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# Comparison of the Host Searching and Oviposition Behaviors of the Tephritid (Diptera) Parasitoids *Aganaspis pelleranoi* and *Odontosema anastrephae* (Hymenoptera: Figitidae, Eucoilinae)

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**Abstract** We compared the host-searching and oviposition behaviors of two Neotropical figitid parasitoids (Hymenoptera) that exploit the same resource: ripe fruit infested by fruit fly larvae (Tephritidae) that have fallen to the ground. Sexually mature *Aganaspis pelleranoi* (Brèthes) and *Odontosema anastrephae* Borgmeier females were exposed individually, under no choice conditions, to four types of fruit: 1) Clean, intact guavas, *Psidium guajava* L. (no fruit fly larvae, no perforations); 2) clean, with artificial perforations; 3) artificially infested (with larvae), no perforations; 4) infested with artificial perforations. A behavioral transition matrix and sequence diagram of the following behaviors was constructed: walking on fruit, detection of larvae via the antennae, tarsi or aculeus, fruit perforation and penetration, and oviposition. Overall, we found that infested fruit (intact and with artificial perforations) elicited the most activity in the females of both species and that *A. pelleranoi* females exhibited a significantly more diverse behavioral repertoire (i.e., more transitions) and were significantly more active than *O. anastrephae* females. Females of both species penetrated the fruit in search of larvae by biting through the epi- and mesocarp, but *O. anastrephae* remained inside for significantly longer periods (up to eight hours). *A. pelleranoi* females used both their antennae and tarsi to detect larvae but the use of these structures varied depending on context:

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in infested fruit tarsi were used preferentially (usually while standing still) while in uninfested fruit, antennae were mainly used (usually while walking). In the case of *O. anastrephae* females the reverse pattern was usually observed with antennae most commonly used to detect larvae in infested fruit. We discuss our findings in light of their evolutionary, ecological and practical implications.

**Keywords** Host-search behavior · oviposition · *Aganaspis pelleranoi* · *Odontosema anastrephae* · Figitidae · Tephritidae

## Introduction

There is ample evidence that arthropod predators and parasitoids compete, that competitors can displace one another and that this displacement is a significant factor in the structure of natural enemy guilds (Reitz and Trumble 2002). Thus, it can be a challenge to theory to see closely related parasitoid species sharing hosts and habitats. One reason such coexistence might persist is that there are unrecognized microhabitats that one of the potential competitors exploits and the other does not. In some cases, there are morphological clues to a subdivision of the niche. For example, ovipositor length in sympatric braconid parasitoids of tephritid fruit flies may reflect foraging for larval hosts in additional fruits or portions of fruits (Sivinski et al. 2001). There may also be behavioral differences in foraging that allow a species better access to hosts under particular circumstances and thus to escape the full impact of an adjacent competitor (e.g., Vet and Bakker 1985, García-Medel et al. 2007).

Foraging has several stages where host location is determined with increasing precision and differences between parasitoids at any of these might underlie the capacity to subdivide a niche. Hassel and Southwood (1978) postulated that a foraging animal perceives the environment at three hierarchical levels: the habitat, the patch and the food item. In the case of Hymenoptera parasitoids, the host, once localized, is recognized and accepted by means of a series of elaborate mechanisms that involve visual, chemical and mechanical cues (Doutt 1959; Vinson 1976, 1998; van Alphen and Vet 1986; Vet et al. 1992). Many times, the host has to be detected inside a stem, leaf or fruit. In some cases these hidden hosts can be located directly through infrared radiation (Richerson and Borden 1972) or vibrations produced by host movements (Lawrence 1981), or even through vibrations generated by the parasitoid that reveal quiescent hosts with a form of SONAR (Broad and Quike 2000). However, a searching female often needs to respond first to a number of indirect host cues, such as frass (Vet and Dicke 1992), visible plant damage (Faeth 1990), or localized chemical emissions (Steimberg et al. 1992; Tumlinson et al. 1993; Meyhofer et al. 1994; Potting et al. 1995; Ngi-Song et al. 1996). If a suitable egg, larva or pupa is detected, aculeus insertion through plant tissue and oviposition into the host takes place. The entire process of host location, detection and recognition is influenced by previous experience, rate of host encounter, type and condition of substrate (e.g., presence of host marking pheromones), or changes in the intensity of stimuli emitted by the host or plant on which the host is feeding (Bernstein and Driessen 1996).

Fruit fly (Diptera: Tephritidae) parasitoids follow the above steps through a number of adaptations. For example, the larval-prepupal koinobiont *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), uses chemical cues emitted, in part, by the fungi growing in infested fruit (Greany et al. 1977; Leyva et al. 1991; Messing and Jang 1992; Eben et al. 2000). After a female has landed on a fruit, it localizes larvae inside it through the vibrations they generate while moving and feeding (Lawrence 1981). Guimarães and Zucchi (2004) showed that females of the figitid *Aganaspis pelleranoi* (Brèthes) also use vibrotaxis to detect larvae while walking on the surface of a fruit. Earlier, Ovruski (1994a) working with the same species, reported that females are also able to penetrate the fruit and move within the pulp in search of larvae.

*A. pelleranoi* is often found together with another Figitid, *Odontosema anastrephae* Borgmeier. Both species are among the most common parasitoids attacking fruit fly larvae in the New World (Ovruski et al. 2000). In a recent cladistic analysis of the Eucoilinae (Fontal-Cazalla et al. 2002), *A. pelleranoi* and *O. anastrephae* were included in the "Neotropical grade", an unresolved group of unplaced Neotropical taxa. Both eucoiline species are part of a guild of solitary, koinobiont, endoparasitoids that attack third instar larvae of *Ceratitis capitata* Wiedemann and several species in the genus *Anastrepha* Schiner (Ovruski et al. 2000), and have also been recovered from Lonchaeidae (Wharton et al. 1998; Guimarães et al. 1999). They have been found associated with a wide variety of *Anastrepha* host plants in Mexico (Piedra et al. 1993; López et al. 1999; Sivinski et al. 2000), Costa Rica (Wharton et al. 1981; Wharton et al. 1998), Guatemala (Eskafi 1990), Brazil (Matrangolo et al. 1998; Guimarães et al. 1999, 2000, 2003, 2004, 2005), Bolivia (Wharton et al. 1998), Colombia (Yepés and Vélez 1989), and Argentina (Turica 1968; Ovruski 1994b; Ovruski 1995; Ovruski et al. 2004, 2005), but are most often collected in guavas (*Psidium guajava* L.). Even though they can be collected in infested fruit on the tree, they are most commonly found in fallen fruit on the ground (Sivinski et al. 1997, 2000; López et al. 1999; Ovruski et al. 2004). *O. anastrephae* in particular is almost always found parasitizing larvae in fallen fruit that are rarely intact. Immatures of both species can undergo long diapause periods (up to 11 months) under tropical conditions (Aluja et al. 1998) and live up to 33 d as adults under laboratory conditions (Gallegos-Chan 1999).

Our principal aim here was to compare the close-range host location and detection behaviors of *A. pelleranoi* and *O. anastrephae* confronted with four forms of guavas that might be encountered in the field: those with larvae and damaged skin, those with larvae and undamaged skin, and those without larvae and with damaged and undamaged skin. The rationale behind the latter design was that differences in host detection strategies might reflect differences in niche preferences and resource exploitation strategies, which in turn could explain how these related species are able to coexist.

## Materials and Methods

*Study Site and Insects* Experiments were conducted in the laboratories of the Fruit Fly Research Unit of the Instituto de Ecología, A.C. in Xalapa, Veracruz, México.

Environmental conditions were 26±1°C, 65±5 % RH, and a photoperiod of 12:12 h (L:D). Light came from fluorescent bulbs that illuminated the room with 600 lux. The *O. anastrephae* and *A. pelleranoi* specimens used throughout the study, stemmed from newly established colonies ($F_7$-$F_{10}$) with founders collected from infested guavas *Psidium guajava* in Central Veracruz, Mexico. *Anastrepha ludens* (Loew) (Diptera: Tephritidae) third-instar larvae used as parasitoid hosts, stemmed from a colony kept under laboratory conditions and reared in artificial diet. Parasitoid and fly rearing techniques are described in Aluja et al. (2009).

*Experimental Arena* All observations were made using 15×15×15 cm Plexiglas cages containing a single guava in the centers of their floors. Observers were stationed on opposite ends. Guavas were of four types: 1) artificially infested, mechanically punctured, 2) artificially infested, not punctured, 3) clean (non-infested) and punctured, 4) clean, not punctured.

All the guavas used were bought at a local supermarket, were ¾ ripe (80% yellow, 20% green) and weight on average 38±3 g ($n$=120). To artificially infest a guava, we cut the upper part transversally along the peduncle, about ¼ down from proximal end. These sections functioned as "lids" and the remainder of the fruit served as "bases" for filling. Pulp was extracted in the bases to create cavities that could be filled with larvae and diet. We inserted 20, third-instar (7–9 d), lab-reared *A. ludens* larvae mixed with some of the diet they had been reared in (details in Aluja et al. 2009). After filling, the lid was placed on top of the base, and sealed with melted wax. A 5 mm dia metal probe was used to create four equidistant equatorial punctures that simulated larval respiration and exit holes (Aluja et al. 2009). Diet was needed because otherwise the larvae would immediately crawl out of the fruit through the artificial punctures. Puncturing occurred a few minutes prior to bioassay initiation. Non infested, punctured fruit were treated in the same way as infested ones, but only diet was used to fill them.

*Bioassay* Every morning, immediately before the observation period began, we introduced single guavas into the cages. We simultaneously observed eight cages so that both parasitoids and all four guava types were represented in every period. A single, 3–8 d old, mated *A. pelleranoi* or *O. anastrephae* female was gently captured using a glass vial and transferred it to the observation cage where it was released on the cage floor at a predetermined release point. Females were *naïve*, i.e., they had no previous oviposition experience or contact with guavas or fruit fly larvae. They stemmed from cages where aproximately 100 females and 80 males were kept until the moment of emergence (fed with water and honey *ad libitum*). Cage position on the observation table was assigned randomly every morning. Observations were started at 0800 h and ended at 1600 h. A total of 15 replicates were run for each of the eight treatments (two species × four fruit conditions). For each observation, a new guava and a new parasitoid female were always used.

Observations started as soon as a female was released into the cage and ended 1) after the pre-established 8 h cut-off time was reached, 2) if a female left (by walking or flying) the test guava before the 8 h cut-off time had been reached or 3) if a female walked or flew to a fruit, started to forage but then stood motionless for a continuous period of 6 h. Data obtained under scenario 3 were not considered for

analysis and the female was replaced. If a female did not walk or fly to the fruit within 30 min after having been released into the cage, the test was also called invalid, the female discarded and replaced by another. With the help of a handheld stopwatch and data sheets, we recorded the sequence and the duration of the eleven types of behaviors exhibited by females while on the surface of guavas. The operational definitions of these behaviors are provided in Table 1. Based on this information, we then calculated latency before arrival on fruit after release, fruit residency time, time spent inside fruit (out of sight of observer), and time spent ovipositing and detecting (i.e., detection of larvae in fruit by means of tarsal or antennal receptors [or possibly receptors on the aculeus]). We note that we felt justified to consider the "tarsal detection behavior" as such a phenomenon had been

**Table 1** Operational Definitions of the Various Behaviors Exhibited by *A. pelleranoi* y *O. anastrephae* Females while on the Surface of a Guava which could be of Four Types: Infested with and without Punctures and Uninfested with and without Punctures

| Behaviors | Description |
|---|---|
| 1) Walking on fruit (W) | Female walks on fruit with the antennae moving back and forth but never touching fruit surface. |
| 2) Antennation while walking (AW) | Contact of fruit surface with the apex of both antennae while female walks on the surface of fruit. During antennation, the flagellum is moved rhythmically downward and upward in front of the females head with the apex touching the fruit surface. |
| 3) Antennation while rotating body (AR) | Contact of fruit surface with the apex of both antennae while female turns 360 degrees but with no forward, backward or lateral displacement. Turns maintain female in same location. |
| 4) Detection using tarsi (DT) | Female stands still on surface of fruit. Only contact with surface is via the tarsi. Antennae are held in a straight, forward position. |
| 5) Probing with aculeus (PA) | Partial insertion of aculeus through skin of fruit (ca. 50% of total aculeus length is inserted) for periods under one minute. The anntenae remain in a straight position, slightly raised. |
| 6) Perforation with mandibles (PM) | Female uses mandibles to create an opening in fruit (hole) through which she can reach the pulp in search of larvae. |
| 7) Entering into and remaining inside fruit (EnF) | Female enters fruit through holes she created or through already existing cavities. We were not able to ascertain what females did once inside fruit. |
| 8) Exit from fruit (ExF) | Exit from fruit after period inside it. Hole used to enter fruit not always same used to exit it. |
| 9) Oviposition (O) | Total insertion of aculeus through skin of fruit for periods that lasted over a minute. |
| 10) Cleaning (C) | Preening (cleaning) wings and aculeus with hind legs, antennae with front legs and the latter with mouth parts. |
| 11) Resting (R) | Female stays totally immobile on surface of fruit for periods over 10 minutes. |

We note, that the "perforation with mandible" behavior was only recorded in the infested fruit, mainly in the infested without puncture type

previously described/quantified by Vet and Bakker (1985) in the case of eight eucoilid species attacking *Drosophila* larvae.

