

# Are individuals from thelytokous and arrhenotokous populations equally adept as biocontrol agents? Orientation and host searching behavior of a fruit fly parasitoid

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**Abstract** Hymenopteran parasitoids generally reproduce by arrhenotoky, in which males develop from unfertilized eggs and females from fertilized eggs. A minority reproduce by thelytoky, in which all-female broods are derived from unfertilized eggs. Thelytokous populations are potentially of interest for augmentative biological control programs since the exclusive production of females could significantly lower the costs of mass rearing. Behavioral traits are a

major component of parasitoid efficacy. Here, we examined orientation and host searching behavior in thelytokous and arrhenotokous populations of the fruit fly parasitoid *Odontosema anastrephae* Borgmeier (Hymenoptera: Figitidae). Orientation behavior to various odorant sources was studied in a two-choice olfactometer. No major differences were found between thelytokous and arrhenotokous wasps for this behavior. However, when host-searching behaviors were analyzed, some differences were found. Thelytokous females arrived sooner, foraged longer, and remained longer on non-infested guavas than arrhenotokous females. Individuals of both forms exhibited similar stereotyped behavioral sequences vis-à-vis guava treatments, with only slight deviations detected. Our results suggest that individuals from selected thelytokous and arrhenotokous *O. anastrephae* populations have similar abilities to search for tephritid larvae, supporting the use of thelytokous strains for augmentative releases.

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## Introduction

Pest fruit flies infest hundreds of species of fruits and vegetables (e.g., Liquido et al. 1991; Aluja and Mangan 2008), and are responsible for trade

restrictions wherever these infestations occur (Siebert 1999; Siebert and Cooper 1995; Aluja and Mangan 2008). Of particular importance in the New World are the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), and species of *Anastrepha* (e.g., the Mexican fruit fly, *A. ludens* [Loew] and the West Indian fruit fly, *A. obliqua* [Macquart] [Diptera: Tephritidae]) (White and Elson-Harris 1992). Despite the importance of *C. capitata* to export economies, *Anastrepha* spp. are considered of even greater significance throughout Latin America and the Caribbean (Aluja 1994, 1999). Traditional area-wide control for *C. capitata* and *Anastrepha* spp. consists of bait sprays containing a protein-based attractant/food source and an insecticide (Vargas et al. 2001; Burns et al. 2001). In large-scale eradication programs, these sprays are followed by repeated mass releases of sterile males (e.g., Knipling 1979; Rull et al. 1996).

Augmentative releases of tephritid fruit fly parasitoids can also significantly suppress pest populations, with or without accompanying sterile males (Wong et al. 1991, 1992; Sivinski et al. 1996; Montoya et al. 2000), and hold promise as an additional technique for area-wide control. Such releases may be particularly attractive in urban or environmentally sensitive areas where chemical controls, i.e., the commonly used insecticide-bait sprays, are difficult or impossible to employ (Rendon et al. 2006). Agricultural action agencies such as USDA-APHIS and MOSCAMED have questioned the technique because of its perceived expense relative to both chemical and sterile insect technique (SIT) controls (JS, personal communication). Further reductions in cost would make the technique increasingly attractive, and dramatic savings could be achieved by the colonization of thelytokous populations (Stouthamer 1993).

While the majority of Hymenoptera reproduce through arrhenotoky (i.e. males develop from unfertilized eggs and females from fertilized eggs) (Godfray 1994; Heimpel and de Boer 2008), a significant minority are thelytokous (all-female broods derived from unfertilized eggs) (Luck et al. 1993; Stouthamer 1997). Thelytoky is often the result of infection with cellular endosymbionts, particularly strains of the bacterium *Wolbachia* ( $\alpha$ -proteobacteria) (Stouthamer et al. 1999). These bacteria induce parthenogenesis through the abortion of anaphase during the first mitotic division (Stouthamer and Kazmer 1994;

Pannebakker et al. 2004), changing haploid males into diploid females capable of vertically transmitting the bacteria in the egg cytoplasm to their offspring (Floate et al. 2006).

Among the parasitic hymenopteran families that include natural enemies of tephritid larvae (see Ovruski et al. 2000), the Figitidae have four described thelytokous species, all of which have also been found to be infected with *Wolbachia*. These are parasitoids of *Drosophila* spp. and agromyzid leaf-miners (Ara-kaki et al. 2001; Pannebakker 2004; Pannebakker et al. 2004, 2005), as well as two recently described cases of fruit fly parasitoids (Ovruski et al. 2007; Copeland et al. 2010). Such thelytokous populations could be important resources for augmentative biological control programs because of potential advantages such as reduced production costs (a parthenogenetic population of equal fecundity and efficacy would be half as expensive to produce) and easier establishment of populations (Aeschlimann 1990; Stouthamer 1993, 2003).

However, before considering mass rearing of thelytokous wasps, their potential for success as biological control agents should be compared with that of their arrhenotokous counterparts by measuring differences in traits that could impact efficiency. Some of these traits are morphological or physiological, such as fecundity, longevity, adult size, and parasitism rates (Grenier and De Clercq 2003). Reductions in parasitism rates, emergence, progeny production, and survivorship of *Wolbachia*-infected *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae) have been reported (Miura and Tagami 2004; Hohmann et al. 2001). On the other hand, other parameters such as numbers of mature ovarian eggs at emergence of *T. kaykai* (Miura and Tagami 2004) are similar in infected and uninfected strains. In the case of *T. kaykai*, thelytokous populations have greater longevity and a higher intrinsic rate of natural increase (Miura and Tagami 2004). Thus, it is difficult to generalize concerning the effects of thelytoky on these parameters and the examination of new species/populations should be done on an individual basis.

