

5. *Utetes anastrephae* females were less likely to oviposit into hosts previously attacked by *D. areolatus* than *vice versa*. This was a second case of the relatively rare phenomenon of inter-specific discrimination of a previously exploited host within the opiine braconid parasitoids of frugivorous tephritids.

Key words. Competitor-free space, fruit flies, multiparasitism, natural enemy guilds, tephritidae

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Introduction

Multiple parasitioid species often exploit a particular host species (Hawkins, 1994) and the co-existence of potential competitors can depend on selection for resource partitioning and their occupations of 'competitor-free spaces'. Thus, niche differences can imply divergence and a history of competition avoidance (e.g. Connell, 1980; Tscharntke, 1992). The basis of this inference, a history of severe interference competition (Hawkins, 2000), can be illustrated by the rapid changes of species composition that can occur when parasitiods are sequentially introduced into novel situations. Classic cases are opiine braconid parasitoids of exotic tephritid fruit flies in Hawaii and Florida, where flourishing natural enemies were quickly replaced over large portions of their ranges by introductions of new species (Wang *et al.*, 2003; Eitam *et al.*, 2004). In addition to competition causing spatial displacement, the introduction of a competitor can also result in host shifts. *Diachasmimorpha tryoni* (Cameron), an opiine braconid, was introduced into Hawaii where it commonly attacked the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Later a superior competitor, another opiine *Fopius arisanus* (Sonan), was established and subsequent to that *D. tryoni* was rarely recovered from *C. capitata* (Wang & Messing, 2003). However, *D. tryoni* did become an increasingly common parasitoid of the lantana gall fly (*Eutreta xanthochaeta* Aldrich) a non-frugivorous tephritid not attacked by *F. arisanus* (Messing & Wang, 2009).

In the Neotropics and subtropics a guild of opiine braconid koinobiont parasitoids, both native and introduced, attack a variety of *Anastrepha* spp. (Tephritidae) infesting both native and exotic fruit species (López *et al.*, 1999). While some of these parasitoids are separated to one degree or another by environmental factors such as altitude, latitude, and host preferences (Sivinski *et al.*, 2000), two widespread and often abundant native species are generally sympatric and we here address the question of how these competitors coexist. *Utetes anastrephae* (Viereck) and *Doryctobracon areolatus* (Szépligeti) both attack late-instar *Anastrepha* spp. larvae inside fruit (hosts in laboratory colonies ~ 8 days of age; Aluja *et al.*, 2009). They complete development in the host prepupa and emerge from the puparium. Both naturally co-occur from Mexico to Argentina (Wharton & Marsh, 1978), have similar altitudinal distributions (Sivinski *et al.*, 2000), and are capable of third-instar diapause (Aluja *et al.*, 1998). In addition, they have been recovered from the same fruits and fruit fly hosts (Table 1; data set available from J.S.), although as will be addressed they are not similarly abundant in all fruit species.

Part of the solution to the problem of their co-existence may be the longer ovipositor of *D. areolatus* which permits it to attack larvae in larger fruit than *U. anastrephae* (Sivinski, 1991; Sivinski *et al.*, 1997, 2001). Greater ovipositor length increases its potential host range and provides it *U. anastrephae*-free space. Host proportioning to avoid competition through different ovipositor lengths has previously been hypothesized to allow the exploitation of the same host by three large *Megarhyssa* spp. (Ichneumonidae) (Heatwole & Davis, 1965). Their siricid host tunnels throughout rotting tree trunks and they select larvae at depths equal to the lengths of their ovipositors. In this particular case, intra-specific competition may have led to polymorphic ovipositors and ultimately to sympatric speciation (Gibbons, 1979).

The host choices facing inter-specifically competing Neotropical opiines are more various, as are their opportunities to avoid competition. On first examination the central and southern Mexican host ranges of the two parasitoids appear to overlap almost completely (Table 1), but further scrutiny reveals that the mere presence of *U. anastrephae* in all these combinations of host larvae and fruit is misleading. In smaller fruit both species can be abundant, inflicting summed parasitisms that exceed 80%; e.g. in one set of *Spondias mombin* (Anacardiaceae) samples *D. areolatus* parasitised a mean (SE) of 41.8 (7.0)% of the *Anastrepha obliqua* (Macquart) contained and *U. anastrephae* 34.7 (6.8)%; López *et al.*, 1999). However, in others only *D. areolatus* is common, and with the 15 samples of 7 tree species from which López *et al.* (1999) recovered both species we have calculated that there was a significant positive correlation between mean fruit weight and the proportion of parasitism inflicted by *D. areolatus* [$r = 0.66$; $P = 0.008$; overall mean (SE) % parasitisms by *D. areolatus* and *U. anastrephae* were 24.6 (5.6) and 15.8 (4.6) respectively]. In Florida, where biological control introductions have reunited the two species, only *D. areolatus* was found in *Psidium guajava* L. (Myrtaceae) although both species occurred in the much smaller *Eugenia uniflora* L. (Myrtaceae) (Sivinski *et al.*, 1998). Even in this one species the mean size of fruit containing *U. anastrephae* was smaller than that of fruits containing *D. areolatus*. At a still finer scale, within the fruits of a single tree, *U. anastrephae* can be concentrated into portions of the canopy where fruit tend to be smaller, e.g. the central core of *S. mombin* (Sivinski *et al.*, 1997). Several surveys carried out in Argentina (Ovruski *et al.*, 2004, 2008) and Brazil (Canal & Zucchi, 2000; Aguiar-Menezes *et al.*, 2001) also found *U. anastrephae* to be most common in small fruits.

A longer ovipositor, one that allows *D. areolatus* access to hosts beyond the reach of *U. anastrephae*, only provides a possible solution to how *D. areolatus* can avoid competition. Unresolved is how *U. anastrephae*, with a shorter ovipositor (1.6 mm vs. 3.8 mm), smaller mean egg-load (11.8 vs. 47), smaller egg (0.5 mm in length vs. 1.1 mm) (Sivinski *et al.*, 2001) and shorter adult lifespan (~ 12 days for females on an optimal diet vs. 20 days) (Stuhl *et al.*, 2011) can flourish in small fruit in the presence of *D. areolatus*. After all, a long ovipositor does not preclude it from accessing tephritid larvae in little fruit (Sivinski, 1991; Sivinski *et al.*, 2001). It would appear that *U. anastrephae* must be able to out compete *D. areolatus* in the hosts that it is able to reach.

The outcome of interactions among parasitoids can be resolved by *direct* or *indirect* means (Boivin & Brodeur, 2006). Females that discriminate against already parasitised hosts, typically by sensing an Oviposition Deterring Pheromone (= ODP), an internal marker or some cue such as faeces, deposited by a previously ovipositing female, are avoiding competition *indirectly*. While conspecific, even individual, ODP recognition is common and widespread, recognition of

Table 1. The host ranges and numbers of potentially co-evolved competitors of *Doryctobracon areolatus* and *Utetes anastrephae* in south-central Mexico as they occur in native tephritids infesting native plants.