*Statistical and Behavioral Sequence Analyses* Information on total fruit residency time and latency before arrival to fruit was analyzed through a three-way ANOVA, and means separated with a Tukey honestly significant difference (HSD) test ($P<0.05$) (Stat-Soft 1995). Time spent detecting with the antennae was analyzed by means of a three-way ANOVA using a type III SS regression for unequal sample sizes. Time spent detecting with the tarsi and the aculeus, as well as residence time inside fruit were analyzed by means of a two-way ANOVA also using a type III SS regression. Then means were separated with a Tukey HSD test for unequal sample size ($P<0.05$) (Stat-Soft 1995). Comparison of fruit perforation time (biting into interior of fruit) by *O. anastrephae* and *A. pelleranoi* females in unperforated, infested fruit and length of oviposition bouts by *A. pelleranoi* females in perforated and unperforated, infested fruit were analyzed using a *t*-test ($P<0.05$) (Stat-Soft 1995). Given lack of normality, data were rank transformed prior to being analyzed (Conover and Iman 1981), but untransformed means (± SD) were used in all tables and figures. If sample size (n) (i.e., number of events of a particular behavior) was lower than 10, data were not subjected to a formal analysis (ANOVA). In these cases, the information is presented to the reader as mean values (± SD).

Transition frequencies of one behavior to another were first tabulated and then consolidated into a transition probability matrix using only first-order, preceeding-following behavioral transitions (Lehner 1996). For each *A. pelleranoi* and *O. anastrephae* individual (15 females per treatment), a transition matrix was built. Furthermore, for each treatment, another global transition matrix was built by summing all values obtained from the 15 females (i.e., overall frequency of each behavioral transition per treatment). Self-transitions (i.e., repetitions of the same behavior) were not included in these matrices. Using overall transition matrices, we selected the ten most frequent transitions per treatment to compare observed versus expected values in individual matrices (i.e., per female basis) by means of a Wilcoxon Matched Pairs test at $P=0.005$ (Stat-Soft 1995). Expected transition frequencies were calculated as follows: *Expected = (row sum × column sum) / (grand sum)* (Slater and Ollason 1972). To determine whether significant differences varied between species and fruit condition (e.g., infested and punctured vs infested unpunctured), a three-way ANOVA was run. Means were separated with a Tukey HSD test ($P<0.05$) (Stat-Soft 1995). Prior to analysis, data were rank transformed since they were not normally distributed. Nevertheless, in Table 4b untransformed values are presented. Finally, we generated a behavioral flow chart for each parasitoid species considering only the one treatment in which the most behavioral transitions were recorded.

We note, that we did not apply commonly used sequence analysis methods such as Pearson's Chi-Square analysis and related statistics (Lehner 1996), because as aptly noted by Kramer and Schmidhammer (1992) these tests are not appropriate for all frequency-type, ethological data. These authors showed that lack of independence between observations, such as the number of times a particular behavior is exhibited (many times repeatedly), can lead to an artificially inflated or deflated chi-square statistic. Based on the latter, in this study, we used a Wilcoxon Matched Pairs test.

Because the significance level was set at $P=0.005$ (details in Kramer and Schmidhammer 1992), many transitions which would have otherwise been found to be significant were left out.

## Results

We first describe general patterns such as time to arrival on fruit, fruit residency time (surface), time spent inside fruit searching for larvae, time spent detecting larvae by using the antennae, tarsi and aculeus, time spent biting into fruit to penetrate it and time spent ovipositing through the epicarp. All interactions resulting from the two- and three-way ANOVA`s are reported in Table 2. The results of the post hoc multiple mean comparisons (Tukey HSD test, $P<0.05$), are reported directly in Figs. 1, 2, 3, 4, 5 and 6. We also describe behavioral sequences exhibited by both *A. pelleranoi* and *O. anastrephae* while searching for larvae. Such sequences were built with the 11 clearly distinguishable behaviors we identified during the process of searching for a host (i.e., larvae) by females of both parasitoid species (Table 1).

*Time to Arrival on Fruit* In general, the time between release into the cage and arrival on all types of fruit differed significantly between the two parasitoid species (Table 2, Fig. 1). Overall (i.e., independent of fruit condition), *O. anastrephae* females took twice as long as *A. pelleranoi* females (68.8±78.1 vs 32.1±61.8 min). There were significant interactions between species and both puncture and non-puncture fruit condition, and infested and non-infested fruit condition (Table 2). In the case of *A. pelleranoi*, females reached punctured fruit 5.2 times faster than unpuctured fruit (9.6±13.9 vs 51.5±81.6 min) and 1.3 times faster infested vs non infested fruit (26.5±40.0 vs 34.6±53.4 min). In contrast to this, *O. anastrephae* females reached unpunctured fruit 1.2 times faster than punctured fruit (63.8± 76.5 min vs 73.8±60.1) and noninfested fruit 2.4 times faster than infested (41.1± 40.2 vs 96.5±95.9 min). In a multiple comparison (Tukey HSD test) considering all treatments, significant differences were only found between species in the case of infested, punctured fruit (Fig. 1). Under these conditions, *A. pelleranoi* females reach the fruit 30 times faster than *O. anastrephae* (3.6±3.5 vs 106.5±77.2 min).

*Total Residence Time on Fruit A. pelleranoi* females remained significantly longer on the fruit than *O. anastrephae* females (155.2±132.3 vs 78.6±101.3 min) (Table 2). In both species, we found that fruit residence time was significantly influenced by the presence of larvae (Table 2). Females of both species remained approximately three times as long on infested vs non infested fruit (229.4±136.9 vs 80.9±77.2 and 121.4±108.4 vs 35.8±50.9 min for *A. pelleranoi* and *O. anastrephae*, respectively). Presence or absence of punctures in infested fruit had no significant influence on fruit residence time in either species (Table 2). We found a significant interaction between the three independent variables (species, punctured vs unpunctured fruit and infested vs non infested fruit) and fruit residence time (Table 2). Independent of presence or absence of punctures in fruit, *A. pelleranoi* females spent significantly more time on infested vs non infested fruit (Fig. 2). In the

**Table 2** Results of Two and Three-way Anova's Run to Determine Significant Differences in Behavioral Patterns Exhibited when *A. pelleranoi* and *O. anastrephae* Females were Exposed to Two Fruit Conditions Between Treatments

| Factors and their interactions | Behavioral Pattern | | | | | |
|---|---|---|---|---|---|---|
| | Time to arrival on fruit | Residence time on fruit | Time spent inside fruit | Time spent detecting with tarsi | Time spent detecting with aculeus | Time spent detecting witn antennae |
| 1 | $F(1,112)=24.57$ | $F(1,112)=27.57$ | $F(1,192)=5.05$ | $F(1,238)=60.66$ | $F(1,168)=49.42$ | $F(1,597)=82.59$ |
| | **$P<0.001$** | **$P<0.001$** | **$P=0.025$** | **$P<0.001$** | **$P<0.001$** | **$P<0.001$** |
| 2 | $F(1,112)=1.11$ | $F(1,112)=0.19$ | $F(1,192)=1.69$ | $F(1,238)=0.98$ | $F(1,168)=0.49$ | $F(1,597)=0.09$ |
| | $P=0.294$ | $P=0.664$ | $P=0.194$ | $P=0.323$ | $P=0.482$ | $P=0.767$ |
| 3 | $F(1,112)=0.92$ | $F(1,112)=41.41$ | ——— | ——— | ——— | $F(1,597)=10.12$ |
| | $P=0.340$ | **$P<0.001$** | | | | **$P<0.001$** |
| 1×2 | $F(1,112)=8.56$ | $F(1,112)=0.02$ | $F(1,192)=0.01$ | $F(1,238)=0.16$ | $F(1,168)=0.17$ | $F(1,597)=4.84$ |
| | **$P=0.004$** | $P=0.886$ | $P=0.935$ | $P=0.692$ | $P=0.679$ | **$P=0.028$** |
| 1×3 | $F(1,112)=7.73$ | $F(1,112)=0.05$ | ——— | ——— | ——— | $F(1,597)=4.20$ |
| | **$P=0.006$** | $P=0.821$ | | | | **$P=0.041$** |
| 2×3 | $F(1,112)=0.26$ | $F(1,112)=2.01$ | ——— | ——— | ——— | $F(1,597)=52.01$ |
| | $P=0.614$ | $P=0.158$ | | | | **$P<0.001$** |
| 1×2 ×3 | $F(1,112)=1.73$ | $F(1,112)=7.62$ | ——— | ——— | ——— | $F(1,597)=45.30$ |
| | $P=0.191$ | **$P=0.006$** | | | | **$P<0.001$** |

The interaction analysis considered the following factors: 1=parasitoid species, 2=punctured vs unpunctured fruit; 3=infested vs uninfested fruit. Significant probabilities are denoted using bold characters.

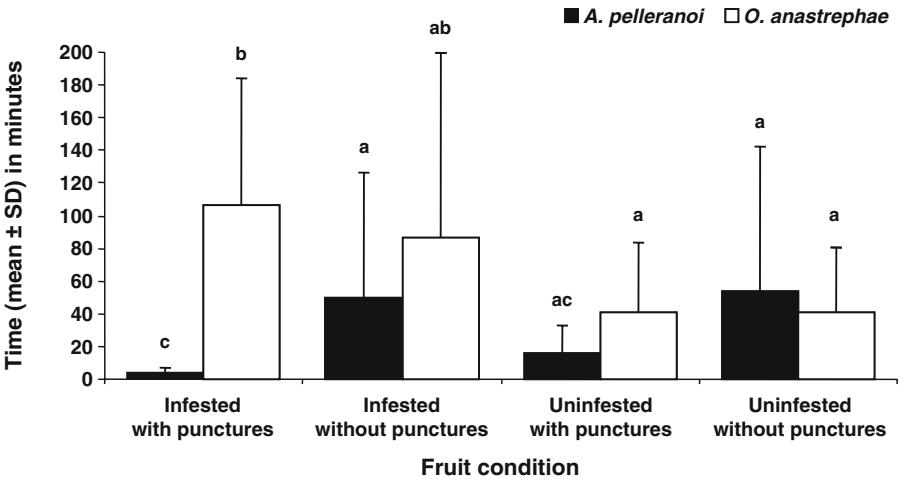


**Fig. 1** Mean time (in min) spent by female *A. pelleranoi* and *O. anastrephae* before arrival on *Psidium guajava* fruit of four different conditions ($n$=15 females per condition). Bars (mean ± SD) with different lower case letters are statistically significant at $P$<0.05 (Tukey HSD test).

case of *O. anastrephae* females, their tenure was statistically similar to that of *A. pelleranoi* only on infested fruit with punctures (Fig. 2).