There are other components to assess parasitoid efficacy; namely, behavioral components. Studies of behavioral traits such as host searching and host location could help to better estimate thelytokous vs. arrhenotokous populations' potential as biological control agents (Lewis et al. 1990; van Lenteren

1986; Grenier and De Clercq 2003). Silva et al. (2000) found that thelytokous females of *Trichogramma cordubensis* Vargas and Cabello moved more under greenhouse conditions, and concluded that these wasps have good biocontrol potential in spite of their relatively lower fecundity. Ardeh et al. (2005a, b) found no differences between arrhenotokous and thelytokous *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) with respect to host discrimination. Amat et al. (2006) saw no differences in the oviposition behaviors of arrhenotokous and thelytokous *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae), although arrhenotokous females exhibited longer patch residence times. Subsequently, Amat et al. (2009) found similar responses by both populations to parasitized hosts, but thelytokous females adjusted their patch residence time by using cues from conspecifics while arrhenotokous wasps did not. In general, these studies found that although there may be some behavioral differences between thelytokous and arrhenotokous populations during initial movement after release, both populations display similar host selection, acceptance, and handling behaviors.

Most available information for *Odontosema anastrephae* Borgmeier (Hymenoptera: Figitidae) corresponds to the arrhenotokous population, though a limited amount of genetic sequence information is available for both the arrhenotokous and thelytokous strains (Copeland et al. 2010). This species is described as a solitary, koinobiont endoparasitoid that attacks third instar larvae of *C. capitata* and several species of the genus *Anastrepha* (Ovruski et al. 2000). It is most often associated with guavas (*Psidium guajava*), and individuals are most commonly found in fallen fruit (Sivinski et al. 1997; Lopez et al. 1999). *Odontosema anastrephae* can undergo diapause for up to 11 months (Aluja et al. 1998) and adults live up to 46 and 50 days (females and males, respectively) under laboratory conditions (unpubl. data). As recently shown by Aluja et al. (2009a), *O. anastrephae* females exhibit a stereotyped sequence of behaviors in the proximity of infested guavas.

Prior to host selection and acceptance, other behavioral steps such as orientation and host searching are crucial for successful parasitism. To our knowledge, these behaviors have not been studied for thelytokous and arrhenotokous parasitoids, but could eventually be part of rapid initial bioassays to estimate relative potentials. The objective of this study was to

determine whether thelytokous and arrhenotokous females exhibited differences during orientation and host-searching behaviors. The orientation behavior of thelytokous females to various odorant sources was compared with the behavior exhibited by arrhenotokous females. Host-searching behaviors were also compared between thelytokous and arrhenotokous *O. anastrephae* females. We discuss our findings with regard to the competence of thelytokous *O. anastrephae* for biological control.

## Materials and methods

### Parasitoids

The original arrhenotokous population of *O. anastrephae* was collected in 1998 from guavas infested with *A. striata* (Schiner) and *A. fraterculus* (Wiedemann) larvae in Llano Grande and Tejería, Veracruz, México (Aluja et al. 2009b). [Note: though some researchers have proposed a name change for this species, this remains controversial, and the generally accepted name for this parasitoid remains *O. anastrephae*. (See Copeland et al. 2010)]. Thelytokous *O. anastrephae* were also collected from guavas, infested mainly with *A. striata*, in 2000 in La Mancha, Santiago Tuxtla and in San Andrés Tuxtla, in Veracruz State, México, and in the vicinity of Tapachula, Chiapas State, México (Aluja et al. 2009b). Colonies of arrhenotokous and thelytokous *O. anastrephae* were started with small numbers of insects (no more than 60 each) and maintained under laboratory conditions at the facilities of the Unidad de Entomología Aplicada, INECOL, Mexico, as described by Aluja et al. (2009b). Infection of thelytokous *O. anastrephae* with *Wolbachia* endosymbionts was confirmed by PCR analyses and females exposed to antibiotics revert to the production of males (unpubl. data). Parasitoids of both populations were reared using third-instar *Anastrepha ludens* Loew larvae, at  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  r.h. and an L12:D12 photoperiod. At emergence, adults of both strains were transferred into plastic containers ( $11.5 \times 17 \times 16$  cm) and provided with honey and water *ad libitum* until used in experiments. Arrhenotokous parasitoids were caged at a 1:1 [male:female] sex ratio after emergence and held thus until use (i.e. females had ample opportunities to mate and were presumably mated). Prior to bioassay, parasitoids were

not exposed to either fruit fly larvae or guavas (i.e., they were “naïve”). Individuals were tested when they were four to eight days old.

### Odor sources

As odor sources, we used guavas at a stage when the surfaces were ~80% yellow, 20% green with a mean weight ( $\pm$ SE) of  $52.8 \pm 0.32$  g. Artificially infested guavas (hereafter “infested”) were prepared by cutting transversally along the peduncle, about  $\frac{1}{4}$  of the length, from the proximal end. These sections served as ‘lids’ and the rest of the fruit served as ‘bases’ for filling. Pulp was extracted from the bases and replenished with 20 third-instar (7–8 day old) lab-reared *A. ludens* larvae mixed with artificial diet (Aluja et al. 2009b). After filling, we placed the lids on top of the bases, and sealed them with a strip of Parafilm®. We used a 2 mm metal probe to make six artificial punctures (opposite sides) that enabled larval respiration (Aluja et al. 2009b). Artificial diet was absolutely necessary because otherwise larvae would immediately crawl out of the fruit through the artificial punctures. Puncturing occurred a few minutes prior to bioassay initiation. Non-infested guavas (hereafter “non-infested”) followed the same treatment as infested ones, but no *A. ludens* larvae were employed.