	1	2	3	4	5	6	7	8	9
A	*Da(1)*	*Ua(1)*							
B			*Da(1) Ua(1)*						
C			*Da(2) Ua(1)*						
D									*Da(2) Ua(2)*
E									*Da(1) Ua(2)*
F				*Da(1) Ua(1)*					
G	*Da(16) Ua(1)*				*Da(16) Ua(1)*				
H	*Da(1)Ua(1)*				*Da(1) Ua(1)*				
I	*Da(1) Ua(1)*				*Da(1) Ua(1)*				
J						*Da(1) Ua(1)*			
K							*Da (1)*		
L		*Da(10) Ua(7)*							
M		*Da(10) Ua(2)*							
N		*Da(4) Ua(3)*							
O		*Da(1) Ua(1)*							
P								*Da(3) Ua(2)*	
Q			*Da(1)*						

Tephritid species are coded as numbers across the upper horizontal row of the table and host fruits on the first vertical column (see codes below). Within the blocks: *Da* refers to the published recovery of *D. areolatus* from a particular fly and host fruit and numbers in parentheses immediately after indicate the number of published records; Likewise, *Ua* refers to *U. anastrephae* and the numbers immediately following in parentheses to the numbers of published records. Plant codes are as follows: A = *Ampelocera hottlei*, B = *Bumelia sebonlana*, C = *Manilkara zapota*, D = *Crataegus mexicana*, E = *Crataegus rosei*, F = *Malmea guameri*, G = *Psidium guajava*, H = *Psidium guineense*, I = *Psidium sartorianum*, J = *Quararibea funebris*, K = *Schoephia schreberi*, L = *Spondias mombin*, M = *Spondias purpurea*, N = *Spondias radlkoferi*, O = *Tapirira mexicana*, P = *Ximenia americana*, Q = *Calocarpum mammosum*. Tephritid codes are as follows: 1 = *Anastrepha fraterculus*, 2 = *A. obliqua*, 3 = *A. serpentina*, 4 = *A. bahiensis*, 5 = *A. striata*, 6 = *A. crebra*, 7 = *A. spatulata*, 8 = *A. alveata*, 9 = *Rhagoletis 'pomonella'*.

heterospecific ODPs is relatively rare (Boivin & Brodeur, 2006). *Direct* competition is *extrinsic* when foraging females confront each other over access to hosts or host locations, e.g. by aggressively defending a parasitised host (Griffiths & Godfray, 1988). Subsequent to oviposition, direct competition can occur after multiple eggs-larvae occupy a host. This *intrinsic* competition may be resolved by females adding substances that make the host physiologically unsuited for other eggs or larvae (Silvers & Nappi, 1986), or more rarely by killing competitors directly with paralysing venom introduced during oviposition (Wang & Messing, 2004). Larvae may intrinsically compete directly with other larvae or destroy eggs prior to hatching by either inducing physiological changes in the host that starve, suffocate or poison potential competitors (Fisher, 1961), or through physical attacks with powerful mandibles or armoured caudal appendages typical of certain motile first-instar larvae (Salt, 1961).

In the present study we determined the outcomes of direct and indirect competition between *U. anastrephae* and *D. areolatus*. Specifically we predicted that: (i) *U. anastrephae* will be a superior *direct-intrinsic* competitor and able to eliminate *D. areolatus* from multiparasitised hosts; and (ii) should such a competitive asymmetry occur, females of the weaker of the competitors, presumably *D. areolatus*, would have evolved *indirect* means to recognise the presence of its stronger rival and avoid placing its offspring in peril. Finally we address how such information can be useful for biological control of pestiferous fruit flies.

Methods

Insect rearing

Parasitoids and host fruit fly larvae were reared at the Instituto de Ecología, A.C., Xalapa, Mexico. *Doryctobracon areolatus* (= Da) and *U. anastrephae* (= Ua) stemmed from colonies that had been maintained for 15 and 10 generations, respectively, and whose founders had been collected from *Spondias purpurea* L. and *S. mombin* L. fruit in central Veracruz state (Mexico) (Aluja *et al.*, 2009). The parasitoid colonies were kept at $25 \pm 1\,^{\circ}$C, $65 \pm 5\%$ RH, and a photoperiod of LD 12:12 h. Artificial diet-reared third-instars of *Anastrepha ludens* (Loew) originating from a colony kept under separate laboratory conditions ($27 \pm 1\,^{\circ}$C, $70 \pm 5\%$ RH, 12 : 12 h photoperiod) were used as hosts for all parasitoid species. Parasitoid and fly-rearing techniques are described in Aluja *et al.* (2009).

Experimental conditions

All bioassays were conducted inside a room at $26 \pm 1\,^{\circ}$C, $65 \pm 5\%$ RH, and a photoperiod of LD 12 : 12 h. Light came from 600 lux daylight fluorescent tubes. The observations were made in artificial arenas consisting of $30 \times 30 \times 30$ cm Plexiglas cages covered with fiberglass and containing plastic Petri dish oviposition units of different diameters and heights according to the experiment (see below). These oviposition

units contained third-instar (8 day-old), lab-reared *A. ludens* larvae mixed with some of the diet they had been reared on (details in Aluja *et al.*, 2009), and covered with a taught piece of Parafilm or with organdy cloth depending on the parasitoid species under investigation (Ua with Parafilm and Da with organdy). All tested parasitoid females were 6–7 days old, mated, and with previous oviposition experience. While Ua's mean lifespan is shorter than that of Da the ages used are well within the range of peak maturity for both (Stuhl *et al.*, 2011).

Influence of intrinsic competition on adult parasitoid emergence

This experiment was carried out to test the following: (i) is one species dominant over the other in a competitive situation (species effect); (ii) what is the influence of oviposition order and elapsed time between two ovipositions on parasitoid adult emergence (oviposition order effect and time effect, respectively); and (iii) what is the interaction between oviposition order and oviposition time (order × time interaction effect).

Ten treatments were compared: (T1) = a single Ua parasitised the host and was then removed. A Da adult was immediately put in the cage until it was observed to parasitise the host; (T2) = the inverse of T1, a Da was put into the test cage then followed by a Ua female; (T3) = a Ua parasitised a host and was removed. After 24 h a Da female was introduced in the cage and remained until it parasitised the host; (T4) = the inverse of T3. A Da was put in the cage first and a Ua replaced it 24 h later; (T5) = a Ua parasitised the host and was removed. Another Ua was immediately introduced and remained until it also parasitised the host; (T6) = similar to T5, but the second Ua was placed in the cage 24 h later; (T7) (control) = a Ua parasitised the host and was removed; (T8) = a Da parasitised the host and was removed to be immediately replaced by a second Da which remained until it parasitised the host; (T9) = similar to T8, except that the second Da was placed in the cage 24 h later; and (T10) (control) = a Da parasitised the host and was then removed.