*Time Spent Inside Fruit* One hundred and 67% of *A. pelleranoi* and *O. anastrephae* females, respectively, penetrated the infested, punctured fruit, presumably in search of the larvae inside. Mean ± SD time spent inside a fruit is graphically represented in Fig. 3. Considering repeated measures on the same individual, we observed a total of 127 and 27 penetration events for *A. pelleranoi* and *O. anastrephae*, respectively, in

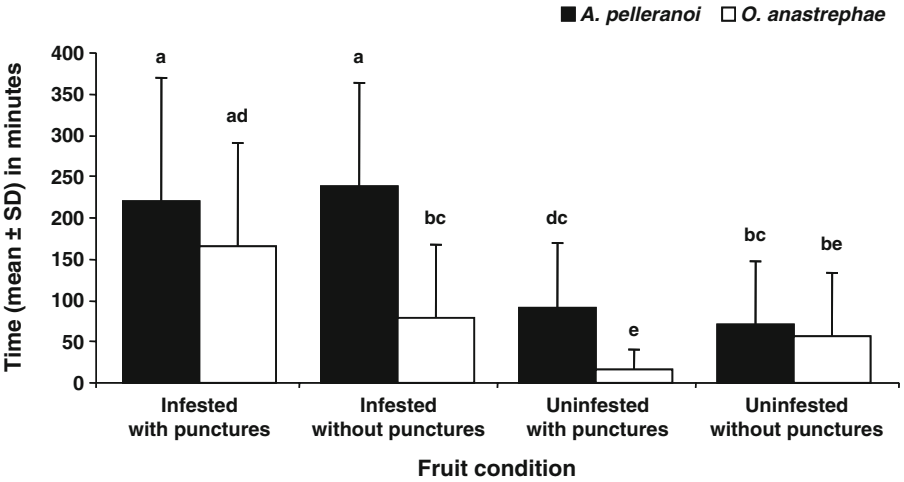


**Fig. 2** Mean time (in min) spent by female *A. pelleranoi* and *O. anastrephae* on *Psidium guajava* fruit of four different conditions ($n$=15 females per condition). Bars (mean ± SD) with different lower case letters are statistically significant at $P$<0.05 (Tukey HSD test).

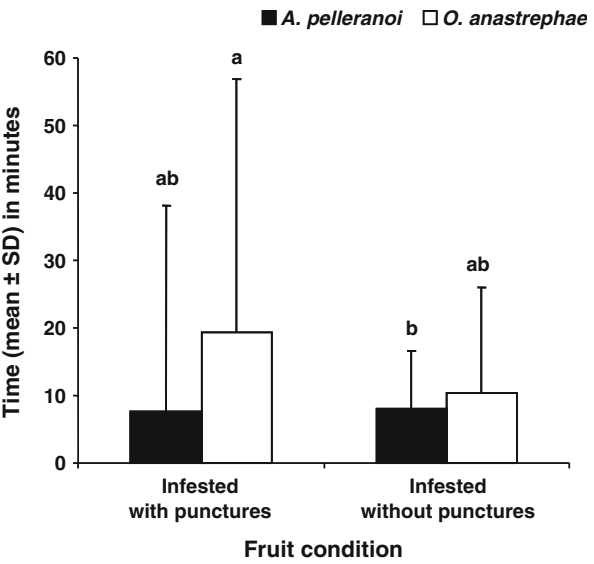


**Fig. 3** Mean time (in min) spent by female *A. pelleranoi* and *O. anastrephae* inside of *Psidium guajava* fruit of two different conditions ($n$=127 and 31 in case of *A. pelleranoi* for infested, punctured fruit and infested, intact fruit (without punctures), respectively; $n$=27 and 11 in case of *O. anastrephae* for infested, punctured fruit and infested, intact fruit, respectively). Bars (mean ± SD) with different lower case letters are statistically significant at $P<0.05$ (Tukey HSD for unequal N test).

infested fruit with punctures. Similarly, 93 and 53% of females (same order as before) penetrated the fruit if they were non-infested but had punctures. In these cases, events lasted 11.3±44.4 and 1.2±0.6 min for *A. pelleranoi* and *O. anastrephae*, respectively ($n$=32 and 7, respectively). Interestingly, seven and four *A. pelleranoi* and *O. anastrephae* females, respectively, penetrated infested, unpunctured fruit (considering repeated measures on same individual, 31 and 11 events, respectively). In these cases, females would start biting off the skin and pulp, until a cavity was formed through which they could enter the interior part of the fruit filled with larvae and pulp. No such activity was ever observed in the case of non

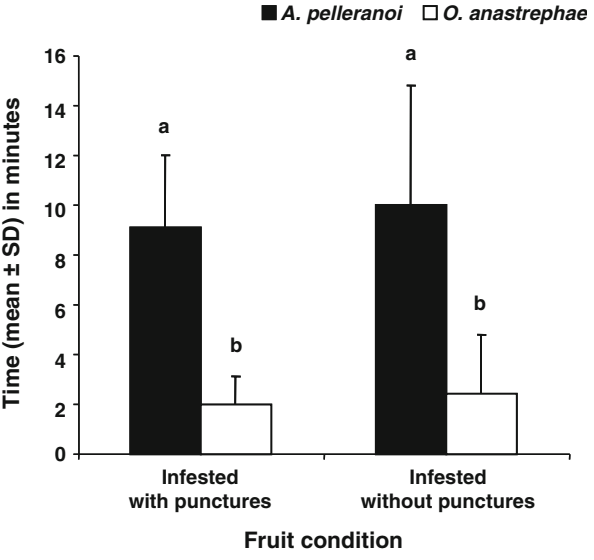


**Fig. 4** Mean time (in min) spent by female *A. pelleranoi* and *O. anastrephae* detecting larvae with tarsi in *Psidium guajava* fruit of two different conditions ($n$=136 and 81 in case of *A. pelleranoi* for infested, punctured fruit and infested, intact fruit (without punctures), respectively; $n$=14 and 11 in case of *O. anastrephae* for infested, punctured fruit and infested, intact fruit, respectively). Bars (mean ± SD) with different lower case letters are statistically significant at $P<0.05$ (Tukey HSD for unequal N test).

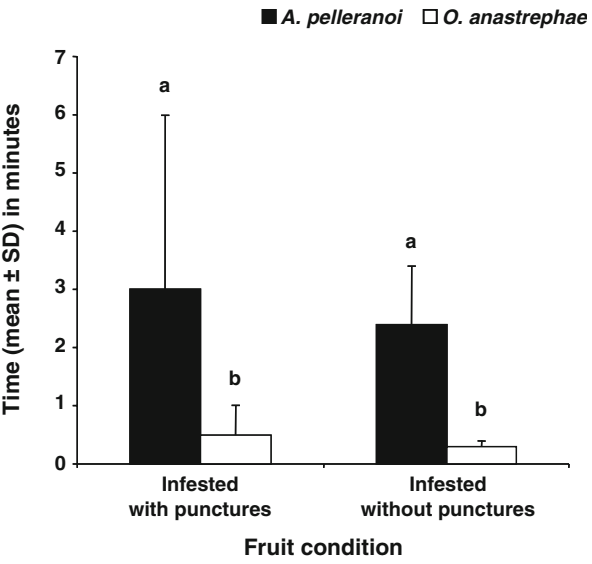


**Fig. 5** Mean time (in min) spent by female *A. pelleranoi* and *O. anastrephae* detecting larvae with ovipositor in *Psidium guajava* fruit of two different conditions ($n$=53 and 74 in case of *A. pelleranoi* for infested, punctured fruit and infested, intact fruit, respectively); $n$=11 and 34 in case of *O. anastrephae* for infested, punctured fruit and infested, intact fruit, respectively. Bars (mean ± SD) with different lower case letters are statistically significant at $P<0.05$ (Tukey HSD for unequal N test).

infested, unpunctured fruit. Females of both species spent double the amount of time inside infested, punctured fruit than in clean, punctured fruit (13.5±34.1 vs 6.3± 21.5 min, respectively). *O. anastrephae* females spent significantly more time (2.4-fold difference) inside infested fruit without holes (females penetrated them by creating an entrance hole themselves) than *A. pelleranoi* females did (Table 2,

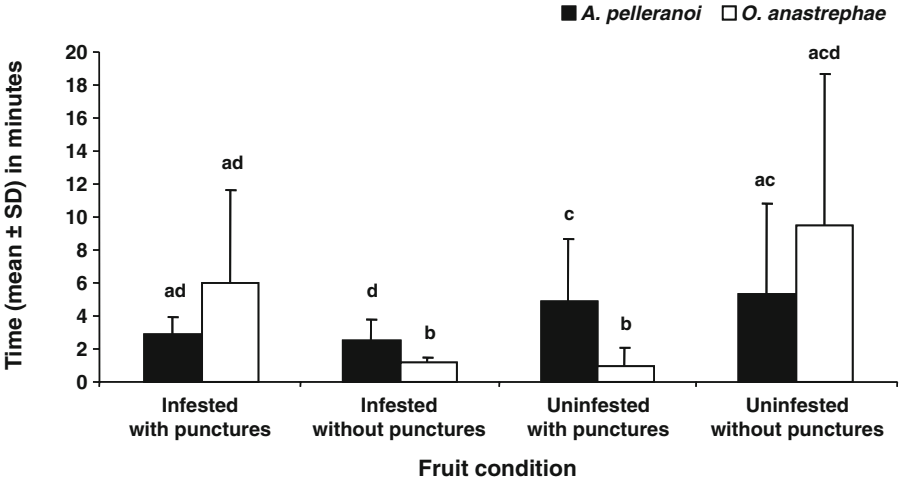


**Fig. 6** Mean time (in min) spent by female *A. pelleranoi* and *O. anastrephae* detecting larvae with antennae in *Psidium guajava* fruit of four different conditions ($n$=113, 213, 68, and 78 in case of *A. pelleranoi* for 1) infested, punctured fruit, 2) infested, intact fruit, 3) clean (uninfested), punctured fruit and 4) clean, intact fruit (without punctures), respectively; $n$=33, 71, 12, and 17 in case of *O. anastrephae* for infested, punctured fruit, infested, intact fruit, clean (uninfested), punctured fruit and clean, intact fruit, respectively). Bars (mean±SD) with different lower case letters are statistically significant at $P<0.05$ (Tukey HSD test).

Fig. 3). In the case of infested fruit that were already punctured, no statistically difference was found when comparing both species (Fig. 3).

*Time Spent Detecting with the Tarsi* Sixty and 53% ($n$=15), respectively, of the females of both species used their tarsi to detect the presence of larvae inside infested, unpunctured fruit (considering repeated measures on same individuals, we observed a total of 136 and 14 such events in *A. pelleranoi* and *O. anastrephae*, respectively). Similarly, 60 and 33% ($n$=15) of the females of the latter species, exhibited tarsal detection behavior in infested, punctured fruit, respectively (81 and 11 events, respectively). In sharp contrast, only 27 and 20% ($n$=15) of the *A. pelleranoi* and *O. anastrephae* females, respectively, exhibited such behavior when walking on the surface of a non infested, unpunctured fruit (eight and four events, respectively). In the case of non infested, punctured fruit 93 and 40% ($n$=15) of *A. pelleranoi* and *O. anastrephae* females, respectively, exhibited tarsal detection behavior (25 and ten events, respectively).

Independent of fruit puncture condition, *A. pelleranoi* females spent significantly more time than did *O. anastrephae* females detecting with their tarsi in infested fruit (9.8±4.3 vs 2.2±1.9 min, respectively) (Table 2, Fig. 4). Nevertheless, the total time spent exhibiting such a behavior was not significantly influenced by the presence or absence of punctures in infested fruit (Table 2, Fig. 4). When comparing female response to both fruit conditions at the species level, we found that *A. pelleranoi* females spent significantly more time detecting with their tarsi in infested punctured fruit, than in non infested, punctured fruit ($t$=7.68, df=104, $P$<0.0001). No significant differences were found in the case of *O. anastrephae* ($t$=0.93, df=9, $P$=0.375).