### Experiment 1: response to odor sources

Experiments were performed in a controlled-environment room at  $26 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  r.h. and a light intensity of 215 lux. A two-choice olfactometer was used to determine if *O. anastrephae* females used distance cues, presumably chemical, to locate host larvae. This bioassay device was similar to that described by Sivinski et al. (1998) and consisted of an arena and two collecting jars. The arena was a plastic storage container,  $19.5 \times 13.5 \times 4.5$  cm, with its lid replaced with fine-mesh screen. At the center of its floor, a hole (2.5 cm diameter) served as a release point. At opposite ends of the arena, two circular, 6 cm diameter holes were made and the mouths of collecting jars were inserted in each hole to be flush with the arena-floor. These collecting jars (6 cm diam  $\times$  13.5 cm height) had their bottoms replaced with fine-mesh screen, and contained (or, in the case of blank controls, did not contain) an odor source. When the bioassay device was lifted on blocks above the

surface of a table, air flow from the top of the container through the bottom of the collecting jars, and vice versa, was possible. A plastic transparent sheet was placed between jars to avoid odors passing from one jar to another. After each replicate, the olfactometers were washed with water and cleaned with ethanol and the positions of the odorized jars were changed to prevent any directional bias.

For each parasitoid population, the following treatments were tested: (i) blank vs. infested guava, (ii) blank vs. non-infested guava, and (iii) infested vs. non-infested guava. “Blank” refers to an empty collecting jar with no odor source; “infested” and “non-infested” refer to collecting jars containing an infested or non-infested guava, respectively. In the present experiment, all guavas were covered with fine-mesh net to prevent parasitoid contact with the guava’s surface. There was concern that such contact might result in the deposition of parasitoid-derived pheromones and so potentially change the volatiles emitted by the odor source. After each replicate, new guavas were used to avoid pseudoreplication.

Each morning at 08:00, all treatments were prepared to be run simultaneously. One hour later, ten female parasitoids per treatment were released in the olfactometer through a plastic container inserted at the release point. Females could freely move into the arena and go down into the collecting jars or remain in the arena (no-response). To establish if, during the first few hours, thelytokous and arrhenotokous females responded to odor sources in a similar way, the number of females in the collecting jars was recorded every hour (from 10:00 to 16:00). The numbers of thelytokous and arrhenotokous females responding to odorant sources during the seven hours following release were compared by using a repeated-measures mixed effects model. The fixed component was the population nested within the treatment, with the hour as covariate, and the random component defining the repeated measures was the time factor, modeled as a function of the individual assay. Pos-hoc tests were performed with contrasts derived from the linear model (Pinheiro and Bates 2000).

To determine if, after a longer exposure period, thelytokous and arrhenotokous females responded similarly to odor sources, the number of parasitoids in the collecting jars (responding) and in the arena (not responding) was recorded 24 h after the initial wasp release. For each treatment, the mean numbers of

wasps in the collecting jars, as well as those that did not respond, were compared between thelytokous and arrhenotokous females via a generalized linear model (GLM) with Poisson error a Log link function (Crawley 2007). Comparisons among treatment combinations were done with contrasts based on the linear predictor of the model. All treatments were replicated 11 times and a total of 330 females per population were tested.

#### Experiment 2: close-range host foraging behavior

Observations were performed under the same environmental conditions as those in Experiment 1. In plastic cages (17 cm diameter  $\times$  14.5 cm height), a treated guava was placed in the center of the floor. The following treatments were examined: (1) infested guavas, (2) non-infested guavas, and (3) cleaned guavas (i.e., fruits were washed, rinsed, and used once). Cleaned guavas were used as a negative control because presumably there is no production of arresting cues from host larvae, such as vibratory signals (Meyhofer and Casas 1999) or related products (e.g., frass) (Turling et al. 1991).

Every day at 08:00, the three guava treatments were simultaneously placed in separate experimental arenas. Following the methods described in Aluja et al. (2009a), an hour later, one female parasitoid, either arrhenotokous or thelytokous, per guava-treatment was introduced via an open glass vial (4  $\times$  2 cm height and diameter) into the arenas. Females could freely move into the arena, move onto the guava, or remain in the vial (no response). Observations were performed from 09:00 to 17:00 and behavior was continuously recorded. An observation started when the female was released and ended when the female left the guava. If a female remained motionless inside the vial for three hours, the test was considered invalid and the female was removed and replaced by another. Cage position on the observation table was assigned randomly every morning. Each guava treatment was replicated 15 times per parasitoid reproductive population. Thus, a total of 45 females per population were analyzed. A new guava and a new parasitoid female were always used for each replicate.

We recorded the time spent to reach the guava (finding time), the time spent on the guava (residence time), and the time spent foraging inside the guava (time inside). The finding time, residence time, and

time inside the fruit were compared between arrhenotokous and thelytokous populations through a generalized linear model (GLM) with the population nested within the treatments. The GLM was performed with a gamma error and an inverse link function. Pos-hoc tests were performed with contrasts derived from the linear model.

