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Larval feeding substrate and species significantly influence the effect of a juvenile hormone analog on sexual development/performance in four tropical tephritid flies

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ABSTRACT

The juvenile hormone (JH) analog methoprene reduces the amount of time it takes laboratory-reared *Anastrepha suspensa* (Caribbean fruit fly) males to reach sexual maturity by almost half. Here, we examined if methoprene exerted a similar effect on four other tropical *Anastrepha* species (*Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha serpentina* and *Anastrepha striata*) reared on natural hosts and exhibiting contrasting life histories. In the case of *A. ludens*, we worked with two populations that derived from *Casimiroa greggii* (ancestral host, larvae feed on seeds) and *Citrus paradisi* (exotic host, larvae feed on pulp). We found that the effects of methoprene, when they occurred, varied according to species and, in the case of *A. ludens*, according to larval host. For example, in the case of the two *A. ludens* populations the effect of methoprene on first appearance of male calling behavior and number of copulations was only apparent in flies derived from *C. greggii*. In contrast, males derived from *C. paradisi* called and mated almost twice as often and females started to lay eggs almost 1 day earlier than individuals derived from *C. greggii*, but in this case there was no significant effect of treatment (methoprene) only a significant host effect. There were also significant host and host by treatment interactions with respect to egg clutch size. *A. ludens* females derived from *C. paradisi* laid significantly more eggs per clutch and total number of eggs than females derived from *C. greggii*. With respect to the multiple species comparisons, the treatment effect was consistent for *A. ludens*, occasional in *A. serpentina* (e.g., calling by males, clutch size), and not apparent in the cases of *A. obliqua* and *A. striata*. Interestingly, with respect to clutch size, in the cases of *A. ludens* and *A. serpentina*, the treatment effect followed opposite directions: positive in the case of *A. ludens* and negative in the case of *A. serpentina*. We center our discussion on two hypotheses (differential physiology and larval-food), and also interpret our results in light of the life history differences exhibited by the different species we compared.

1. Introduction

Sexual signaling systems of most insects are correlated with adult reproductive maturity. Therefore, the endogenous mechanisms that regulate the signaling systems are often coordinated with the factors responsible for controlling sexual maturity. Juvenile hormone (JH) has been shown to coordinate both sexual maturity and sexual signaling in groups as diverse as cockroaches and moths (Schal et al., 1994, and references therein; Cusson and McNeil, 1989a,b; Cusson et al., 1994; Gadenne, 1993; Picimbon et al., 1994) and in some economically important tephritid fruit flies (Pereira, 2005).

The ability of JH to accelerate both reproductive development and sexual signaling in tephritid fruit flies was first reported for *Anastrepha suspensa* (Loew), the Caribbean fruit fly (Teal et al., 2000). These studies showed conclusively that JH or the JH mimics, methoprene and fenoxycarb, accelerated reproductive development by as much as 4–5 days and suggested that hormone "therapy" using JH or its analogs might effectively improve efficacy of the Sterile Insect Technique – SIT (Teal et al., 2007). Work by Pereira (2005), using flies recently introduced to laboratory culture, showed that male *A. suspensa* treated with methoprene not only mature earlier but also significantly out perform untreated males in obtaining successful matings throughout their lives in both laboratory and field cage assays.

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Although JH is an important hormone regulating reproductive development and subsequent sexual signaling in some tephritids, other factors can have similar consequences (Pereira et al., 2006). For example, a protein-rich adult diet in *A. suspensa* has a positive effect on the sexual success of males that is statistically indistinguishable from that of methoprene (Pereira, 2005; Teal et al., 2007), and protein-enhanced adult diets have significant influences on reproductive behavior of other tephritids as well (Blay and Yuval, 1997; Warburg and Yuval, 1997; Papadopoulos et al., 1998; Kaspi and Yuval, 2000; Kaspi et al., 2000; Yuval et al., 2002; Aluja et al., 2001a,b). While a proteinaceous adult diet was found to have the same effect as methoprene on the reproductive success of *A. suspensa* (Pereira, 2005; Teal et al., 2007), there are no comparable data available regarding possible interactions between the natural larval diet and methoprene. Gaining insight into the latter, as well as to compare the effect of methoprene on the reproductive behavior of various other *Anastrepha* species placed in two different infrageneric groups and exhibiting contrasting life histories (details in Table 1), was our aim in the present study. We hypothesized that (1) the response to the JH analogue methoprene would vary among species exhibiting different natural histories and species-specific physiologies (physiology hypothesis) and (2) larval diet plays a role in the adult response to methoprene (larval-food hypothesis).