*Time Spent Detecting with the Aculeus O. anastrephae* females exhibited a lower tendency than *A. pelleranoi* to detect host larvae with the aculeus (13 vs 38%, respectively, $n$=60). Many *A. pelleranoi* females exhibited host detection behavior with the aculeus ("probing") in infested fruit with or without holes (67 and 53%, respectively, $n$=15) while only a few did so in non infested fruit with or without holes (20 and 13%, respectively, $n$=15). The total number of "probing" events recorded under the latter two conditions, were 53 vs 74 and 14 vs 7, respectively. *O. anastrephae* nearly always exhibited ovipositor probing in infested fruit (only one event that lasted 0.5 min was observed in non infested fruit). The difference in "probing" time in infested fruit between *A. pelleranoi* and *O. anastrephae* females was seven-fold and statistically significant (2.7±2.0 vs 0.4±0.3 min, Table 2). But the latter behavior was not influenced in neither of the two species by the presence or lack of holes in the fruit (Table 2, Fig. 5). *A. pelleranoi* females spent significantly more time detecting with the aculeus in non infested, punctured fruit than in infested punctured fruit ($t$=6.07, df=86, $P$<0.0001; $n$=53 in infested punctured fruit and $n$= 14 in non infested punctured fruit).

*Time Spent Detecting with the Antennae* Similar numbers of *A. pelleranoi* females were observed detecting with antennae in the four fruit conditions: nine females (60%, $n$=15) with 113 events in infested punctured fruit, ten females (67%, $n$=15) with 68 events in non infested punctured fruit, 12 females (80%, $n$=15) with 213

and 78 events in infested unpunctured fruit and in non infested unpunctured fruit, respectively. In the case of *O. anastrephae*, we observed four (27%, $n$ =15) and five (33%, $n$=15) females performing 33 and 71 events, in infested fruit with and without holes, respectively, and eight (53%, $n$=15) and seven (47%, $n$=15) females with 12 and 17 events in non infested fruit with and without holes, respectively. Formal analyses showed that there are statistically significant differences when comparing both parasitoid species with respect to the variable "time spent detecting with the antennae" (Table 2). In particular, differences were found only in the case of two treatments: infested fruit without punctures and uninfested fruit with punctures. In both treatments *A. pelleranoi* use their antennae more time than *O. anastrephae* (Fig. 6). Interestingly, *A. pelleranoi* females spent significantly more time detecting with antennae on non infested fruit than on fruit with larvae (5.1±4.7 vs 2.3± 1.2 min) (Table 2, Fig. 6), whereas in *O. anastrephae* no significant differences were found (5.3±5.2 vs 3.7±3.9 min) (Fig. 6).

*Fruit Puncturing Behavior by Parasitoid Females* A similar number (eight and seven respectively) of *A. pelleranoi* and *O. anastrephae* females were observed puncturing (i.e., biting into) infested fruit. Nevertheless, *A. pelleranoi* females only punctured fruit without holes (mean duration of each event: 3.6±2.5 min, $n$=27). In the case of *O. anastrephae*, 71.4% ($n$=5) of the females punctured infested fruit without holes (mean duration of each event: 1.5±0.5 min, $n$=22), and 28.6% ($n$=2) did so in infested fruit with holes (mean duration of each event: 0.5±0.4 min.). There was no significant difference when comparing both species with respect to puncturing duration in the case of non infested, already punctured fruit ($t$=1.34, df=47, $P$=0.187). Attempts at puncturing non infested, unpunctured fruit were never recorded.

*Egg-laying Activity* Larvae were not dissected to ascertain if they had eggs inside them. Nevertheless, the females we recorded as laying eggs went through all the motions typical of egg-laying behavior in these insects (Table 1). Overall (i.e., considering both infested fruit conditions) and the fact that we could only record oviposition behavior on the surface of fruit (once females started to forage inside fruit we could not record their behavior), *A. pelleranoi* females oviposited qualitatively more often that did *O. anastrephae* females (a formal statistical analysis comparing both species was not possible because oviposition behavior outside fruit by *O. anastrephae*, independent of treatment, was rare [$n$=7 including repeated measures on same individual]). For example, in infested fruit that had been previously punctured, 73% of all *A. pelleranoi* females observed ($n$=15) exhibited oviposition-type behavior (operational definitions in Table 1). Taking into account repeated measures by the same individual, we recorded a total of 87 oviposition events that lasted 2.6±1.3 min. In sharp contrast, only one *O. anastrephae* female (6.7%, $n$=15) oviposited on the surface of infested, punctured fruit (bout lasted 3.9 min). In the case of infested, unpunctured fruit, all *A. pelleranoi* females exhibited oviposition behavior ($n$=15; considering repeated measures, a total of 224 oviposition events were recorded that lasted on average 3.1±1.6 min). Again, in sharp contrast, only four *O. anastrephae* females exhibited oviposition behavior (27%, $n$=15; total of six oviposition events considering repeated measures on same

individual), with a mean duration of 2.8±1.2 min. Time employed by *A. pelleranoi* females to oviposit in infested, unpunctured fruit was significantly higher than in punctured fruit ($t$==2.10, df=329, $P$=0.040).

*Analysis of Behavioral Sequences while Females were Foraging on Fruit* Operational definitions of the types of behaviors we considered for formal analysis are provided in Table 1. Behaviors considered were: walking on fruit surface (W), detection using antennae while walking (AW) or by rotating body in same place (AR), detection with tarsi (DT), probing with partial insertion of aculeus (PA), oviposition (O), perforation of fruit (PM), entrance into (EnF) and exit (ExF) from fruit, cleaning (C) and resting on fruit surface (R).

The number of preceding = following behavioral transitions recorded varied significantly among treatments (one way ANOVA, $F$=27.82; df=7, 112; $P$<0.0001). As shown in Fig. 7, the most transitions (46.5% of the total) were observed in infested, unpunctured fruit (36.4% in infested punctured fruit, 9.5% in non-infested punctured fruit, and 7.6% in non-infested unpunctured fruit), even though many did not occur significantly more often than expected by chance alone (details follow) (Tukey HSD test). Considering the fact that females of both parasitoid species exhibited the richest behavioral repertoire in infested, unpunctured fruit (i.e., all behaviors described in Table 1 were recorded, among them "Perforation" [PM]), we selected this treatment to illustrate patterns of behavior by females of both species. Once females reached the fruit after having been released in the experimental arena, most of them (80 and 60%, in the cases of *A. pelleranoi* and *O. anastrephae*, respectively) walked on the fruit surface before engaging in another behavior (Figs. 8a and 8b). Comparing both species, *O. anastrephae* females tended to leave the fruit more often and sooner (Figs. 2, 8a and 8b). The latter pattern is clearly illustrated when looking at the proportion of females that left the fruit before exhibiting between one and three behavioral transitions (of any type). In the case of

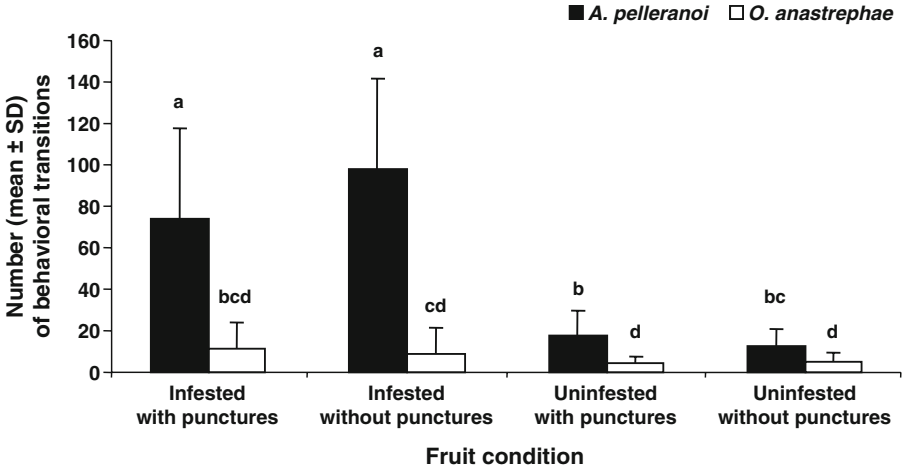


**Fig. 7** Mean number of behavioral transitions exhibited by *A. pelleranoi* and *O. anastrephae* in *Psidium guajava* fruit of four different conditions (i.e., infested, punctured fruit, infested, intact fruit, clean (uninfested), punctured fruit and clean, intact fruit, respectively).

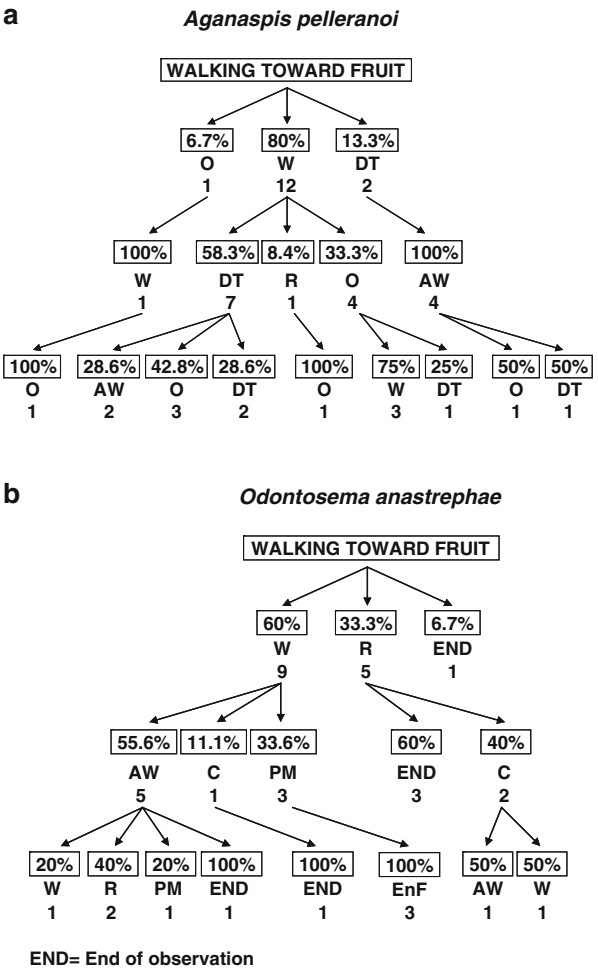


**Fig. 8** Proportion of behavioral transitions exibited by *A. pelleranoi* (**a**) and *O. anastrephae* (**b**) females after having been released in the experimental arena containing an infested guava without punctures.

infested fruit (with and without punctures), 100% of the *A. pelleranoi* females observed (total of 30 individuals [15 per treatment]) remained on the fruit, while only 47 and 40% of *O. anastrephae* females (infested fruit with and without punctures, respectively), left the fruit after exhibiting one to three behavioral transitions (Fig. 9). To provide the reader with a broad picture of the types of behavioral transitions exhibited by the females of both species of parasitoids and also illustrate the overall flow of events, we built behavioral transition matrices in which observed and expected values are represented (Tables 3 and 4) and a behavioral flow chart using only transitions that occurred at a frequency>0.1 (Figs. 10a and 10b). The latter, also illustrated behavioral transitions on infested, unpunctured fruit conditions because that was the treatment where most perforation activity was observed.

Using overall transition matrices (i.e., overall frequency of each behavioral transition per treatment calculated by summing up all values obtained from the 15

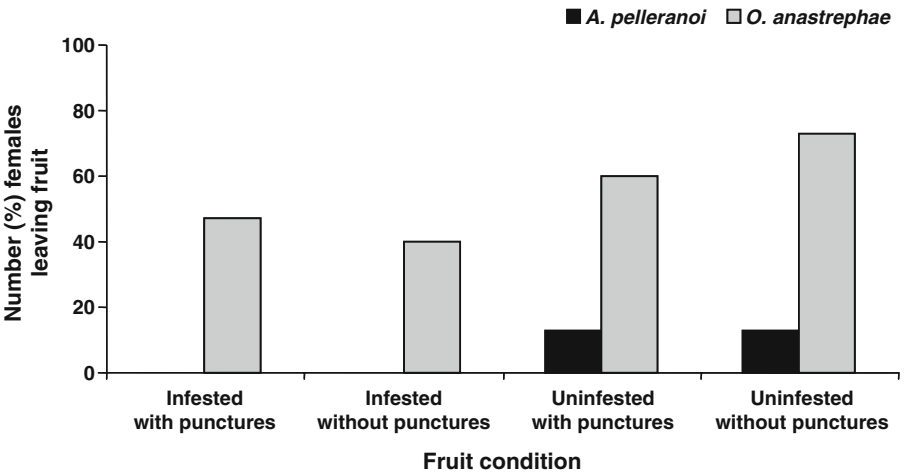


**Fig. 9** Proportion of *A. pelleranoi* and *O. anastrephae* females that left the fruit (guava) before exhibiting between one and three behavioral transitions.

replicates [females] per treatment), we determined which of the first order sequences occurred significantly more often than expected by chance alone (Wilcoxon Matched Pairs test, $P$=0.005). In the case of *A. pelleranoi*, results look as follows: 1) infested, punctured fruit, C followed by W (C-W) ($T$=0.0, $Z$=2.93, $P$=0.003, $n$=15), W-EnF ($T$=6.0, $Z$=3.07, $P$=0.002, $n$=15), ExF- C ($T$=0.0, $Z$=3.18, $P$=0.001, $n$=15), and EnF-ExF ($T$=0.0, $Z$=3.40, $P$=0.0006, $n$=15); 2) infested unpunctured fruit, W-DT ($T$=0.0, $Z$=2.80, $P$=0.005, $n$=15); 3) non-infested punctured fruit, W-EnF ($T$=2.0, $Z$=3.17, $P$=0.001, $n$=15) and EnF=ExF ($T$=1.0, $Z$=3.23, $P$=0.001, $n$=15); 4) non-infested unpunctured fruit, AW-W ($T$=1.0, $Z$=2.84, $P$=0.004, $n$=15) and W-AW ($T$=0.0, $Z$=2.93, $P$=0.003, $n$=15).