Based on preliminary observations and behavioral studies by Aluja et al. (2009a), the searching behavior of *O. anastrephae* was characterized and a behavioral catalogue of eleven distinctive elements was built (Table 1). This catalogue was used for both populations because they displayed similar behaviors. Frequencies of each type of behavior were analyzed using a GLM with a Poisson error and the populations were nested within the treatments. When significant differences were detected, we performed pos-hoc contrasts based on *t* tests with standard errors estimated from the linear predictor (Crawley 2007). Then we applied a sequential Bonferroni criterion (Rice 1989) to avoid overestimating significant differences (type I errors).

Frequencies of transitions from one behavior to another were first tabulated and then consolidated into a transition probability matrix using only first-order, preceding-following behavioral transitions (Lehner 1996). Self-transitions (i.e., repetitions of the same behavior) were not included in these matrices. A transition matrix was created for each thelytokous and arrhenotokous individual (15 females per reproductive population). Furthermore, for each population within treatments, another global transition matrix was created by pooling values from all 15 females. The principal diagonal elements of these matrices were logical zeros since behaviors could not follow themselves in our records. Expected transition frequencies were calculated as follows:  $\text{Expected} = (\text{row sum} \times \text{column sum}) / (\text{grand sum})$  (Lehner 1996). The statistical significance of the overall table was evaluated using a log-likelihood ratio (*G*) test. Yates correction for continuity was applied throughout. When deviations in the overall table were found to be statistically significant, significant transitions were found by collapsing the tables into 2  $\times$  2 matrices around each transition and performing a *G* test. The significance of these individual tests was adjusted to a table-wide level of 5% using the sequential Bonferroni method (Rice 1989). The results of analyses of behavioral transition matrices are presented graphically in flow diagrams (Field and Keller 1993; Mondor and Roland 1998).

**Table 1** Catalogue of behaviors of arrhenotokous and thelytokous *Odontosema anastrephae* females considered/analyzed in this study

Event	Symbol	Description
Walk	WK	The wasp moves along the guava surface, usually in several directions
Rotation	RO	The wasp rotates on the same axis on the guava surface, usually spinning only once
Antennate walking	AW	The wasp palpates the guava surface with both antennal tips while moving along the guava surface
Detection with tarsi	DT	The wasp remains motionless on the guava surface, presumably using tarsi to detect concealed larvae, antennae are extended and tilted forward
Entering	EN	Penetration into the guava by the wasp
Exit	EX	Resurfacing from the interior of the guava after penetration and residence for a determined period of time
Oviposition	OV	Insertion of the ovipositor completely inside the fruit for periods of time longer than one minute
Groom	GR	Any cleaning of the body, including stroking the wings, head, or antennae with the legs, rubbing the legs together, or cleaning the legs with mouthparts
Head rubbing	HR	Rubbing of the wasp's head on the surface of the guava, usually between walking events
Detection with tarsi & antennae	DTA	Detection with tarsi combined with frequent palpation of the guava surface with both antennal tips
Probing	PR	When wasp incompletely inserts its ovipositor into fruit for less than a minute

## Results

### Experiment 1: response to odor sources

Overall, the numbers of thelytokous and arrhenotokous females responding to odorant sources during the seven hours following release were significantly different ( $F = 3.484$ ; 3,390 df;  $P = 0.016$ ). However, pos-hoc comparisons showed marginal and non-significant differences. The first choice test (i.e., blank vs. infested guava) found no significant differences in responses ( $t = 1.900$ ; 390 df;  $P = 0.058$ ) (Fig. 1a). The second (i.e., blank vs. non-infested guava) also found no significant differences in responses ( $t = 1.859$ ; 390 df;  $P = 0.063$ ) (Fig. 1b). When the experimental arena contained infested vs. non-infested guavas, no significant differences in the response to infested guavas were observed between thelytokous and arrhenotokous wasps ( $t = 1.838$ ; 390 df;  $P = 0.066$ ) (Fig. 1c).

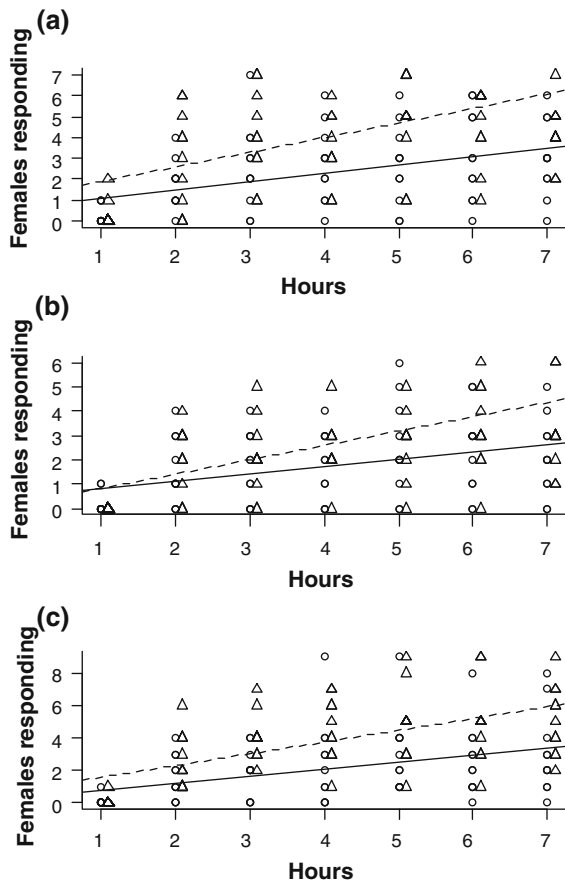
Over a 24 h exposure to blanks vs. infested guavas, females of both population types responded significantly more to the fruit-odor source (Fig. 2a), but no significant difference was found between thelytokous and arrhenotokous females. When females were offered blank vs. non-infested guavas (Fig. 2b), and non-infested vs. infested guavas (Fig. 2c) only thelytokous females responded significantly more to the odor sources. However, these responses were not

significantly higher than those of arrhenotokous females (Fig. 2b, c).