Oviposition units (OU) 2.5 cm in diameter and 0.5 cm high were used in all treatments. In T1 and T3, the OU was initially covered with Parafilm to facilitate parasitism by Ua, but once the Ua female parasitised the host larva the Parafilm was removed and changed to an organdy cover prefered by Da females for oviposition. Covers in T2 and T4 were the inverse of T1 and T2. Only Parafilm covers were used in T5, T6, and T7, whereas only organdy covers were used in T8, T9, and T10.

Each procedure was repeated 100 times for every treatment. A new host larva and female parasitoid were provided with a new OU for every exposure. After being parasitised, each host larva was placed separately in a 200-ml plastic container with artificial diet. The larva stayed in the container for 2 days. The food was then removed and the larva was placed in another 200-ml plastic container to pupate and complete development. The bottom of this container was covered by 1 cm^3 of damp sterilised vermiculite. After 20–25 days, the eclosion of an adult parasitoid or fly was recorded.

Influence of intrinsic competition on survival of parasitoid eggs and larvae prior to adult eclosion

The experiment followed the procedures described above; however, it consisted of only four treatments, which corresponded to the first four treatments of Experiment 1 (last section). However, in this second set of observations the host was not allowed to develop until adult emergence, but was dissected 72 h after its last exposure to parasitism. After this period, first-instar larvae of each parasitoid species were found without difficulty. The presence and condition (alive or dead) of larvae and/or eggs of both parasitoid species were determined after a period of potential competition. A Ua or Da larva was considered dead when it either did not move or was damaged. Eggs were considered dead either when no embryo was observed or when the egg was collapsed without having hatched. This experiment assessed whether the survival of larvae or eggs under multiparasitism conditions was influenced by heterospecific oviposition order and/or the amount of time that passed prior to a subsequent heterospecific oviposition. There were four possible outcomes: both opiine species live or die, Ua lived but Da died, and Ua died but Da lived. Note that while the prior experiment measured adult emergence and that only one adult ever emerged, this second measured egg/hatchling survival and it was possible for multiple immature to be alive at the time of dissection.

Influence of prior parasitism on oviposition

Two treatments were conducted to determine heterospecific host discrimination ability in Da and Ua. **T1** = two OU (10 cm in diameter and 0.9 cm in height) covered with Organdy cloth were placed in a cage. One unit contained an artificial diet with 20 *A. ludens* larvae parasitised 24 h earlier by Ua (Condition 1); the other contained an artificial diet with 20 non-parasitised *A. ludens* larvae (Condition 2). The OUs were placed 8 cm apart from each other, 5 cm away from the back wall and 15 cm away from the front wall. Ten Da females were released in the front part of the cage floor and allowed to oviposit for 3 h. **T2** = this treatment was similar to T1, except that the OUs (10 cm in diameter; 0.4 cm in height) were covered with Parafilm (Aluja *et al.*, 2009). One of the units contained 20 *A. ludens* larvae parasitised by Da 24 h earlier (Condition 1) and the other contained 20 non-parasitised host larvae (Condition 2). Ten Ua females were introduced simultaneously in each test cage and allowed to oviposit for 3 h. Each treatment was repeated 10 times and the OU and the parasitoids were changed in each replicate and never reused. During the observation period, the numbers of wasp visits on ovipositor probes in both OUs were recorded. A 'probe' was confirmed when a parasitoid female elevated its metasoma and inserted the tip of its ovipositor through the Parafilm or organdy cloth for at least 3 s (Duan & Messing, 1999). After the observation period, the larvae of each OU were removed and placed in separate 200-ml plastic containers with an artificial diet. They remained 2 days after which dead larvae were separated from the living and counted. Live larvae were then placed in 500-ml plastic containers with 1 cm^3 of dampened, sterilised vermiculite in the

bottom to serve as a pupation medium. After 20–25 days, the number of parasitoids and flies that had emerged was recorded, as was the number of puparia from which no adult insects had emerged.

Influence of parasitoid female density on host parasitisation and mortality rates

In this experiment, the host density was kept constant, but female parasitoid density varied. The OUs used were the same as for Experiment 3. Five treatments for each parasitoid species were examined. In each, host density was 25 *A. ludens* larvae per OU and the OU stayed inside the experimental cage for 8 h. The treatments were as follows: T1 = 5 parasitoid females (Ua or Da); T2 = 8 females; T3 = 25 females; T4 = 75 females; and T5 = 125 females. Ten replicates per treatment were carried out. The variables analysed were prevalence of parasitism and host mortality.

Influence of host density on parasitism and mortality rates

In this experiment, female parasitoid density was kept constant, but host density varied: T1 = 5 *A. ludens* larvae; T2 = 8 larvae; T3 = 25 larvae; T4 = 75 larvae; and T5 = 125 larvae. The OUs used were the same as in Experiment 3. Groups of 25 female parasitoids per species were placed in the experimental cages, and the OUs remained for 8 h. Ten replicates per treatment were carried out. The variables analysed were host parasitism and mortality.

Data analysis

A generalised linear model (Crawley, 1993) was used to evaluate the effect of competing parasitoids on the emergence of the first species to occupy a host. In this model, the factors were: (i) 'original' species, with two levels, Ua and Da; and (ii) 'subsequent' species, i.e. the second parasitoid species to occupy the host, with two levels, present and absent. If both factors 1 and 2 were Ua or both Da, then the subsequent competing species was absent. However, when factors 1 and 2 were different a competitor was present. A third factor ('time') considered the effect of when the subsequent parasitoid oviposited into the host. This time factor had two levels, 0 and 24 h. Given the binomial nature of the response variable, 0 = non emergence and 1 = emergence, a binomial error with logit link-function was specified.

To evaluate the effect of con- and heterospecific competition on egg and/or larval survival, a generalised linear model was used similar to the above. In this model, the factors were: (i) order of oviposition (with two levels, Ua or Da), and (ii) time of oviposition (0 and 24 h). These two factors were tested for both Ua and Da survival (univariate models) using a binomial response with logit link-function. The condition of eggs/larvae under competitive situations was described as either live or dead. To assess if this condition was dependent or not on the presence of second ovipositing species, a log-linear analysis model with three categorical factors (condition, species, and time) was used, and the frequency for each combination was the response.

The number of wasp visits and the number of ovipositor probes in alternative OUs containing non-parasitised larvae and parasitised hosts was compared using a G-test of goodness of fit to the equal proportion hypothesis, with Yates' correction for continuity (Sokal & Rohlf, 1995). Evaluation of the effect of prior parasitism of the host larva by a heterospecific parasitoid on emergence rate of the second species to oviposit and on the host mortality rate was by a generalised linear model. In the model, OU condition when exposed to parasitoids was a fixed factor with four levels: (i) = OU containing parasitised host larvae by *Ua* and exposed to Da (HpUa-Da); (ii) = OU containing unparasitised host larvae exposed to Da females (Hnp-Da); (iii) = OU containing parasitised host larvae by *Da* and exposed to Ua (HpDa-Ua); and (iv) = OU containing unparasitised host larvae exposed to Ua females (Hnp-Ua). Three response variables were separately analysed: (i) = Da emergence rate; (ii) = Ua emergence rate; and (iii) = host mortality rate. All these dependent variables were associated with a normal distribution with a link log function. This decision was based on the Scaled Deviance values, which varied from 1.08 to 1.10 and which indicated the selected error was appropriate.