If the probability level was relaxed to $P$=0.05, then the following new first-order behavioral transitions occurred significantly more often than expected by chance in *A. pelleranoi*: 1) infested punctured fruit, W-DT ($T$=0.0, $Z$=2.66, $P$=0.007, $n$=15), DT-W ($T$=2.0, $Z$=2.59, $P$=0.009, $n$=15), W-AW ($T$=6.0, $Z$=1.95, $P$=0.05, $n$=15), O-W ($T$=10.0, $Z$=2.93, $P$=0.04, $n$=15); 2) infested unpunctured fruit, DT-W ($T$= 7.0, $Z$=2.09, $P$=0.03, $n$=15), $W$ = DA ($T$=13.0, $Z$=2.27, $P$=0.02, $n$=15), DA-W ($T$=11.0, $Z$=2.19, $P$=0.02, $n$=15), DA-O ($T$=10.0, $Z$=2.27, $P$=0.02, $n$=15), O-W ($T$=15.5, $Z$=2.52, $P$=0.01, $n$=15); and 3) non-infested punctured fruit, DA=W ($T$= 1.0, $Z$=2.19, $P$=0.02, $n$=15), W-DA ($T$=6.0, $Z$=2.19, $P$=0.02, $n$=15), $W$ = DT ($T$= 0.0, $Z$=2.02, $P$=0.04, $n$=15), ExF-W ($T$=2.0, $Z$=3.17, $P$=0.02, $n$=15).

Based on the above, the following significant transitions were recorded for every treatment: 1) infested punctured fruit, W → EnF → ExF → C→ W → DT → (W → AW) or (W → O); 2) infested unpunctured fruit, W → DT → W→ AW → O → W; 3) non-infested punctured fruit, W → EnF → ExF → W→ AW → W → DT; 4) non-infested unpunctured fruit, W → AW → W.

In the case of *O. anastrephae*, no first-order behavioral transition occurred significantly more often than expected by chance. However, we note that the latter is the result of the stringent $P$ value used ($P$=0.005, Kramer and Schmidhammer

**Table 3** Behavioral Transition Matrix for *A. pelleranoi* on Infested Fruit without Punctures

| Preceeding Behavior | Following behavior[a] | | | | | | | | | | | Row sum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W | AW | PA | DT | AR | C | R | PM | O | EnF | ExF | |
| W | — | 150 (75.3) | 27 (19.4) | 76 (44.2) | 0 (0.35 | 15 (17.3) | 2 (1.7 | 19 (10.0) | 91 (68.1) | 6 (7.6) | 0 (8.6) | 386 |
| AW | 150 (77.1) | — | 6 (11.2) | 5 (25.6) | 0 (0.2) | 1 (2.4) | 1 (1.0) | 2 (5.8) | 57 (39.3) | 1 (4.4) | 0 (4.9) | 223 |
| PA | 30 (18.7) | 7 (10.5) | — | 5 (6.2) | 0 (0.05) | 0 (2.4) | 0 (0.2) | 1 (1.4) | 10 (9.5) | 1 (1.1) | 0 (1.2) | 54 |
| DT | 63 (44.2) | 10 (24.9) | 7 (6.4) | — | 0 (0.1) | 11 (5.7) | 0 (0.6) | 3 (3.3) | 31 (22.6) | 3 (2.5) | 0 (2.9) | 128 |
| AR | 0 (0.3) | 0 (0.2) | 0 (0.05) | 0 (0.1) | — | 0 (0.04) | 0 (0.0) | 1 (0.03) | 0 (0.2) | 0 (0.02) | 0 (0.02) | 1 |
| C | 28 (15.5) | 2 (8.8) | 5 (2.2) | 6 (5.2) | 0 (0.04) | — | 1 (0.2) | 0 (1.2) | 3 (7.9) | 0 (0.9) | 0 (1.0) | 45 |
| R | 0 (0.3) | 0 (0.2) | 0 (0.05) | 0 (0.1) | 0 (0.0) | 0 (0.04) | — | 0 (0.03) | 1 (0.2) | 0 (0.02) | 0 (0.02) | 1 |
| PM | 10 (8.9) | 0 (5.1) | 1 (1.3) | 2 (2.9) | 0 (0.02) | 2 (1.2) | 0 (0.1) | — | 1 (4.6) | 10 (0.5) | 0 (0.6) | 26 |
| O | 103 (70.8) | 49 (40.0) | 10 (10.3) | 32 (23.5) | 1 (0.2) | 4 (9.2) | 1 (0.9) | 4 (5.3) | — | 1 (4.0) | 0 (4.6) | 205 |
| EnF | 0 (8.6) | 0 (4.9) | 0 (1.3) | 0 (2.9) | 0 (0.02) | 0 (1.1) | 0 (0.1) | 0 (0.6) | 0 (4.4) | — | 25 (0.6) | 25 |
| ExF | 2 (7.9) | 0 (4.5) | 0 (1.2) | 2 (2.6) | 0 (0.02) | 17 (1.0) | 0 (0.1) | 0 (0.6) | 2 (4.1) | 0 (0.59) | — | 23 |
| Column sum | 386 | 218 | 56 | 128 | 1 | 50 | 5 | 29 | 197 | 22 | 25 | 1117 |

[a] Top value in each cell indicates the observed frequency of that particular behavioral transition based on 15 females; the bottom value indicates the expected frequency.

**Table 4** Behavioral Transition Matrix for *O. anastrephae* on Infested Fruit without Punctures

| Preceeding Behavior | Following behavior[a] | | | | | | | | | | | Row sum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W | AW | PA | DT | AR | C | R | PM | O | EnF | ExF | |
| W | — | 28 (18.9) | 7 (7.5) | 7 (2.6) | 0 (0.8) | 13 (7.8) | 0 (0.3) | 10 (5.9) | 2 (5.4) | 3 (2.3) | 0 (2.1) | 70 |
| AW | 29 (16.3) | — | 17 (7.4) | 0 (2.6) | 0 (0.8) | 4 (7.6) | 0 (0.3) | 11 (5.9) | 6 (5.4) | 2 (2.3) | 0 (2.0) | 69 |
| PA | 8 (7.8) | 15 (8.9) | — | 0 (1.2) | 1 (0.4) | 2 (3.7) | 0 (0.1) | 0 (2.8) | 7 (2.6) | 0 (1.1) | 0 (0.9) | 33 |
| DT | 7 (4.5) | 4 (5.1) | 0 (2.0) | — | 1 (0.2) | 3 (2.1) | 0 (0.07) | 1 (1.6) | 3 (1.5) | 0 (0.6) | 0 (0.5) | 19 |
| AR | 0 (0.5) | 1 (0.5) | 0 (0.2) | 0 (0.07) | — | 1 (0.2) | 0 (0.01) | 0 (0.1) | 0 (0.2) | 0 (0.07) | 0 (0.06) | 2 |
| C | 13 (6.1) | 8 (7.0) | 0 (2.8) | 0 (1.0) | 1 (0.3) | — | 1 (0.1) | 0 (2.2) | 3 (2.0) | 0 (0.9) | 0 (0.8) | 26 |
| R | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | — | 0 (0.0) | 1 (0.0) | 0 (0.0) | 0 (0.02) | 0 |
| PM | 2 (5.2) | 10 (5.9) | 2 (2.3) | 2 (0.8) | 0 (0.2) | 2 (2.4) | 0 (0.1) | — | 0 (1.7) | 4 (0.7) | 0 (0.6) | 22 |
| O | 3 (3.3) | 7 (3.8) | 3 (1.5) | 0 (0.5) | 0 (0.2) | 0 (1.6) | 0 (0.05) | 1 (1.2) | — | 0 (0.5) | 0 (0.4) | 14 |
| EnF | 0 (1.9) | 0 (2.2) | 0 (0.9) | 0 (0.3) | 0 (0.1) | 0 (0.9) | 0 (0.03) | 0 (0.7) | 0 (0.6) | — | 8 (0.2) | 8 |
| ExF | 2 (1.9) | 0 (2.2) | 0 (0.9) | 1 (0.3) | 0 (0.09) | 5 (0.9) | 0 (0.03) | 0 (0.7) | 0 (0.6) | 0 (0.3) | — | 8 |
| Column sum | 64 | 73 | 29 | 10 | 3 | 30 | 1 | 23 | 21 | 9 | 8 | 271 |

[a] Top value in each cell indicates the observed frequency of that particular behavioral transition based on 15 females; the bottom value indicates the expected frequency.

1992). If we relaxed the probability level to $P$=0.05, then the following first=order behavioral transitions occurred significantly more often than expected by chance in *O. anastrephae*: 1) infested, punctured fruit, C-W ($T$=4, $Z$=1.96, $P$=0.04, $n$=15), EnF=ExF ($T$=1, $Z$=2.66, $P$=0.007, $n$=15), ExF-C ($T$=1, $Z$=2.54, $P$=0.01, $n$=15); 2) infested unpunctured fruit, W-AW ($T$=1, $Z$=2.02, $P$=0.04, $n$=15), C-W ($T$=1, $Z$=2.37, $P$=0.01, $n$=15), O-W ($T$=1, $Z$=1.99, $P$=0.04, $n$=15), W-PM ($T$=1, $Z$=1.92, $P$=0.04, $n$=15), EnF-ExF ($T$=1, $Z$=2.37, $P$=0.02, $n$=15); 3) non-infested punctured fruit, W-ExF ($T$=1, $Z$=2.02, $P$=0.04, $n$=15), EnF-ExF ($T$=1, $Z$=2.21, $P$=0.03, $n$=15), ExF-W ($T$=1, $Z$=2.02, $P$=0.04, $n$=15).

Based on the above, the following significant transitions were recorded for every treatment in the case of *O. anastrephae*: 1) infested punctured fruit, EnF → ExF → C→ W; 2) infested unpunctured fruit, C or O → W → AW or (W → PM → EnF → ExF); 3) non-infested punctured fruit, W → EnF → ExF → W.