### Experiment 2: close-range host foraging behavior

When fruit-finding time was analyzed (Fig. 3a), thelytokous wasps required significantly less time than arrhenotokous wasps to reach non-infested guavas ( $t = 2.148$ ; 84 df;  $P = 0.034$ ) and cleaned guavas ( $t = 2.187$ ; 84 df;  $P = 0.031$ ). However, both populations required similar amounts of time to find infested guavas (Fig. 3a). In terms of residence time (Fig. 3b), thelytokous wasps remained significantly longer than arrhenotokous wasps ( $t = 1.998$ ; 84 df;  $P = 0.048$ ) on non-infested guavas. There were no significant differences between population types in time spent on infested and cleaned guavas. Thelytokous wasps spent significantly more time inside non-infested fruits than arrhenotokous wasps ( $t = 2.507$ ; 84 df;  $P = 0.014$ ) (Fig. 3c). This difference was not observed in infested and cleaned guava treatments (Fig. 3c).

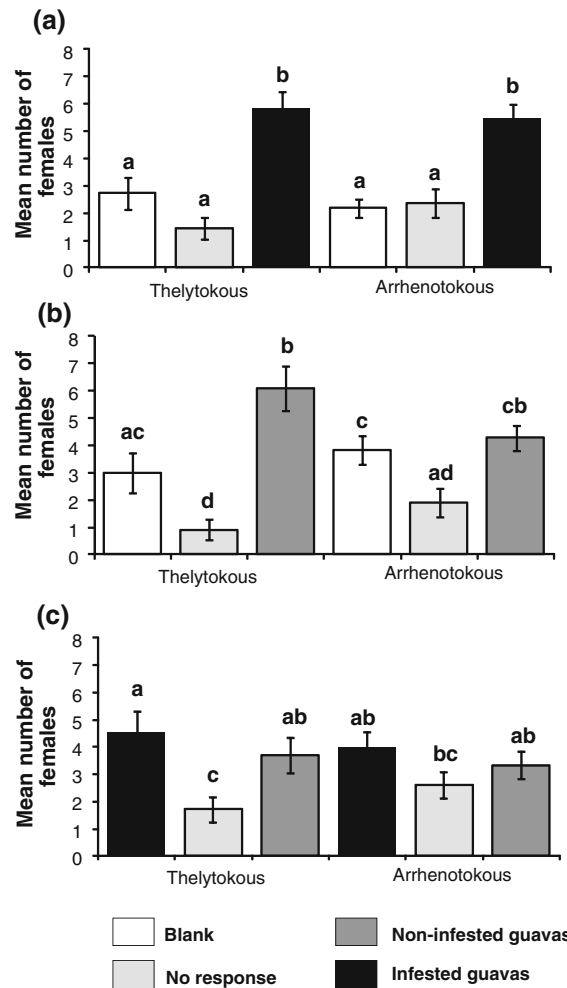
When frequencies of the 11 recorded behaviors were analyzed, a significant difference between thelytokous and arrhenotokous females was found in two behaviors. These behaviors, 'antennate' and 'head rubbing' were displayed significantly more often by arrhenotokous females than by their thelytokous counterparts (Table 2).



**Fig. 1** Mean number of *Odontosema anastrephae* females (arrhenotokous are indicated by empty circles and thelytokous by empty triangles) responding to odorant sources at the indicated hours following release. Three experimental setups are shown: **(a)** blank vs. infested guavas (showing only responses to infested guavas [ $t = 1.900$ ; 390 df;  $P = 0.058$ ]), **(b)** blank vs. non-infested guavas (showing only responses to non-infested guavas [ $t = 1.859$ ; 390 df;  $P = 0.063$ ]) and **(c)** infested vs. non-infested guavas (showing only the responses to infested guavas [ $t = 1.838$ ; 390 df;  $P = 0.066$ ])