A generalised linear model was used to examine the effects of host larva and parasitoid female density on parasitism by Da and Ua and host mortality rates. In the models, density variations (host larva density or parasitoid female density) and opiine parasitoid species (Da and Ua) were fixed factors. The density × species interaction was also included. Because the Scaled Deviance values were 1.11, the three dependent variables were associated with a normal distribution with a link log function.

Per cent parasitism was calculated by dividing the total number of emerged and unemerged parasitoids by the total number of larvae exposed in the UO. Unemerged puparia were dissected to check for the presence of larvae, pupae and/or pharate adult parasitoids. The parasitoid emergence rate was calculated as the total number of emerged adult parasitoids divided by the total number of larvae exposed in the UO. Host mortality rate was calculated as the total number of pupae that did not yield flies or parasitoids divided by the total number of emerged and unemerged pupae. The numbers of dead pupae used to calculate the pupal mortality rate excluded all the pupae that were dissected or cases when pupae contained a parasitoid adult, or parasitoid pupa or prepupa.

These and all previous analyses were performed with Statistica 7 (StatSoft, Inc., 2004).

Discrimination of eggs and first-instar larvae of U. anastrephae *and* D. areolatus

To assign each parasitoid egg/larva to a given species several host puparia were removed 48 or 72 h after parasitism and dissected in physiological serum in depression slides. Parasitoid larvae (Ua or Da) were removed from each host

paparium and fixed in Carnoy's solution for 24 h, and then transferred into 96% ethanol for later examination under a light microscope. Drawings of parasitoid first-instar larvae were made with the help of a Zeiss-Stemi SV6 stereo-microscope. Parasitoid eggs were fixed in 4% glutaraldehyde and 0.2 M phosphate buffer, dehydrated through an alcohol series, and then were placed in 100% acetone. Parasitoid eggs were critical-point dried prior to examination by scanning electron microscopy. Images of parasitoid eggs were taken using a JEOL Model JSM-5600LV microscope.

First-instar larvae of both Ua (Fig. 1) and Da (Fig. 2) were distinguished by three morphological structures, the size of both cephalic hood and mandibles and the shape of caudal abdominal segment. In Da first-instar larvae the cephalic hood is 2.5–2.7 times smaller than the cephalic hood of Ua first-instar larvae, Da's sickle-like mandibles are 3.1–3.4 times smaller than the first-instar larval mandibles of Ua, and the caudal abdominal segment of Da is 2.4–2.5 times longer than its 12th body segment and has a finger-shaped projection. In Ua first-instar larva the caudal segment is 1.3–1.4 longer than the 12th body segment and it has a horseshoe-like shape with two recurved tooth-like extensions. Over 20 first-instar larvae of each parasitoid species were examined.

Parasitoid eggs of Ua (Fig. 3) and Da (Fig. 4) 48 h after oviposition were distinguished by the caudal body shape. The caudal body of the Ua egg has a thumb-like projection, whereas this is absent in Da eggs (20 eggs of each opiine species examined).

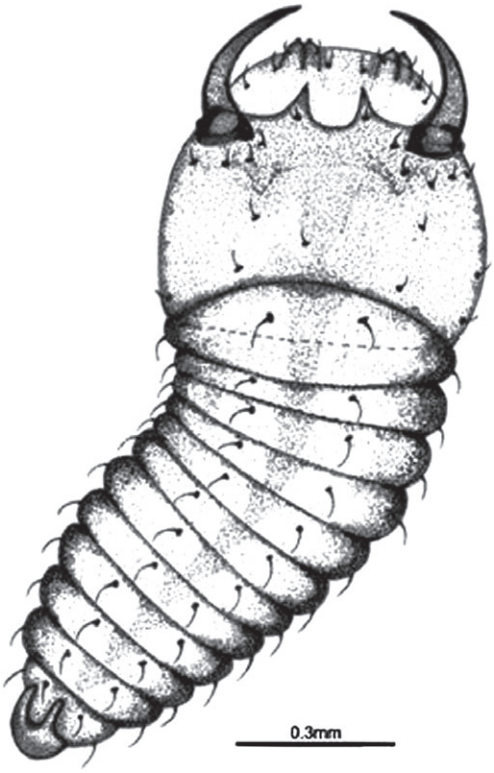

Fig. 1. First-instar larva of *Utetes anastrephae*.

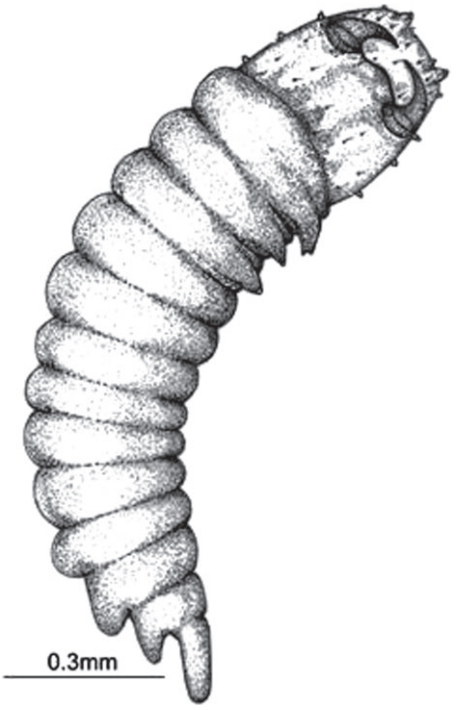

Fig. 2. First-instar larva of *Doryctobracon areolatus*.

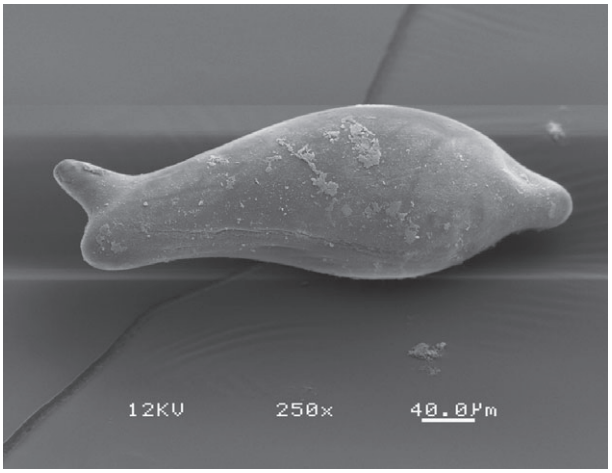

Fig. 3. Forty-eight-hour-old egg of *Utetes anastrephae*.