To test these hypotheses, we selected four species within *Anastrepha* (*Anastrepha ludens* [Loew], *Anastrepha obliqua* [Macquart], *Anastrepha serpentina* [Wiedemann] and *Anastrepha striata* Schiner) exhibiting differences in reproductive investment schedules (i.e., egg vs. time limited species), clutch size, life expectancy and host use patterns (Table 1). We predicted that species that invest early and heavily in large numbers of eggs that are laid quickly to exploit an ephemeral fruit (e.g., *A. obliqua* [Aluja and Birke, 1993; Díaz-Fleischer and Aluja, 2003a]) will use larval-acquired resources as quickly as possible and as a result, the addition of JH may have relatively little effect on their already rapid maturation. On the other hand, we predicted that in species that produce eggs over an extended fruiting season (e.g., *A. ludens* [Díaz-Fleischer and Aluja, 2003a]) may have evolved particularly long pre-reproductive food-foraging periods and as a consequence, additional JH will result in noticeably accelerated maturation. Finally, we predicted that species exhibiting nuptial gifts (e.g., *A. striata* [Aluja et al., 1993]) would be resilient to environmental stimuli accelerating sexual maturation as they may need to accrue resources over a minimum period to be able to offer high quality nuptial gifts.

A. ludens is a long-lived, time-limited species (i.e., dies before being able to lay all eggs produced; Díaz-Fleischer and Aluja, 2003a), polyphagous species whose purported ancestral hosts are *Casimiroa greggii* (S. Wats.) and *Casimiroa edulis* La Llave & Lex. (both Rutaceae) (Aluja et al., 2009). In the case of *C. greggii*, females lay eggs into seeds and larvae feed on them (Aluja et al., 2000a), a behavior also occasionally seen in the case of *C. edulis* (Díaz-Fleischer, personal communication). It is considered one of the most important fruit fly pests of citrus, particularly *Citrus paradisi* Macfadyen (grapefruit). It also commonly infests mangoes (Anacardiaceae), peaches (Rosaceae), and peppers (Solanaceae), among many other fruit species (Norrbom, 2004; Thomas, 2003, 2004; Birke et al., 2006). Because larvae can variably feed on pulp or seeds in nature, larval acquired resources may differ among eclosing individuals with potential effects on adult developmental schedules and possibly influencing the effect of JH in adults fed a methoprene-supplemented diet. Its propensity to lay eggs over time may select for slower (compared to its congeneric *A. obliqua*) adult maturation and long adult life spans with an emphasis on the importance of adult feeding. As such, accelerated adult maturation resulting from JH consumption might limit an important resource-gathering period with a subsequent negative effect on early reproductive performance.

A. serpentina Wiedemann is placed within the *serpentina* species group with 10 other species including *A. striata*, *Anastrepha bistrigata*, *Anastrepha ornata* and *Anastrepha anomala* (Norrbom, 2004). It is also a clutch layer that preferentially infests fruit within the Sapotaceae (e.g., *Manilkara sapota* [L.] Van Royen, *Chrysophyllum cainito* L., *Chrysophyllum mexicanum* Brandegee ex Standl., *Calocarpum mammosum* [L.] P. Royen, *Bumelia sebolana* Lundell, *Pouteria campechiana* [Kunth] Baehni) (Norrbom, 2004). Conse-

Table 1
Overview of the most important life history characteristics/behavioral attributes plus native host availability patterns for *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha serpentina* and *Anastrepha striata* (highly modified from Aluja et al., 2001a,b).