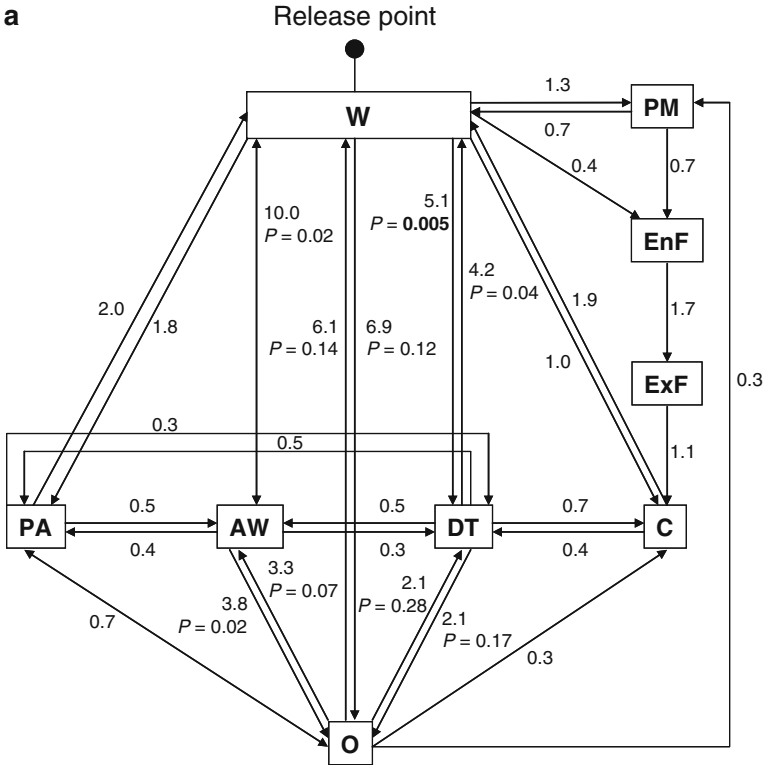


**Fig. 10** Representative example of sequence analysis performed to illustrate patterns of behavior exhibited by *A. pelleranoi* (**a**) and *O. anastrephae* (**b**) during study (infested guava without punctures chosen as it elicited the richest behavioral response by females). Arrows indicate the direction of the behavioral sequence. Operational definitions of behaviors are provided in Table 1. Numbers represent frequencies of each first-order preceding-following behavioral transition per 15 observed females. Behavioral transitions occurring at $P$=0.1 were not included. Probabilities of ten behavioral transitions selected for statistical analysis are given. Significant probability is bold denoted (Wilcoxon Matched Pairs test, $P$=0.005). The W behavior, which had the highest interaction level with other behavioral events, is denoted by a larger rectangle.

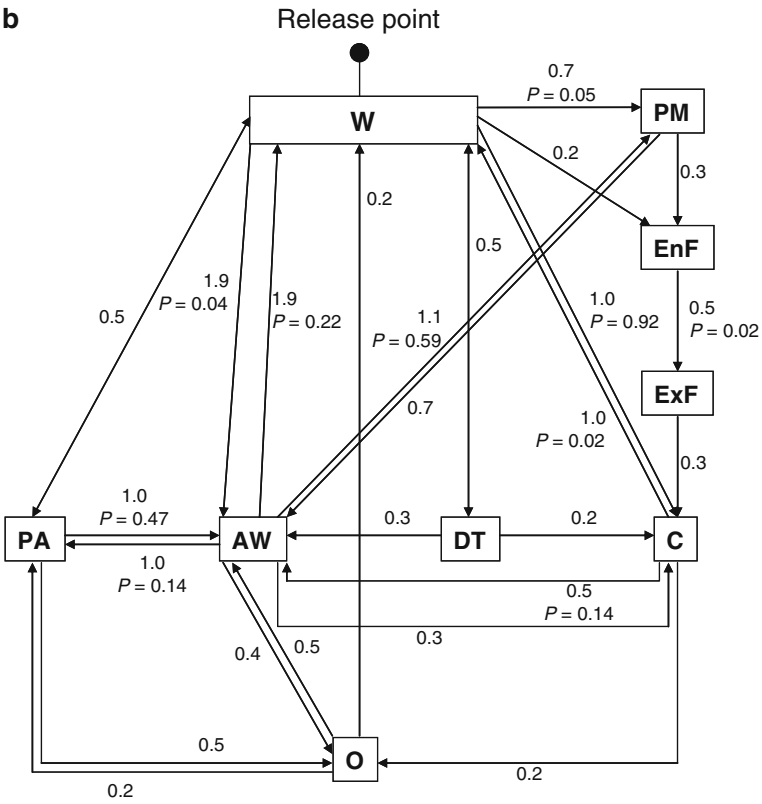


**Fig. 10** (continued).

Finally, using the seven behavioral transitions that ended up being highly significant (i.e., more often than would be expected by chance alone, Wilcoxon Matched Pairs test, $P$=0.005) in the case of *A. pelleranoi*, we sought out differences between species and fruit condition (e.g., infested and punctured vs infested unpunctured). In general terms, a clear pattern emerges indicating that infested fruit elicited the most activity in the females of both species. Furthermore and as already noted before, *A. pelleranoi* females were significantly more active than *O. anastrephae* females (details in Tables 5 and 6).

## Discussion

Several questions concerning the evolution of eucoiline foraging are raised by the present research. These are: 1) what is the adaptive significance of the different foraging techniques of the two species?; 2) do differences in foraging reflect tactics that allow co-existence?; and 3) if so, have tactics diverged as a consequence of recent competition with one another or have the various tactics evolved under other, allopatric, circumstances, but now permit sympatry?

**Table 5** Results of Three-way ANOVA Run to Determine Whether the Mean Number of Transitions Varied between Parasitoid Species and Fruit Condition

| Behavioral transitions | Interactions | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1×2 | 1×3 | 2×3 | 1×2 ×3 |
| W—DT | $F(1,112)$ =8.13 | $F(1,112)$ =0.18 | $F(1,112)$ =12.45 | $F(1,112)$ =0.01 | $F(1,112)$ =5.36 | $F(1,112)$ =0.33 | $F(1,112)$ =0.06 |
| | ***P*=0.005** | $P$=0.670 | ***P*<0.001** | $P$=0.929 | ***P*=0.02** | $P$=0.565 | $P$=0.813 |
| W—AW | $F(1,112)$ =20.91 | $F(1,112)$ =5.07 | $F(1,112)$ =0.01 | $F(1,112)$ =3.58 | $F(1,112)$ =0.76 | $F(1,112)$ =0.02 | $F(1,112)$ =0.02a |
| | ***P*<0.001** | ***P*=0.026** | $P$=0.894 | ***P*=0.041** | $P$=0.384 | $P$=0.884 | $P$=0.899 |
| W—EnF | $F(1,112)$ =16.79 | $F(1,112)$=75.42 | $F(1,112)$ =17.41 | $F(1,112)$ =8.15 | $F(1,112)$ =0.05 | $F(1,112)$ =0.20 | $F(1,112)$ =2.12 |
| | ***P*<0.001** | ***P*<0.001** | ***P*<0.001** | ***P*=0.005** | $P$=0.830 | $P$=0.653 | $P$=0.148 |
| AW—W | $F(1,112)$ =21.06 | $F(1,112)$ =6.61 | $F(1,112)$ =0.05 | $F(1,112)$ =0.76 | $F(1,112)$ =1.03 | $F(1,112)$ =1.01 | $F(1,112)$ =0.10 |
| | ***P*<0.001** | ***P*=0.011** | $P$=0.823 | $P$=0.383 | $P$=0.311 | $P$=0.965 | $P$=0.752 |
| C—W | $F(1,112)$ =2.67 | $F(1,112)$ =0.73 | $F(1,112)$ =35.69 | $F(1,112)$ =0.56 | $F(1,112)$ =6.91 | $F(1,112)$ =0.13 | $F(1,112)$ =0.06 |
| | $P$=0.105 | $P$=0.394 | ***P*<0.001** | $P$=0.453 | ***P*=0.009** | $P$=0.723 | $P$=0.800 |
| EnF—ExF | $F(1,112)$ =31.52 | $F(1,112)$=52.49 | $F(1,112)$ =36.16 | $F(1,112)$=10.13 | $F(1,112)$ =2.44 | $F(1,112)$ =2.63 | $F(1,112)$ =0.76 |
| | ***P*<0.001** | ***P*<0.001** | ***P*<0.001** | ***P*=0.001** | $P$=0.121 | $P$=0.107 | $P$=0.386 |
| ExF—C | $F(1,112)$ =7.35 | $F(1,112)$=10.95 | $F(1,112)$ =59.61 | $F(1,112)$ =1.39 | $F(1,112)$ =4.79 | $F(1,112)$ =3.75 | $F(1,112)$ =0.43 |
| | ***P*=0.008** | ***P*<0.001** | ***P*<0.001** | $P$=0.241 | ***P*=0.031** | ***P*=0.050** | $P$=0.512 |

Only seven behavioral transitions were used for this analysis as they represent the only ones that were determined to be significant (i.e., more often than would be expected by chance alone). Interaction analysis (1=parasitoid species: *A. pelleranoi* vs *O. anastrephae*; 2=first fruit condition: punctured vs unpunctured (clean) fruit; 3=second fruit condition: infested vs uninfested fruit), significant probabilities are denoted in bold

**Table 6** Comparison of the Mean (± SD) Number of Significant Behavioral Transitions between Parasitoid Species (*A. pelleranoi* [*A. p.*] and *O. anastrephae* [*O. a.*]) and Fruit Condition (e.g., Infested with (IP) or without (IW) Punctures vs. Uninfested with [UP] or without [UW] Punctures)

| Parasitoid species and fruit conditions | Behavioral transitions[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | W—DT | W—AW | W—EnF | AW—W | C—W | EnF—ExF | ExF—C |
| *A. p.* IP | 3.1±4.7a | 5.8±13.6ab | 3.8±2.7a | 4.5±10.5ab | 4.4±4.1a | 6.4±4.6a | 4.1±3.6a |
| IW | 5.1±5.9a | 10.0±14.8c | 0.4±0.5bc | 10.0±16.3a | 1.9±1.8a | 1.6±1.7b | 1.1±1.5b |
| UP | 0.8±1.8b | 3.5±5.5ab | 1.6±1.5b | 3.3±5.5ab | 0.3±1.0a | 2.0±1.6b | 0.2±0.6b |
| UW | 0.5±1.1b | 5.2±4.7a | 0.0 | 4.9±5.7a | 0.1±0.3a | 0.0 | 0.0 |
| *O. a.* IP | 0.4±0.8b | 1.1±2.5bc | 1.2±1.3bc | 1.1±3.1b | 1.0±1.5a | 1.3±1.6b | 0.9±1.0b |
| IW | 0.5±0.8b | 1.9±4.9bc | 0.2±0.4c | 1.9±5.9ab | 0.9±1.2a | 0.5±0.6b | 0.3±0.5b |
| UP | 0.2±0.4b | 0.3±0.6b | 0.4±0.7bc | 0.1±0.3b | 0.3±0.6a | 0.5±0.7b | 0.1±0.3b |
| UW | 0.2±0.4b | 0.4±0.8b | 0.0 | 0.5±0.9b | 0.3±0.6a | 0.0 | 0.0 |

[a] Means in same column followed by same letter are not significantly different (Tukey HSD test, $P<0.05$).

*Adaptive Significance* In general, the *short range* orientation of *A. pelleranoi* females towards the host appears to have been mediated by a combination of chemical stimuli related to the host larvae and host fruit. When punctured, infested guavas were offered to the *A. pelleranoi* females, they moved from the release site to the fruit in a significantly shorter time when compared to non = infested, unpunctured fruit. In contrast, *O. anastrephae* did not react as strongly to infested fruit, since females walked significantly faster to non-infested fruit. When punctured fruit contained hosts, it took 30 times longer for *O. anastrephae* than *A. pelleranoi* to reach the fruit, and three times longer than it took conspecifics to reach non-infested fruit. Given the relative specialization of *O. anastrephae* on tephritid larvae inside *Psidium* spp. fruits, this "leisurely" response is unexpected, and perhaps reflects either a genuine caution in approaching fruits due to associated risks (e.g., predation) or was partially an artifact of the experimental procedure. Vet and Bakker (1985) found that an apparent "inefficiency" in two species of eucoiline parasitoids of drosophilids in the laboratory was due to confused orientation caused by unnaturally high and homogeneous concentrations of host-kairomones in the observation arena possibly affecting *O. anastrephae* but not *A. pelleranoi*. In addition, we note that the infested fruit contained larvae mixed with some of the diet they were reared in, and perhaps *O. anastrephae* females, in contrast to those of *A. pelleranoi*, took longer to recognize the mixture of guava and diet odors. Once on a fruit, *O. anastrephae* females exhibited a wide behavioral repertoire (details follow).