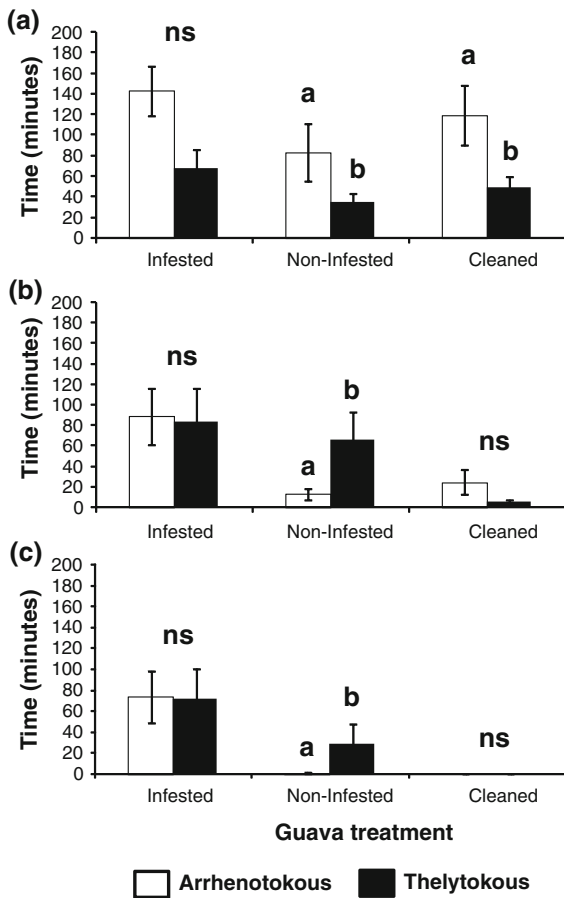
Host searching behavior of arrhenotokous and thelytokous females in the three experimental conditions is summarized in Figs. 4, 5 and 6. Females of both populations initially arrived at the guavas (by flying or walking) and then exhibited a stereotyped sequence of behaviors that included walking-detection with tarsi-groom. Overall, arrhenotokous females exhibited a more complex behavioral repertoire than their thelytokous counterparts.

When females of both populations were exposed to infested guavas (Fig. 4), they initially walked on the guava’s surface and then frequently exhibited detection with tarsi. However, after this sequence,



**Fig. 2** Mean ( $\pm$ SE) numbers of *Odontosema anastrephae* females (thelytokous and arrhenotokous) responding or not to combinations of odorant and blank sources in a two-way olfactometer over a 24 h period. Three treatments are shown: **(a)** blank vs. infested guavas, **(b)** blank vs. non-infested guavas and **(c)** non-infested vs. infested guavas. No response refers to insects that did not move into collection chambers. For each treatment, the mean numbers of wasps were compared via a GLM with Poisson error a Log link function. Comparisons among treatment combinations were done with contrasts based on the linear predictor of the model. Means sharing a letter are not significantly different ( $P = 0.05$ )

arrhenotokous females engaged in antennating, groom, or probing behaviors (Fig. 4a) whereas thelytokous females engaged frequently in groom and walking behaviors (Fig. 4b). Arrhenotokous females frequently displayed sequences such as detection with tarsi-antennating, antennating-antennate & walking, and entering-exiting (Fig. 4a), whereas their thelytokous counterparts did not (Fig. 4b).



**Fig. 3** (a) Mean ( $\pm$ SE) finding times (in minutes) for arrhenotokous and thelytokous females confronted with infested, non-infested and cleaned guavas, (b) Mean residence times ( $\pm$ SE) and (c) mean times inside the fruit ( $\pm$ SE). Different letters indicate significant differences between populations ( $P = 0.05$ ); ns not significantly different (GLM analysis with a gamma error and an inverse link function)

During host searching on non-infested guavas (Fig. 5), the pattern of behavior displayed by individuals of both types was less complex relative to that on infested guavas (Fig. 4). Individuals of both populations displayed the stereotyped sequence walking-detection with tarsi-groom and only arrhenotokous females displayed an additional sequence of behaviors: walking-antennate & walking-detection with tarsi-walking (Fig. 5a).

Finally when wasps were confronted with cleaned guavas (Fig. 6), thelytokous females exhibited a “random” behavioral pattern (Fig. 6b). This is interpreted as “random” because the overall transition table was not significantly different from the expected

transition table. In contrast, arrhenotokous females displayed the stereotyped sequence: walking-detection with tarsi-groom followed by other significant sequences such as detection with tarsi-antennating-detection with tarsi and detection with tarsi-head rubbing sequences (Fig. 6a).

## Discussion

As yet, no general patterns of increased or decreased fitness parameters in thelytokous populations have been firmly established. Some studies have reported parasitism rates, emergence, and progeny production to be low in thelytokous organisms when compared with their arrhenotokous counterparts (Miura and Tagami 2004; Hohmann et al. 2001). However, other characteristics, such as mature ovarian eggs at emergence, can be similar (Miura and Tagami 2004) and thelytokous wasps may have relatively greater longevity and higher intrinsic rates of natural increase (Miura and Tagami 2004), as well as more rapid responses to hosts (Silva et al. 2000). Interpretation of the present comparison of thelytokous and arrhenotokous *O. anastrephae* was complicated by the allopatric origins of the contrasted strains. Thus, any differences may have nothing to do with *Wolbachia* infection or thelytoky, but might be the result of different selection pressures in different original environments. Since *Wolbachia*-induced thelytoky tends to be fixed in populations (Huigens and Stouthamer 2003), this will be a common dilemma. A possible solution to this problem would be to “cure” a single population of endosymbiont infection through antibiotic treatment and then colonize a bisexual culture from the treated parental stock to compare with the original thelytokous population. However, even this strategy assumes that only the sex ratio distorting bacteria would be eliminated and that no beneficial members of the microflora would be inadvertently eradicated at the same time (Nogge and Gerresheim 1982; Wilkinson 1998; Visotto et al. 2009). Alternative explanations for behavioral differences could include genetic drift or bottlenecks during colonization. The numbers of original founders might reflect the upper level of genetic diversity that was likely to have been included in the colonies. But in our case, it seems difficult to estimate what significance these laboratory population sizes could have either in