Natural history/behavioral attribute and host availability	*A. ludens*	*A. obliqua*	*A. serpentina*	*A. striata*
Intrageneric group[a]	*fraterculus*	*fraterculus*	*serpentina*	*serpentina*
Host breadth[b]	Polyphagous	Polyphagous	Oligophagous	Stenophagous
Plant part eaten by larvae[b]	Pulp or seed	Pulp	Pulp (rarely seed)	Pulp
Native host availability	Stable (2–3 months)	Highly ephemeral (2–3 weeks)	Stable (2–3 months)	Ephemeral (1 month)
Mean ± life expectancy (days)	51.7 ± 2.2♀, 71.9 ± 6.6♂[c]	39.9 ± 22.4♀, 38.5 ± 21.7♂[d]***	52.8 ± 5♀, 44.6 ± 8.2♂[e]	83.3 ± 16.4♀[f]
Sexual, pre-maturation period (days)[g]	10–15	7–13	10–16	15–20
Clutch size (eggs)[h]	1–40	1	1–40	1–3
Egg resorption	No	Probably	?	?
Calling hour (time of day)	Single peak in late afternoon/dusk	Bimodal pattern (morning and afternoon)	Single peak during midday	From 10 to 17 h (peak between 13 and 15 h
Calling modality	Single or in lek	Single or in lek	Single or in lek	Almost always single, leks uncommon, trophallaxis present
Nuptial gift from male to female	No	No	No	Yes
Mean (±S.E.) copulation duration (min)[i]	73.4 ± 6.6	47.1 ± .09	31.1 ± 1.4	36.4 ± 2.1

*Original data in weeks.
[a] Norrbom et al. (2000).
[b] Aluja et al. (2000a).
[c] Dávila (1995).
[d] Bressan and da Costa Teles (1991).
[e] Jácome et al. (1999).
[f] Aluja et al. (2008, 2009).
[g] M.A. (unpublished information).
[h] Aluja et al. (2000a).
[i] Pérez-Staples and Aluja (2004).

quences of JH may be as above, with the exception that larval diets are mostly limited to pulp so that within species variance in resources at emergence were predicted to be more homogeneous.

In sharp contrast, *A. obliqua* invariably lays a single egg per oviposition bout (Aluja et al., 2000a; Díaz-Fleischer and Aluja, 2003a, specializing in the Anacardiaceae (e.g., *Spondias purpurea* L., *Spondias mombin* L., *Tapirira mexicana* Marchand [all native], *Mangifera indica* L. [exotic]), but also able to attack fruit within the Myrtaceae (e.g., *Myrciaria floribunda* [H. West ex Willd.], Aluja et al., 2000b). *A. obliqua* is a short-lived, egg-limited species (i.e., not enough eggs given the opportunities to oviposit during lifetime) (Díaz-Fleischer and Aluja, 2003b). As such, there may be less opportunity for a JH effect on maturation and fecundity due to an already rapid utilization of larval-acquired resources.

Finally, *A. striata* is a stenophagous species only attacking fruit within the Myrtaceae (e.g., *P. guajava*, *P. guineense* Sw., *P. sartorianum* (O. Berg), *P. cattleianum* Sabine) (Aluja et al., 2000b). It lays one to three eggs per oviposition bout and distinguishes itself from other *Anastrepha* species because males exhibit labellum-to-labellum contacts transferring materials to females (most likely trophallaxis) during courtship (Aluja et al., 1993). Because of the potentially high nutritive demands made on males that offer nuptial gifts, an accelerated male development that limits the ability to sequester resources through adult feeding may negatively affect its reproductive performance.

2. Materials and methods

2.1. Study site and experimental setup

All experiments were run at the headquarters of the Instituto de Ecología, A.C. in Xalapa, Veracruz, México. Xalapa is located at 19°30′N and 96°57′W, has a mean annual rainfall of 1517 mm and mean annual temperature of 18 °C. During the experiment, the temperature fluctuated between 21 and 26.5 °C (mean 22.9 °C), and relative humidity between 82.5 and 92.5% (mean 86.37%). Observations were made inside a 12 m × 12 m × 3 m nylon field cage surrounded by native vegetation. The cage was totally protected by a fiber glass roof that allowed natural light to go through. Inside the cage, we placed eight 2 m × 0.78 m × 0.4 m iron shelves with four individual shelves at different heights. In each of the four shelves, we placed two Plexiglas cages with flies that were rotated every day to overcome possible position effects (details follow).