Our data on *A. pelleranoi* support the results of a recent study by Guimarães and Zucchi (2004), who with the aid of a four-way olfactometer, demonstrated that *A. pelleranoi* and another eucoilid (*Diceratasphis grenadensis* Ashmead) were principally attracted by the volatiles emitted by guavas containing larvae of *Anastrepha* spp. and *Zaprionus indianus* Gupta (Drosophilidae), respectively. Such attraction to host and host fruit volatiles are widespread among tephritid parasitoids. In the braconids *Psyttalia fletcheri* (Silvestri) (Messing et al. 1996) and *D. longicaudata*

(Nishida and Napompeth 1974; Greany et al. 1977; Leyva et al. 1991; Messing and Wong 1992; Messing and Jang 1992), odors from infested fruit (as opposed to clean, uninfested fruit) significantly influence host finding behavior. More recently, Eitam et al. (2003) suggested that chemical stimuli emitted from the skin of host fruit play a role in the localization of *Anastrepha* larvae by another braconid, *Doryctobracon areolatus* (Szépligeti).

Once females of both eucoilinid species arrived at the guava, they explored the fruit for a significantly longer period, and showed a more complex sequence of behavioral transitions, if the fruit was infested (punctured or not) than if it was without larvae. This suggests that stimuli associated with the host (chemical and/or physical), probably played an important role in this second phase of host searching behavior. Chemical stimuli that originate from the host are frequently used by parasitoids in the short-range host location (Weseloh 1981; Vinson 1984; 1998; Vet and Dicke 1992; Godfray 1994). Females of the pteromalid *Halticoptera laevigata* Thoms., a parasitoid of *Myoleja lucida* Fallén (Diptera: Tephritidae), can distinguish chemical stimuli from non infested and infested fruit and/or those with host marking pheromone (Hoffmeister and Gienapp 1999). Furthermore, Mattiacci et al. (1999), found that females of the eulophid *Hyssopus pallidus* (Askew), a parasitoid of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), remained longer in infested fruit than in non-infested ones, and also directs its searching pattern toward the area of the fruit where host larvae were located.

*A. pelleranoi* females used both their antennae and tarsi to detect larvae but the use of these structures varied depending on context: in infested fruit tarsi were preferentially used (usually while standing still) while in uninfested fruit, antennae were mainly used (usually while walking). In the case of *O. anastrephae* females, the reverse pattern was usually observed with antennae most commonly used to detect larvae in infested fruit (Fig. 10b). In general terms, *O. anastrephae* exhibited simpler behavioral sequences that *A. pelleranoi* (Fig. 10a). Our findings differ from Guimarães and Zucchi (2004) who recently reported that *A. pelleranoi* and *O. anastrephae* females mainly use their antenae to detect larvae. Possibly, tarsi are employed as sensorial receptors of mechanical stimuli, for example to perceive vibrations produced by the host during feeding or moving (Vinson 1998). Comparative studies of drosophilid eucoiline parasitoids found that those species which locate hosts through larval movement, typically stand still and employ tarsal sensing (Vet and Bakker 1985).

One possibility these differences suggest, is that *A. pelleranoi* females may be employing a more general means of monitoring that is used to locate a fruit or an area on a fruit where tarsal sensing can then be used to precisely locate a host. In some species of fruit fly parasitoids, as for example *Psyttalia concolor* (Szépligeti) (Hymenoptera: Braconidae), antennae (in particular the last antennomeres) seem to be the main structures involved in stimuli perception during host larvae location (Canale and Raspi 2000). Antennation in hymenopteran parasitoids can be employed both to detect chemicals coming from host kairomones, and to perceive physical stimuli (Vinson 1976). According to Isidoro et al. (1996), certain gustatory and mechanosensory sensilla, in combination with some accessory glands in the hymenopteran parasitoids antennae, could be functionally responsible of host recognition and discrimination during the host searching process.

Vibrations could be an important cue to host location for parasitoids attacking concealed host, as in some species that attack endophytic leafminer and fruit-borer hosts (reviewed by Meyhöfer and Casas 1999). In the case of larval endoparasitoid species of fruit-infesting Tephritidae there is also evidence of the latter. For example, *D. longicaudata* females attacking *Anastrepha* spp. (Lawrence 1981), and *Diachasma alloeum* (Muesebeck) attacking *Rhagoletis pomonella* (Walsh) (Glas and Vet 1983) orient to the vibrations made by feeding larvae. Similarly, larval endoparasitoids species of Drosophilidae, such as *Ganaspis xanthopoda* (Ashmead) (Figitidae, Eucoilinae) (Vet and Bakker 1985), *Leptopilina longipes* (Hartig) (Figitidae, Eucoilinae) (van Dijken and van Alphen 1998), and *Asobara tabida* (Nees) (Braconidae, Alysiinae) (van Alphen and Drijver 1982; Vet and van Alphen 1985; Sokolowski and Turlings 1987) also detect hosts through their movements.

When motionless, both *A. pelleranoi* and *O. anastrephae* inserted their ovipositors into fruit to detect hosts, mainly in fruit containing larvae. The ovipositor in Hymenoptera is often involved in host location through detection of chemical or physical stimuli. Typically, this is most important during the process of host acceptance (Vinson 1976), and is commonly observed in the eucoiline parasitoids of drosophilids (Vet and Bakker 1985).

While females of both species penetrated the fruit in search of larvae, *O. anastrephae* remained inside for significantly longer periods (up to eight hours). We note that penetration into fruit through punctures, was previously observed by Turica (1968) and Ovruski (1994a) in the case of *A. pelleranoi* females but had not been properly quantified for *O. anastrephae*. "Swimming in the pulp" is not unique to eucoilines. Silvestri (1913) described in graphic detail similar behaviors by the gregarious eulophid *Aceratoneuromyia indica* (Silvestri) as it attacked third instar *C. capitata* larva, as did Mattiacci et al. (1999) in the case of *H. pallidus* seeking out *C. pomonella* larvae.

In the absence of a suitable puncture, females of both eucoiline species are able to break open the skin of the fruit with their mandibles to excavate an entrance into the pulp. This may give an advantage to *A. pelleranoi* and *O. anastrephae* females, in comparison with most of the other larval parasitoid in the same guild. Braconids search for host larvae only by inspecting the surface of the fruit, and oviposition only occurs through the epicarp. This can be ineffective if larvae are feeding deep inside the fruit, or if a fruit is of considerable dimensions, such as is often the case in exotic species like *Citrus* spp. and *Mangifera indica* L (Sivinski et al. 1997; López et al. 1999). Based on the original description by Van Lenteren et al. (1998) and the more recent work by Buffington (2007), the fact that both *A. pelleranoi* and *O. anastrephae* females are able to successfully attack mobile larvae inside a fruit, could be partly related to an ovipositor clip that allows parasitoids to hold on to escaping hosts, even if dragged along in pulpy fruit tissue.

*Coexistence* Do the different foraging techniques and tactics of *A. pelleranoi* and *O. anastrephae* promote their co-existence in the same habitat? Specifically, would their behaviors suggest they are exploiting a different set of fruit fly larvae? The host-produced cues perceived through the tarsi are likely to be vibrational (e.g., Vet and Bakker 1985), and the extensive use of the antennae on uninfested fruit suggests that these are used to perceive chemical cues in the absence of vibrations. If so, it would

appear that *A. pelleranoi* spends more of its foraging time searching for moving/feeding larvae and *O. anastrephae* uses a chemical-based sensory system that might be capable of locating immobile larvae.

Are "moving" and "still" larvae sufficiently large subsets of the potential host pool to support two parasitoids, one specialized on each host-state? Little is known about the larval behavior of tephritids. Larvae of *A. suspensa* fed at any time during the day or night (Webb and Landolt 1984), but individual larvae are active ~70% of the time (R. Mankin, unpublished data). Thus there could be a substantial number of potential hosts that would not be detected through tarsal-searching.

*The Evolutionary Divergence of Foraging Behaviors* Let us hypothesize that the differences in the foraging behavior of *A. pelleranoi* and *O. anastrephae* are critical to their co-existence. If so, have these differences resulted from a history of competition between the two, selection favoring further subdivision of the niche? Or alternatively, are these differences ancestral, and though evolved in allopatry still allow habitat sharing by species that later moved into sympatry? These questions compelled Vet and Bakker (1985) and Vet and van Alphen (1985), to undertake comparative studies of the larval parasitoids of drosophilids, including substantial numbers (25 species) of eucoilines. They determined that parasitoids could be divided in groups according to the patterns of host search behavior, and that these patterns were related to the taxonomic position of species. For example, most species of the genus *Leptopilina* (Eucoilinae) used their ovipositor as means of detection while walking on the substrate; all the species of genus *Ganaspis* (Eucoilinae), as well as the *Asobara* and *Aphaerata* genera (Alysiinae), reacted to host larvae movements showing a characteristic sequence of walks and stops, followed, in some cases, by ovipositor insertion; all the species of genus *Trybliographa* (Eucoilinae), *Leptopilina fimbriata* (Kieffer), and the alysiine *Tanycarpa punctata* van Achterberg used their antennae and exhibited intensive oviposition attempts during long periods of stillness; while species of *Kleidotoma* (Westwood) (Eucoilinae) used different methods of host detection on the substrate's surface. Regardless of different techniques, all of them searched for host larvae in punctures and holes of the substrate. In the case of the two species studied here, we have insufficient observational and phylogentic information (Fontal-Cazalla et al. 2002) to address the evolutionary history of their differences in behavior. We can only point to the drosophilid parasitoid studies (Vet and Bakker 1985 and Vet and van Alphen 1985), and their conclusion that phylogeny was a better predictor of foraging techniques than the type of substrate/habitat being searched.

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## References

Aluja M, López M, Sivinski J (1998) Ecological evidence for diapause in four native and one exotic species of larval-pupal fruit fly (Diptera: Tephritidae) parasitoids in tropical environments. Ann Entomol Soc Am 91:821–833

Aluja M, Sivinski J, Ovruski S, Guillén L, Cancino J, López M, Torres-Anaya A, Gallegos-Chan G, Ruíz L (2009). Colonization and domestication of seven species of native new world hymenopterous larval-pupal and pupal fruit fly (Diptera: Tephritidae) parasitoids. Biocontrol Sci Techn doi: 10.1080/09583150802377373

Bernstein C, Driessen G (1996) Patch-marking and optimal search patterns in the parasitoid *Venturia canescens*. J Anim Ecol 65:211–219

Broad G, Quike D (2000) The adaptive significance of host location by vibrational sounding in parasitoid wasps. Proc R Soc London B 267:2403–2409

Buffington ML (2007) The occurrence and phylogenetic implications of the ovipositor clip within Figitidae (Insecta: Hymenoptera: Cynipoidea). J Nat Hist 41:33–36

Canale A, Raspi A (2000) Host location and oviposition behaviour in *Opius concolor* Szépligeti (Hymenoptera, Braconidae). Entomol Problems 31:25–32

Conover WJ, Iman RL (1981) Rank transformation as a bridge between parametric and nonparametric statistics. Amer Statist 35:124–129

Doutt RL (1959) The biology of parasitic Hymenoptera. Annu Rev Entomol 4:161–182

Eben A, Benrey B, Sivinski J, Aluja M (2000) Host species and host plant effects on performance of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environ Entomol 29:87–94

Eitam A, Holler T, Sivinski J, Aluja M (2003) Use of host fruit chemical cues for laboratory rearing of *Doryctobracon areolatus* (Hymenoptera: Braconidae), a parasitoid of *Anastrepha* spp. (Diptera: Tephritidae). Fla Entomol 86:211–216

Eskafi FM (1990) Parasitism of fruit flies *Ceratitis capitata* and *Anastrepha* (Diptera: Tephritidae) in Guatemala. Entomophaga 35:355–362

Faeth S (1990) Structural damage to oak leaves alters natural enemy attack on a leafminer. Ent Exp Appl 57:57–63

Fontal-Cazalla FM, Buffington ML, Nordlander G, Liljeblad J, Ros-Farré P, Nieves- Aldrey JL, Pujade-Villar J, Ronquist F (2002) Phylogeny of the Eucoilinae (Hymenoptera: Cynipoidea: Figitidae). Cladistics 18:154–199

Gallegos-Chan GJ (1999) *Edad Óptima de Parasitación, Longevidad, Fecundidad y Patrones Diarios de Oviposición de 6 Especies de Parasitoides Nativos y 2 Exóticos de las Moscas de la Fruta del Género Anastrepha (Schiner) (Diptera: Tephritidae)*. BSc Thesis, Universidad Veracruzana. Xalapa, Veracruz, México, p 69