**Table 2** Mean  $\pm$  SE frequency for each type of behavior by thelytokous and arrhenotokous *Odontosema anastrephae*, observed for three different treatments

Behavior	Infested and punctured		Infested and non-punctured		Cleaned	
	Thelytokous	Arrhenotokous	Thelytokous	Arrhenotokous	Thelytokous	Arrhenotokous
Walking	9.26 $\pm$ 2.32	5.20 $\pm$ 1.76	8.20 $\pm$ 3.04	10.53 $\pm$ 5.42	9.13 $\pm$ 3.06	5.80 $\pm$ 1.25
Detection with tarsi	9.60 $\pm$ 2.62	10.13 $\pm$ 3.71	9.86 $\pm$ 3.69	12.73 $\pm$ 6.91	7.20 $\pm$ 2.33	9.80 $\pm$ 2.50
Antennate	2.00 $\pm$ 1.06 a	5.60 $\pm$ 2.37 b	0.80 $\pm$ 0.53 a	4.20 $\pm$ 2.87 b	0.33 $\pm$ 0.23 a	1.33 $\pm$ 0.92 b
Antennate walking	2.00 $\pm$ 1.45	6.20 $\pm$ 2.25	4.13 $\pm$ 2.60	2.40 $\pm$ 1.20	0.53 $\pm$ 0.32	0.93 $\pm$ 0.66
Groom	3.06 $\pm$ 1.03	5.86 $\pm$ 2.81	3.00 $\pm$ 1.09	3.46 $\pm$ 1.65	2.80 $\pm$ 1.13	5.40 $\pm$ 2.07
Head rubbing	0.86 $\pm$ 0.61	1.33 $\pm$ 0.76	1.60 $\pm$ 0.66	0.93 $\pm$ 0.44	0.33 $\pm$ 0.18 a	3.26 $\pm$ 2.00 b
Rotation	0.73 $\pm$ 0.43	2.26 $\pm$ 1.45	1.00 $\pm$ 0.35	3.13 $\pm$ 1.34	0.86 $\pm$ 0.29	1.40 $\pm$ 0.49
Entering	0.66 $\pm$ 0.21	1.60 $\pm$ 0.52	0.13 $\pm$ 0.13	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Exit	0.66 $\pm$ 0.21	1.60 $\pm$ 0.52	0.13 $\pm$ 0.13	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Probing	1.60 $\pm$ 0.66	4.93 $\pm$ 1.86	2.33 $\pm$ 1.44	5.46 $\pm$ 3.37	0.00 $\pm$ 0.00	0.06 $\pm$ 0.06
Oviposition	0.20 $\pm$ 0.14	0.93 $\pm$ 0.74	1.00 $\pm$ 0.72	0.66 $\pm$ 0.39	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

For each behavior, a GLM analysis with a Poisson error was performed (populations were nested within treatments). When significant differences were detected, pos-hoc contrasts were performed. A sequential Bonferroni criterion to avoid overestimating significant differences was applied. Only significant differences are indicated by different letters

terms of genetics or behavior. Nevertheless, a genetic approach to these behaviors could provide additional information to better understand the origin of any differences. It is known that genetic variation, together with environmental factors, can play a role in some parasitoid behavioral traits (Olson and Andow 2002; Wang et al. 2003). In the end, the efficacies of new thelytokous species/populations considered for augmentative release probably need to be examined on an individual basis, perhaps through comparison to a standard, bisexual population. In this way, any shortcomings of various particular populations could be weighed against the larger numbers of females that could be mass-reared and released.

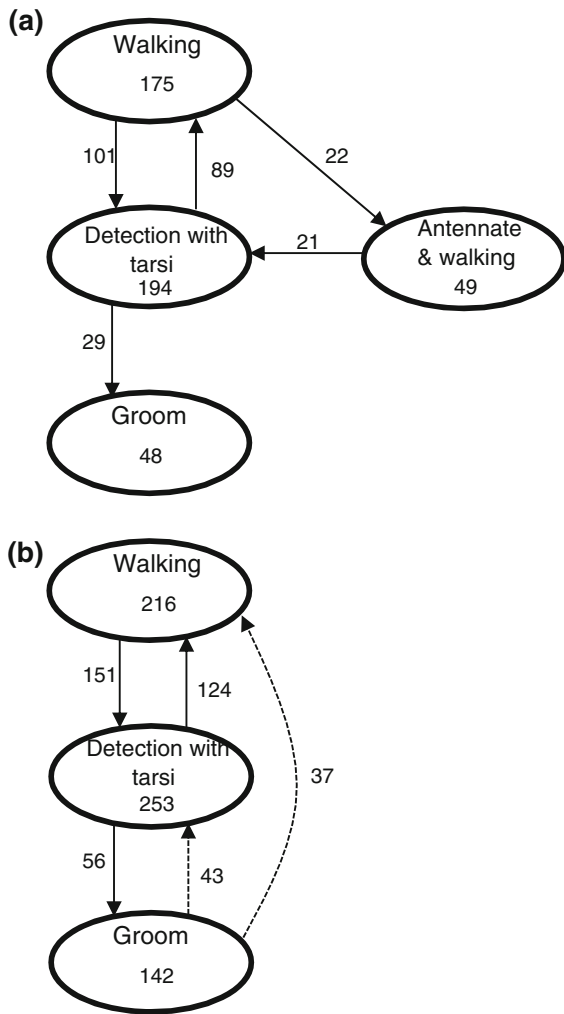
If this were to be done, what should be the basis of comparison? Are there bioassays that might quickly eliminate a thelytokous population from consideration? Behavioral traits are obviously a major component of parasitoid performance. A diminished capacity to perform important initial steps following release, such as moving away from the release site, orientation to target fruit, and foraging, would cast doubts on the agricultural usefulness of the wasps.