Results

Influence of intrinsic competition on adult parasitoid emergence

In inter-specific conflicts, Ua was superior to Da. Of the total number of host larvae used in treatments 1 through to 4, more than 60% resulted in Ua adults (Fig. 5). In treatments 5 and 6 (sequential exposures to Ua, either immediate or after 24 h), Ua emergence were higher than 80% and similar (1.1 times higher) to those of the control treatment. Similarly, Da emergence surpassed 60% after sequential exposures (treatments 8 and

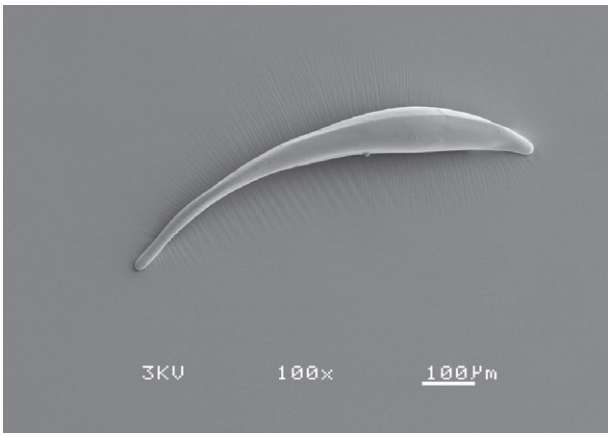

Fig. 4. Forty-eight-hour-old egg of *Doryctobracon areolatus*.

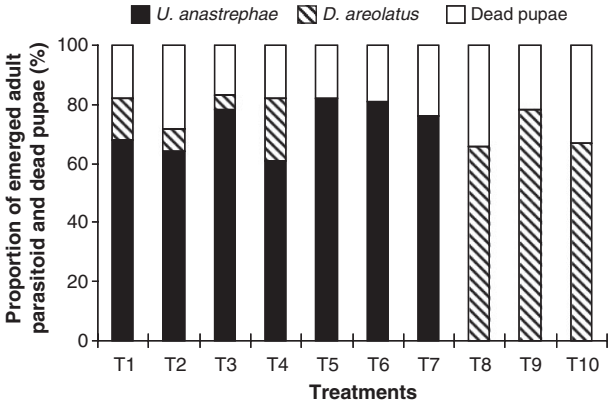

Fig. 5. The proportions of *Utetes anastrephae* (Ua) and *Doryctobracon areolatus* (Da) adults that emerged under different intrinsic competition conditions. The 10 treatments were: **T1** = a single Ua parasitised the host and was then removed. A Da was immediately put in the cage until it was observed to parasitise the host. **T2** = the inverse of T1; a Da was put into the test cage first followed by a Ua female. **T3** = a Ua parasitised a host and was removed. After 24 h a Da female was introduced in the cage and remained until it parasitised the host. **T4** = the inverse of T3; a Da was put in the cage first, and a Ua replaced it 24 h later. **T5** = a Ua parasitised the host and was removed; then another Ua was immediately introduced and remained until it also parasitised the host. **T6** = similar to T5, but the second Ua was placed in the cage 24 h later. **T7** (control) = a Ua parasitised the host and was removed. **T8** = a Da parasitised the host and was removed to be immediately replaced by a second Da which remained until it parasitised the host. **T9** = similar to T8, except that the second Da was placed in the cage 24 h later. **T10** (control) = a Da parasitised the host and was then removed. Each procedure was repeated 100 times for every treatment.

9), and did not differ from the control. The proportion of Ua adults that emerged in the control treatment was not different (1.1 times higher) to the levels observed in the Da control treatment.

In all experiments in which the two parasitoid species were together, Ua adult emergence was significantly higher than that of Da (Wald $\chi^2 = 86.891$, d.f. = 1, $P < 0.0001$). The presence of the subsequent species as well as the time intervals between the two successive ovipositions significantly affected the emergence of the first species occupying the host larva (Wald $\chi^2 = 42.087$, d.f. = 1, $P < 0.0001$ for subsequent-species factor, Wald $\chi^2 = 8.748$, d.f. = 1, $P = 0.0031$ for time factor). Furthermore, the interaction between the two factors original-species × subsequent-species showed that the Da emergence was significantly influenced by the presence or absence of Ua (Wald $\chi^2 = 82.344$, d.f. = 1, $P < 0.0001$). The effect of the time × original species interaction was the same on parasitoid emergence regardless of opiine species (Wald $\chi^2 = 3.029$, d.f. = 1, $P = 0.0817$). Similarly, the time × subsequent-species interaction did not significantly affect the emergence of the first parasitoid species attacking the host larva (Wald $\chi^2 = 0.007$, d.f. = 1, $P = 0.9312$).

Influence of intrinsic competition on survival of parasitoid eggs and larvae prior to adult eclosion

Among the four treatments, 93% of 400 *A. ludens* hosts contained a live larva of only one parasitoid species and 84% of the time this was a larva of Ua. Only 5% of dissected hosts contained live larvae of both species and the remaining 2% had dead larvae and/or eggs of both species (Fig. 6). The following combinations of the dead (Ua egg-dead, Da egg-dead), (Ua egg-dead, Da larva-dead), and (Ua larva-dead, Da larva-dead) accounted for 43%, 28.5%, and 28.5% of the total, respectively. In all treatments the proportions of Ua live eggs/larvae were markedly higher than proportions of Da live eggs/larvae [4.8–6.9 times and 3.1–3.4 times higher in T1–T2 (0 h between first and second oviposition) and T3–T4 (24 h between first and second oviposition), respectively] (Fig. 6). When survival of each species was analysed separately,

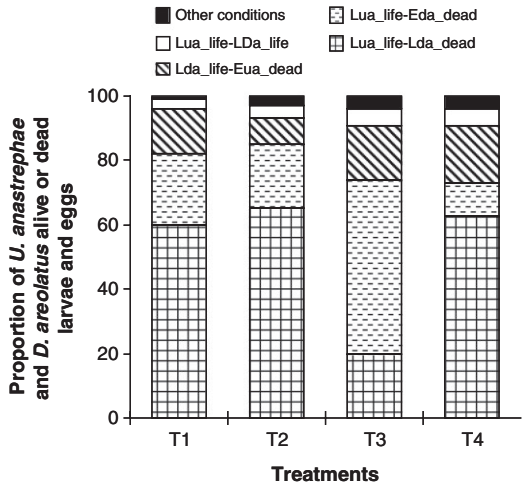

Fig. 6. The proportions of *Utetes anastrephae* and *Doryctobracon areolatus* that were alive or dead as larvae or eggs under different intrinsic competition conditions. Lua_live-Lda_dead = Ua's living larva and Da's dead larva; Lua_live-Eda_dead = Ua's living larva and Da's dead egg; Lda_live-Eua_dead = Da's living larva and Ua's dead egg; Lua_live-Lda_life = Ua's living larva and Da's living larva.