García-Medel D, Sivinski J, Díaz-Fleischer F, Ramírez-Romero R, Aluja M (2007) Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as single- or multiple-species cohorts in field cages: Influence of fruit location and host density. Biol Control 43:12–22

Glas PC, Vet MEM (1983) Host-habitat location and host location by *Diachasma alloeum* muesebeck (Hym.: Braconidae) a parasitoid of *Rhagoletis pomonella* Walsh (Dipt.:Tephritidae). Neth J Zool 33:41–54

Godfray HCJ (1994) Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ

Greany PD, Tumlinson JH, Chambers DL, Boush GM (1977) Chemically mediated host finding by *Biosteres (Opius) longicaudatus*, a parasitoid of Tephritid fruit fly larvae. J Chem Ecol 3:189–195

Guimarães JA, Zucchi RA (2004) Parasitism behavior of three species of Eucolinae (Hymenoptera: Figitidae) fruit fly parasitoids (Diptera) in Brazil. Neotrop Entomol 33:217–224

Guimarães JA, Zucchi RA, Díaz NB, de Souza FMF, Uchoa FMA (1999) Species of Eucoilinae (Hymenoptera: Cynipoidea: Figitidae) parasitoids of frugivorous larvae (Diptera: Tephritidae and Lonchaeidae) in Brazil. Anais Soc Entomol Brasil 28:263–273

Guimarães JA, Diaz NB, Zucchi RA (2000) Parasitoides—Figitidae (Eucoilinae). In: Malavasi A, Zucchi RA (eds) Moscas-das-frutas de Importância Econômica no Brasil. Conhecimento básico e aplicado, Holos Editora Ltda-Me, Riberão Preto, Brasil, pp 127–134

Guimarães JA, Gallardo FE, Diaz NB, Zucchi RA (2003) Eucoilinae species (Hymenoptera: Cynipoidea: Figitidae) parasitoids of fruit-infesting dipterous larvae in Brazil: identity, geographical distribution and host associations. Zootaxa 278:1–23

Guimarães JA, de Souza MF, Raga A, Zucchi RA (2004) Levantamento e interações tritróficas de figítidos (Hymenoptera: Eucoilinae) parasitóides de larvas frugívoras (Diptera) no Brasil. Arq Int Biol Sao Paulo 71:51–56

Guimarães JA, Gallardo FE, Diaz NB (2005) Contribution to the systematic of *Odontosema* Kieffer (Hymenoptera: Cynipoidea, Figitidae). Trans Am Ent Soc 131:457–461

Hassel MP, Southwood TRE (1978) Foraging strategies of insects. Annu Rev Ecol & Syst 9:75–98

Hoffmeister TS, Gienapp P (1999) Exploitation of the host's chemical communication in a parasitoid searching for concealed host larvae. Ethology 105:223–232

Isidoro N, Bin F, Colazza S, Vinson SB (1996) Morphology of antennal gustatory sensilla and glands in some parasitoid Hymenoptera with hypothesis on their role in sex and host recognition. J Hym Res 5:206–239

Kramer M, Schmidhammer J (1992) The chi-squared statistic in ethology: use and misuse. Anim Behav 44:833–841

Lawrence P (1981) Host vibration- a cue to host location by the parasite, *Biosteres longicaudatus*. Oecologia 48:249–251

Lehner PN (1996) Handbook of Ethological Methods. Cambridge Univ, Press, Cambridge, UK

Leyva JL, Browning HW, Gilstrap FE (1991) Effect of host fruit species, size, and color on parasitization of *Anastrepha ludens* (Diptera: Tephritidae) by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environ Entomol 20:1470–1474

López M, Aluja M, Sivinski J (1999) Hymenopterous larval-pupal and pupal parasitoids of *Anastrepha* flies (Diptera: Tephritidae) in Mexico. Biol. Control 15:119–129

Matrangolo WJR, Nascimento AS, Carvalho RS, Melo ED, de Jesus M (1998) Fruit fly parasitoids associated with tropical fruits. Anais Soc Ent Brasil 27:593–603

Mattiacci L, Hütter E, Dorn S (1999) Host location of *Hyssopus pallidus*, a larval parasitoid of the Codling moth. *Cydia pomonella* Biol Control 15:241–251

Messing RH, Jang EB (1992) Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to host fruit stimuli. Environ Entomol 21:1189–1195

Messing RH, Wong TTY (1992) An effective trapping method for field studies of Opiine braconid parasitoids of Tephritid fruit flies. Entomophaga 37:391–396

Messing RH, Klungness LM, Jang EB, Nishida KA (1996) Response of the melon fly parasitoid *Psyttalia fletcheri* (Hymenoptera: Braconidae) to host-habitat stimuli. J Insect Behav 9:933–945

Meyhöfer R, Casas J (1999) Vibratory stimuli in host location by parasitic wasps. J Ins Physiol 45:967–971

Meyhofer R, Casas J, Dorn S (1994) Host location by a parasitoid using leafminer vibrations: Characterizing the vibrational signals produced by the leafmining host. Physiol Entomol 19:349–459

Ngi-Song AJ, Overholt WA, Njagi PGN, Dicke M, Ayertey JN, Lande W (1996) Volatile infochemicals used in host and host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stemborers on graminae. J Chem Ecol 22:307–323

Nishida T, Napompeth B (1974) Trap for tephritid fruit fly parasites. Entomophaga 19:349–351

Ovruski SM (1994a) Comportamiento en la detección del huésped en *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Cynipoidea, Eucoilidae) parasitoide de larvas de *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). Rev Soc Entomol Argent 53:121–127

Ovruski SM (1994b) Immature stages of *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Cynipoidea, Eucoilidae), a parasitoid of *Ceratitis capitata* (Wied.) and *Anastrepha* spp. (Diptera: Tephritidae). J Hym Res 3:233–239

Ovruski SM (1995) Pupal and larval-pupal parasitoids (Hymenoptera) obtained from *Anastrepha* spp. and *Ceratitis* (Diptera: Tephritidae) pupae collected in four localities of Tucumán, Province, Argentina. Entomophaga 40:367–370

Ovruski SM, Aluja M, Sivinski J, Wharton R (2000) Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Int Pest Manag Rev 5:81–107

Ovruski SM, Schliserman P, Aluja M (2004) Indigenous parasitoids (Hymenoptera) attacking *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) in native and exotic host plants in Northwestern Argentina. Biol Control 29:43–57

Ovruski SM, Wharton RA, Schliserman P, Aluja M (2005) Abundance of *Anastrepha fraterculus* (Diptera: Tephritidae) and its associated native parasitoids (Hymenoptera) in "feral" guavas growing in the endangered northernmost Yungas forest of Argentina with an update on the taxonomic status of opiine parasitoids previously reported in this country. Environ Entomol 34:807–818

Piedra E, Zuñiga A, Aluja M (1993) New host plant and parasitoid record in México for *Anastrepha alveata* Stone (Diptera: Tephritidae). Proc Entomol Soc Wash 95:127

Potting R, Vet L, Dicke M (1995) Host microhabitat location by the stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systematically induced plant volatiles. J Chem Ecol 21: 525–539

Reitz S, Trumble J (2002) Competitive displacement among insects and arachnids. Annu Rev Entomol 47:435–465

Richerson J, Borden J (1972) Host finding by heat perception in *Coeloides brunneri* (Hymenoptera: Braconidae). Can Entomol 104:1877–1881

Silvestri F (1913) Viaggio in Africa per cercare parassiti di mosche dei frutti. Boll Lab Zool Gen Agr Portici 8:1–164

Sivinski J, Aluja M, López M (1997) The spatial and temporal distributions of parasitoids of Mexican *Anastrepha* species (Diptera: Tephritidae) within the canopies of fruit trees. Ann Entomol Soc Am 90:604–618

Sivinski J, Piñero J, Aluja M (2000) The distributions of parasitoids (Hymenoptera) of *Anastrepha* fruit flies (Diptera: Tephritidae) along an altitudinal gradient in Veracruz, Mexico. Biol Control 18:258–269

Sivinski J, Vulinec K, Aluja M (2001) Ovipositor length in a guild of parasitoids (Hymenoptera: Braconidae) attacking *Anastrepha* spp. fruit flies (Diptera: Tephritidae) in southern Mexico. Ann Entomol Soc Am 94:886–895

Slater PJB, Ollason JC (1972) The temporal pattern of behavior in isolated male zebra finches: Transition analysis. Behaviour 42:248–269

Sokolowski MB, Turlins TCJ (1987) *Drosophila* parasitoid-host interaction: vibrotaxis and ovipositor searching from the host perspective. Can J Zool 65:461–464

Stat-Soft (1995) Statistica: User Guides. Stat-Soft, Inc, Tulsa, OK, USA

Steimberg S, Dicke M, Vet LEM, Wanningen R (1992) Response of the braconid parasitoid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals: effect of bioassay set-up, parasitoid age and experience and barometrix flux. Exp Appl 63:163–175

Tumlinson JH, Turlings TCJ, Lewis WJ (1993) Semiochemically mediated foraging behaviour in beneficial parasitic insects. Arch Insect Bioch Physiol 22:385–391

Turica A (1968) Lucha biológica como medio de control de las moscas de los frutos. Revista IDIA 241:29–38

van Alphen JJM, Drijver RAB (1982) Host selection by *Asobara tabida* Ness (Braconidae: Alysiinae), a larval parasitoid of fruit-inhabiting *Drosophila* species I. Host stage selection with *Drosophila melanogaster* as host species. J Zool 32:194–214

van Alphen JJM, Vet LEM (1986) An evolutionary approach to host finding and selection. In: Waage J, Greathead D (eds) Insect Parasitoids. Academic Press, FL, pp 23–61

van Dijken MJ, Van Alphen JJM (1998) The ecological significance of differences in host detection behavior in coexisting parasitoid species. Ecol Entomol 23:265–270

van Lenteren JC, Isidoro N, Bin F (1998) Functional anatomy of the ovipositor clip in the parasitoid *Leptopilina heterotoma* (Thompson) (Hymenoptera: Eucoilidae), a structure to grip escaping larvae. Int J Insect Morphol Embryol 27:263–268

Vet LEM, Bakker K (1985) A comparative functional approach to the host detection behaviour of parasitic wasps. 2. A quantitative study on eight eucoilid species. Oikos 44:487–498

Vet L, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Ann Rev Entomol 37:141–172

Vet LEM, van Alphen JJM (1985) A comparative functional approach to the host detection behaviour of parasitic wasps. 1. A qualitative study on Eucolidae and Alysiinae. Oikos 44:478–486

Vet LEM, Wäckers FL, Dicke M (1992) How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. Neth J Zool 41:202–213

Vinson SB (1976) Host selection by insect parasitoids. Ann Rev Entomol 21:109–134

Vinson SB (1984) Parasitoid-host relationships. In: Bell WJ, Cardé RJ (eds) The Chemical Ecology of Insects. Chapman and Hall, NY, USA, pp 205–233

Vinson SB (1998) The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biol Control 11:79–96

Webb JC, Landolt PJ (1984) Detecting insect larvae in fruit by vibrations produced. J Environ Sci Health, Part A Environ. Sci Eng 19:367–375

Weseloh RM (1981) Host location by parasitoids. In: Nordlund DA, Jones RL, Lewis WJ (eds) Semiochemicals: Their role in pest control. John Wiley & Sons, New York, USA, pp 79–95

Wharton RA, Gilstrap FE, Rhode RH, Fischel MM, Hart WG (1981) Hymenopterous egg-pupal and larval-pupal parasitoids of *Ceratitis capitata* and *Anastrepha* spp. (Diptera: Tephritidae) in Costa Rica. Entomophaga 26:285–290

Wharton RA, Ovruski SM, Gilstrap FE (1998) Neotropical Eucoilidae (Cynipoidea) associated with fruit infesting Tephritidae, with new records from Argentina, Bolivia and Costa Rica. J Hym Res 7:102–115

Yépes RF, Vélez R (1989) Contribución al conocimiento de las moscas de la fruta (Tephritidae) y sus parasitoides en el Departamento de Antioquia. Rev Fac Agron Medellín 42:73–98