In the present study, both female types performed similarly during orientation behavior to odor sources, suggesting that both populations would have a similar potential to initially identify potential hosts and host-occupied sites. In terms of short-range foraging, thelytokous wasps reached non-infested guavas more

rapidly than arrhenotokous ones, and spent more time upon the fruits. These differences did not occur with infested fruit, which is consistent with their similar rates of parasitism in culture (Aluja et al. 2009b). This seemingly inappropriate expenditure of time and energy by thelytokous parasitoids may be evidence of poorer sensory capacity or could represent an adaptive response evolved subsequent to *Wolbachia* infection. If, after further investigations in additional species, quicker initial responses to and longer investigations of low-quality oviposition sites turn out to be a widespread phenomenon, then perhaps a slightly negative effect of *Wolbachia* infection and shortened life expectancy has selected for less discrimination and more rapid responses to marginal oviposition opportunities.

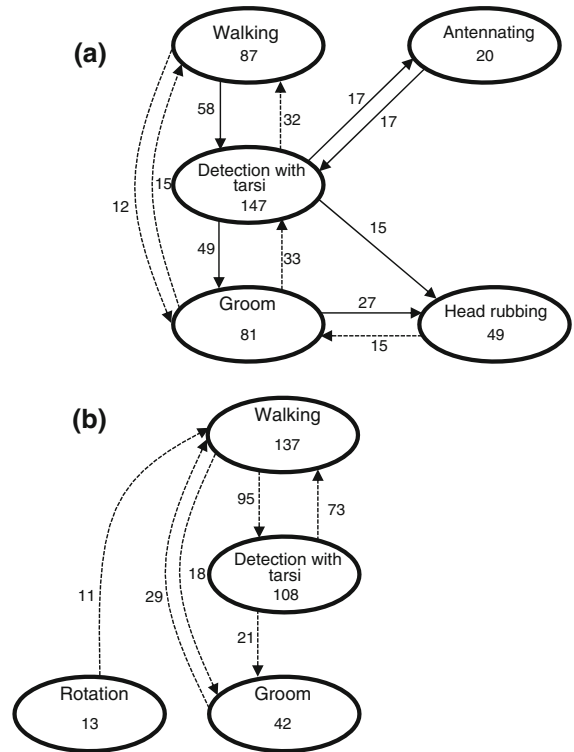
The behavioral repertoire of *O. anastrephae* is relatively simple compared with other figitids, such as *Aganaspis pelleranoi* (Brèthes) (Aluja et al. 2009a), or fruit fly-attacking braconids, such as *Fopius arisanus* (Sonan) (Wang and Messing 2003), and this repertoire was simpler still in thelytokous wasps. Of 11 close-range foraging behaviors and their relationships to one another: (1) two were significantly less practiced by thelytokous wasps (Table 2), and (2) some sequences of behaviors that occurred in arrhenotokous females were absent in thelytokous ones (Figs. 4, 5, 6). A particular behavior not displayed by thelytokous wasps on non-infested guavas (Fig. 5)





**Fig. 5** Flow diagram showing the behavioral sequence of (a) arrhenotokous and (b) thelytokous females of *Odontosema anastrephae* confronted with non-infested guavas. Boxes represent behavioral acts, numbers inside the boxes are behavioral repetitions. Solid arrows represent transitions significantly different from expected (non-random), and dashed arrows represent transitions not significantly different from expected (random). Numbers next to arrows are numbers of transitions. Transitions with a relative frequency lower than 3% are not shown

Overall, however, thelytokous and arrhenotokous *O. anastrephae* females appear to have similar potential for biological control. The differences in behavior that were observed are best described as minor. Both strains oriented equally to odor sources. Thelytokous females invested somewhat more time in investigating fruit without hosts and might have gathered lesser amounts of sensory input because of a simplified behavioral repertoire. It is unclear why



**Fig. 6** Flow diagram showing the behavioral sequence of (a) arrhenotokous and (b) thelytokous females of *Odontosema anastrephae* confronted with cleaned guavas. Boxes represent behavioral acts, numbers inside the boxes are behavioral repetitions. Solid arrows represent transitions significantly different from expected (non-random), and dashed arrows represent transitions not significantly different from expected (random). Numbers next to arrows are numbers of transitions. Transitions with a relative frequency lower than 3% are not shown

these differences occurred, i.e., if they are a debilitating result of *Wolbachia* infection or if they are due to pre- or post-infection selection. While parasitism is similar in both laboratory colonies (Aluja et al. 2009b), the next step is to determine their rates of increase under semi-natural conditions. If placed in a complex environment consisting of infested and uninfested fruit, will thelytokous females' simplified foraging behaviors be as efficient as those of their arrhenotokous competitors? Even if not, would the advantage of all-female broods still result in the eventual thelytokous replacement of arrhenotokous females in mixed-cohorts? If so, then the usefulness of the thelytokous strain for biological control would be supported. The present behavioral analyses suggest that the degree of difference between strains is

inconsequential for choosing biological control candidates. This study could also provide a foundation for the design of bioassays that quickly compare the efficacies of bi- and unisexual parasitoids.

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