survival of Ua or Da was not significantly affected by the order of oviposition (Wald $\chi^2 = 0{,}332$, d.f = 1, $P = 0.5639$ for Ua, Wald $\chi^2 = 0{,}284$, d.f = 1, $P = 0.5939$ for Da), indicating no increased Da survival over Ua when Da was the first parasitoid species that oviposited into the host larva. Survival of the two species was significantly affected by the duration of time between ovipositions (Wald $\chi^2 = 6{,}465$, d.f = 1, $P = 0.0109$ for Ua, Wald $\chi^2 = 4{,}635$, d.f = 1, $P = 0.0313$ for Da). The proportion of live Da eggs/larvae in T3–T4 increased by 55% relative T1–T2. In contrast, the proportion of live Ua live eggs/larvae decreased by 13% compared with the treatments that included 24-h intervals between ovipositions. However, order × time of oviposition interaction did not significantly affect either Ua or Da survival (Wald $\chi^2 = 0{,}622$, d.f = 1, $P = 0.4301$ for Ua, Wald $\chi^2 = 0{,}918$, d.f = 1, $P = 0.3378$ for Da). When the survival of both Da and Ua was assessed, the likelihood that one parasitoid species was alive or dead was dependent on the presence of the other species, regardless of the order or time of oviposition ($G^2 = 213.9$, d.f. = 4, $P < 0.0001$). Overall, results of both statistical tests indicated that Ua was competitively superior to Da.

Influence of prior parasitism on oviposition

Significantly two-times more Da visits were recorded on the OU with non-parasitized host larvae than on the OU with Ua-parasitized hosts ($G_1 = 25.59$, $P < 0.0001$) (Fig. 7). Similarly, the number of Ua visits on the OU with non-parasitised host larvae was 1.6 times significantly greater than that recorded on the OU with Da-parasitised hosts ($G_1 = 19.62$, $P < 0.0001$) (Fig. 7). The number of probes with ovipositor by Da females in OU with non-parasitised larvae was approximately 3.4-times greater than that recorded in OU with parasitised larvae by Ua ($G_1 = 69.0$, $P < 0.0001$) (Fig. 8). Likewise, the number of probes with ovipositor by Ua females in OU with non-parasitised larvae was 2.6-times significantly higher than that recorded in OU with larvae parasitised by Da ($G_1 = 87.39$, $P < 0.0001$) (Fig. 8).

The Da emergence rate recorded from OU with larvae previously parasitised by Da and exposed to Ua was 39 times significantly greater than that recorded from OU with larvae

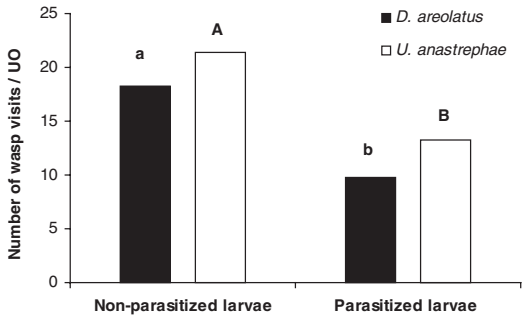

Fig. 7. Number of wasp visits per Oviposition Unit (OU) containing non-parasitised larvae and parasitised larvae by *Utetes anastrephae* (white) or by *Doryctobracon areolatus* (black). Columns followed by the same letter are not significantly different.

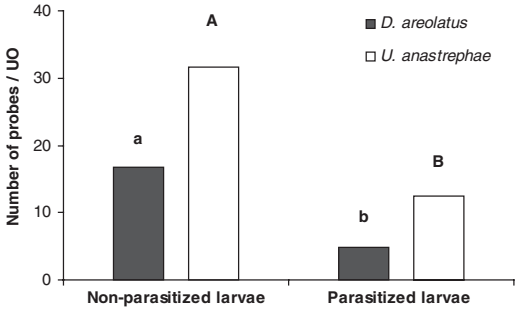

Fig. 8. Number of ovipositor probes per Oviposition Unit (OU) containing non-parasitized larvae and parasitised larvae by *Utetes anastrephae* (white) or by *Doryctobracon areolatus* (black). Columns followed by the same letter are not significantly different.

parasitised by Ua and exposed to Da whereas it was only 1.3 times larger to that obtained from OU containing unparasitised host larvae exposed to Da females (Wald $\chi^2 = 41\,519$, d.f = 2, $P < 0.0001$, Fig. 9). Similarly, the Ua emergence rate recorded from OU with larvae parasitised by Ua and exposed to Da was 4.3-times significantly greater than that recorded from OU with larvae parasitised by Da and exposed to Ua, whereas it was only 1.4-times larger to that found from OU containing unparasitised host larvae exposed to Ua females (Wald $\chi^2 = 36{,}264$, d.f = 2, $P < 0.0001$, Fig. 9). Host mortality rates recorded from OU with larvae previously parasitised by Da or by Ua were 17.3- and 4.2-times significantly greater than those obtained from OU with unparasitised host larvae exposed to Da or Ua, respectively (Wald $\chi^2 = 32{,}288$, d.f = 2, $P < 0.0001$, Fig. 9).

Influence of parasitoid female density on host parasitisation and mortality rates

Parasitoid female density did not significantly influence per cent parasitism (Wald $\chi^2 = 2.847$, d.f. = 4, $P = 0.5837$).

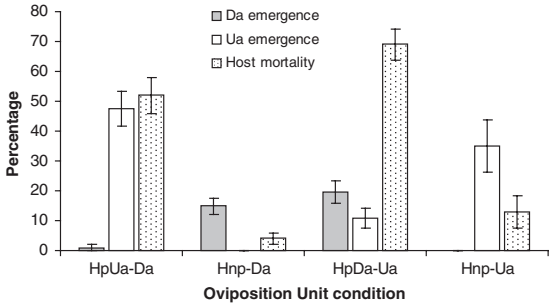

Fig. 9. Mean percentages (± SE) of emerged adults of *Utetes anastrephae* and *Doryctobracon areolatus*, and host mortality recorded from the oviposition units containing parasitised and unparasitised host larvae. HpUa-Da = OU containing host larvae parasitised by Ua and exposed to Da; Hnp-Da = OU containing unparasitised host larvae exposed to Da females; HpDa-Ua = OU containing parasitised host larvae by Da and exposed to Ua; Hnp-Ua = OU containing unparasitised host larvae exposed to Ua females.

Although parasitism by Da was significantly higher than that by Ua (Wald $\chi^2 = 3.895$, d.f. $= 1$, $P = 0.0484$), the density × species interaction was not (Wald $\chi^2 = 2.063$, d.f. $= 4$, $P = 0.7240$) (Fig. 10a). Parasitoid female density significantly influenced host mortality (Wald $\chi^2 = 48.757$, d.f. $= 4$, $P < 0.0001$). Similarly, there was significant differences between the two opiine species in terms of host mortality (Wald $\chi^2 = 5.697$, d.f. $= 1$, $P = 0.0169$). Nevertheless, the parasitoid density × parasitoid species interaction was not significant with regards to host mortality (Wald $\chi^2 = 1.946$, d.f. $= 4$, $P = 0.7455$) (Fig. 10b).

Influence of host density on parasitism and mortality rates

Host density did not significantly affect the per cent parasitism in either species (Wald $\chi^2 = 5.621$, d.f. $= 4$, $P = 0.2292$). Parasitism rates were similar in Da and Ua (Wald $\chi^2 = 1.361$, d.f. $= 1$, $P = 0.2434$) and the parasitoid density × parasitoid species interaction was not significant (Wald $\chi^2 = 2.358$, d.f. $= 4$, $P = 0.6701$) (Fig. 11a). Host density variations did not significantly influence host mortality (Wald $\chi^2 = 6.208$, d.f. $= 4$, $P = 0.1841$). Although, there were significant differences between the two species in terms of host mortality (Wald $\chi^2 = 18.381$, d.f. $= 1$, $P < 0.0001$). The density × species interaction was not significant (Wald $\chi^2 = 1.792$, d.f. $= 4$, $P = 0.7738$) (Fig. 11b).

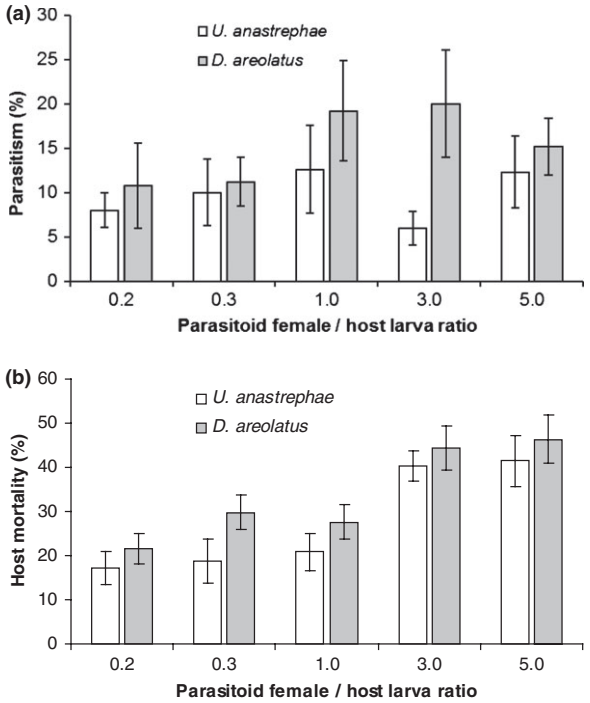

Fig. 10. Influence of *Utetes anastrephae* and *Doryctobracon areolatus* female density on host parasitisation (a) and mortality (b) rates maintaining a host constant density (25 host larvae).

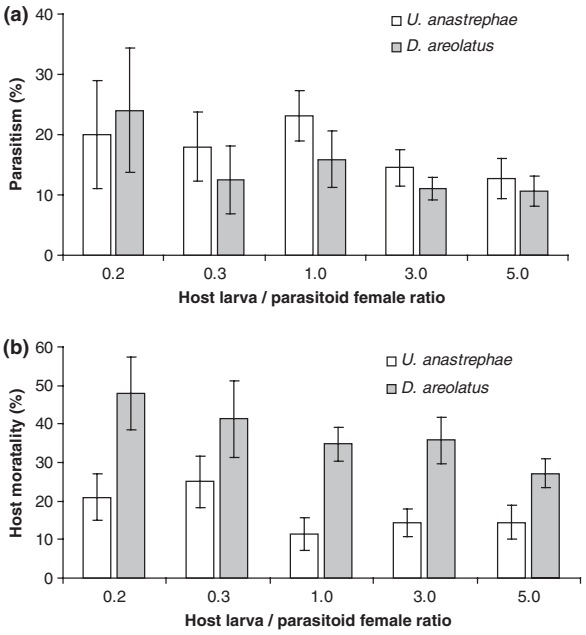

Fig. 11. Influence of host larva density on parasitisation (a) and mortality (b) rates keeping density of *Utetes anastrephae* and *Doryctobracon areolatus* females constant (25 parasitoids).

Discussion

Utetes anastrephae was a superior direct-intrinsic competitor and typically the victorious in competitions with *D. areolatus*. This was consistent with predictions based on the coexistence of *U. anastrephae* in fruit species that were exploitable by *D. areolatus*. Contrary to expectations, both species avoided oviposition units previously exposed to the other. We had hypothesized that the proposed weaker competitor, *D. areolatus*, would be under strong selection to avoid *U. anastrephae* but not vice versa. Moreover, once upon the unit only *U. anastrephae* were significantly less likely to probe its surface with their ovipositors. Given the relatively few eggs available to *U. anastrephae* (Sivinski *et al.*, 2001) perhaps it avoids even heterospecific competitions it is likely, but not inevitably, to win. Because the responses to previous heterospecific parasitism are different in *D. areolatus* and *U. anastrephae* the cues used to recognise heterospecifics may be different in number and kind as well. Both respond to some volatile compound(s), perhaps a component of an ODP, but *U. anastrephae* may also sense a less volatile substance deposited on the surface. While inter-specific discrimination of earlier oviposition is rare it has been previously discovered in another opiine tephritid parasitoid. When *D. tryoni* was presented with host larvae previously parasitised by *F. arisanus*, the ratio of ovipositor-penetration scars to actual ovipositions was double that observed on unparasitised hosts (Wang & Messing, 2003).

The order in which hosts were presented to the two parasitoids had little effect on outcome, *D. areolatus* larvae were more adversely affected by the presence of *U. anastrephae*

than vice versa. However, *D. areolatus* development increased significantly if its eggs had been deposited 24 h prior to exposure to *U. anastrephae*. While *D. areolatus* attacks third-instar *Anastrepha* spp. larvae (Aluja *et al.*, 2009) it is also capable of developing in hosts attacked in their second-instar (Eitam *et al.*, 2003). If the ability to successfully parasitise young hosts is more developed in *D. areolatus* than in *U. anastrephae* then earlier oviposition might be a means to improve *D. areolatus*' competitive situation. That is, it could allow a more mature *D. areolatus* larva a chance to destroy a rivals egg before it hatched or face a younger, smaller, and less formidable larval foe. A subset of such competitive *D. areolatus* in the environment might also explain why *U. anastrephae* has evolved the capacity to discriminate previous ovipositions by *D. areolatus*.

Although we could not identify the precise mechanisms used for elimination of competitors, a possible cause is suggested by the large mandibles of first-instar *U. anastrephae* (Figs. 5 and 6). The comparatively shorter body and head size and the relatively poorly developed and sclerotised mandibles of the first-instar larva of *D. areolatus* may place it at a disadvantage in combats with a first-instar *U. anastrephae*. Physical attacks between immature parasitiods were not directly observed, but broken portions of *D. areolatus* heads and bodies were observed on 10 occasions in dissected host larvae, whereas similar fragments of *U. anastrephae* were never found.

Aggression has been previously recorded or postulated in other braconids attacking fruit flies. Pemberton and Willard (1918) found that first-instar larvae of *Psyttalia humilis* (Silvestri) were eliminated by fighting when it shared the same host with first-instar larvae of *D. tryoni* or *Diachasmimorpha fullawayi* (Silvestri). In multiparasitised hosts that contained *F. arisanus* larvae, the eggs of *D. tryoni*, *Diachasmimorpha kraussii* Fullaway, and *Psyttalia concolor* (Szépligeti) died as the result of physiological inhibition (Wang & Messing, 2002, 2003), but *D. longicaudata* was superior to both *Fopius persulcatus* (Silvestri) and *F. arisanus* when physical competitions took place between first-instar larvae (Palacio *et al.*, 1991). The first-instar larvae of *D. longicaudata* also killed those of its congener *D. tryoni* through physically attacks (Ramadan *et al.*, 1994).

In summary, the continued coexistence of *D. areolatus* and *U. anastrephae* apparently results from *U. anastrephae* being a stronger competitor, both directly and indirectly, and *D. areolatus* occupying *U. anastrephae*-free space provided by fruit larger than *U. anastrephae* can exploit. This raises yet another question. If ovipositor length is the ultimate reason for *D. areolatus*' capacity to thrive, why has *U. anastrephae* not displaced its otherwise inferior competitor through the evolution of a longer ovipositor? The combination of sympatry and common hosts would seem ripe for an 'ovipositor arms-race' with *U. anastrephae* threatening the borders of *D. areolatus*' competitor-free-space and *D. areolatus* in turn investing in the means to escape by attacking formerly inaccessible larvae in still larger fruit. Perhaps these hypothetical larger infested fruit were not available over evolutionary time, but if so an arms race would have presumably ended with both species having ovipositors the length of *D. areolatus*. Any inherent limit in frugivorous fruit fly parasitoid ovipositor length has not been reached by either species. That of the Mexican opiine *Doryctobracon crawfordi* (Viereck), is much longer than those of either (Sivinski *et al.*, 2001).

On the other hand, there may be a cost to having a longer ovipositor that *U. anastrephae* is unable to bear (Sivinski & Aluja, 2003). While braconids such as the Japanaese *Euurobracon yakohamae* Dalla Torre can carry prodigious external ovipositors, up to eight times as long as their bodies (e.g. Townes, 1975), there are advantages to relatively short ovipositors. Hymenopteran ovipositors seldom exceed 1.3× the length of the body because force is greatest when the ovipositor is applied perpendicular to the surface and to do this the abdominal tip must be held at least an ovipositor length above the substrate (van Achterberg, 1986). But even if the optimal position can be attained, too great a force on too thin an ovipositor can cause it to bend (Euhler buckling) and prevent effective penetration (Vincent & King, 1996; Quicke, 1999). While, neither *U. anastrephae* nor *D. areolatus* has an ovipositor even approaching 1.3× body length, that of *U. anastrephae* is significantly shorter than its abdomen, shortening the ovipositor might concentrate force and aid in penetrating particularly tough substrates. But there is no evidence at present that *U. anastrephae* penetrates uniquely hard surfaces. Longer ovipositors can be more brittle. In Torymidae that attack gall-forming Cynipidae, ovipositors are often found broken off in large galls (Askew, 1965). Alternate generations of some species that parasitise different sized galls have correspondingly different ovipositor lengths suggesting that ovipositors are no longer than they need be and so represent some sort cost.

The act of ovipositing into hosts at greater depths is also likely to be more hazardous and costly (Heatwole & Davis, 1965). Females may be less mobile and exposed to predators for longer periods of time when they attempt to drill deeply into fruit. If this is the case, then *U. anastrephae*'s short ovipositor reflects a balance between access to hosts and the risks required to reach them.

Previously mentioned studies of niche segregation based on ovipositor length in *Megarhyssa* spp. did not address why species with longer ovipositors do not cheaply and safely attack shallow larvae as well as deep-feeding hosts. The evidence for depth specificity was complete insertion of the ovipositor into the wood substrate during oviposition. This apparently self-imposed limitation does not seem to be the case in Mexican tephritid-attacking opiines. *Doryctobracon areolatus* is abundantly recovered from smaller fruit species (López *et al.*, 1999; Sivinski *et al.*, 2001), although data do not preclude that it preferentially attacks the deeper dwelling larvae in these small fruit. In both Mexican opiines and *Megarhyssa*, we hypothesise that those species with the shortest ovipositors attack the most vulnerable subset of hosts and so face the greatest number of competitors. They are under the strongest selection to excel at interference competition. If so, then it is the choice of species with longer ovipositors to either risk unequal competition by attacking shallow hosts or endure dangerously protracted ovipositions (up to an hour in the case of *Megarhyssa atrata* (Fab.) ; Heatwole & Davis, 1965).

For whatever reason, perhaps an overwhelming capacity for intrinsic competition by the species with the next shorter ovipositor, *Megarhyssa* spp. would appear to decide to always avoid competition whereas *D. areolatus* may not.

While the evolutionary histories of present ovipositor lengths in *D. areolatus* and *U. anastrephae* are difficult to reconstruct, we suggest that these lengths have consequences for present-day host ranges and distributions, and provide an explanation for the continued coexistence of an inferior intrinsic competitor. This coexistence has also influenced biological control tactics (Serra *et al.*, 2011). *Utetes anastrephae* is native to Hispaniola but *D. areolatus* is not. The later was recently introduced into the Dominican Republic to control the West Indian fruit fly, *A. obliqua*, which infests numerous fruit species, particularly Anacardiaceae and most importantly mango (*Mangifera indica* L.). As on the continental mainland one species was a better intrinsic competitor and the other had a broader host range it was proposed that there would be no negative interactions when the two species were 'reunited' and overall parasitism would increase. Subsequently, there was no evidence of competitive exclusion of *U. anastrephae* by *D. areolatus*.

Acknowledgements

We thank Larissa Guillén for her editorial input. Martín Aluja acknowledges financial support by the Mexican Campaña Nacional Contra Moscas de la Fruta (Dirección General de Sanidad Vegetal–Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación), the Consejo Nacional de Ciencia y Tecnología de México (CONACyT Grant No. 46846) and the United States Department of Agriculture (Agricultural Research Service). Sergio Ovruski acknowledges financial support from the Agencia Nacional de Promoción Científica y Tecnológica de Argentina (ANCyT) through the Fondo Nacional de Ciencia y Tecnología (FONCyT) (Grant PICT/2006 No. 2402, and PICT/2010 No. 0393), Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) (Grants PIP No. 5129/05) and Proyecto de Investigación Conjunta en el marco del Acuerdo de Cooperación Internacional CONICET de Argentina y CONACyT de México (Res. N° 2311/04, 2006–2007). M. Aluja also acknowledges support from CONACyT through a Sabbatical Year Fellowship (Ref. 79449) and thanks Benno Graf and Jörg Samietz (Forschungsanstalt Agroscope Changins-Wädenswil [ACW], Switzerland) for providing ideal conditions to finish writing this paper.

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Accepted 23 April 2013
First published online 14 August 2013