2.2. Insects

A. ludens derived from two populations reared on different hosts (both Rutaceae): *C. greggii* and *C. paradisi* (grapefruit, cv. Ruby Red). *C. greggii* samples were collected in the "Cañón La Oveja", Tamaulipas (24°31′N, 99°43′W). The *C. paradisi* population derived from a semi-wild colony kept in our laboratories (<10 generations in captivity with constant "refreshment" of colony with wild flies). *A. serpentina* were derived from "Zapote Niño" (*C. mexicanum* Brandegee ex Standl.) collected in "Las Cuevas", Teocelo, Veracruz (19°23′N, 96°58′W). *A. obliqua* adults were obtained from tropical plum (*S. purpurea*) collected in Apazapan, Veracruz (19°19′N, 96°42′W). Finally, *A. striata* derived from a semi-wild colony kept in our laboratories on guava (*P. guajava*). Methods for handling fruit collected in the field or kept in the laboratory and processing of pupae are described in Aluja et al. (2000b).

Immediately after the adults emerged, they were placed in clean, sanitized (i.e., walls cleaned with 90% alcohol), screened 30 cm × 30 cm × 30 cm Plexiglas cages (in the case of *A. striata* fewer flies were available, and cages were 22 cm × 22 cm × 22 cm). Water and food was offered *ad libitum*. Type of food depended on treatment (details follow). With the exception of *A. striata*, we placed 15 ♀ and 15 ♂ in every one of the four cages used (details on experimental design follow). In the cases of *A. obliqua* and *A. serpentina* we therefore used a total of 60 ♀ and 60 ♂ individuals per species, respectively. In the case of *A. ludens*, we experimented with a total of 120 ♀ and 120 ♂ individuals as we tested two populations (*C. greggii* and *C. paradisi*, respectively). Finally, in the case of *A. striata*, we had access to fewer individuals and therefore only placed 5 ♀ and 5 ♂ per cage (we experimented with 20 ♀ and 20 ♂ individuals considering the four replicates). In cages containing 15 ♀ (*A. ludens*, *A. obliqua* and *A. serpentina*, respectively), we hung three 3.5 cm diam agar spheres (fruit mimics [Díaz-Fleischer and Aluja, 2003b]) from the roof every morning starting at day 1 (when fly cohorts were placed inside cages). In the case of *A. striata*, only one sphere/cage was hung to maintain the same sphere-to-fly-ratio used with the other species. The next morning, spheres were replaced with new ones. Those that had remained inside the cages for 24 h were dissected to count all the eggs oviposited by females (number of clutches, clutch size and total number of eggs). Each agar sphere was prepared with 0.15 ml of McCormick® green food color (Colorante Artificial Verde McCormick®, Grupo Herdez, San Luis Potosí, Mexico), 45 ml of water and 1.46 g of bacteriologic agar (BD Bioxon, Becton Dickinson de México, Cuatitlán Izcalli, Edo. de Mex., Mexico). Once the agar had hardened, spheres were wrapped in Parafilm "M"® (American National Can, Chicago, IL).

2.3. Treatments and experimental design

Two treatments were tested: hydrolyzed protein (Yeast Hydrolysate Enzymatic, ICN Biomedicals, USA) mixed with sugar (1:3) and the same diet but adding methoprene. Both diets were formulated by adding 20 ml of acetone either containing 10.0 mg of methoprene (treated diet) or not (control diet) to 20 g of dry diet and mixing well. The acetone diet slurries were then subjected to rotary evaporation to remove acetone. The food was provided to flies in the screened cage roof where they could access it *ad libitum*. We used a paint brush to place a thin layer of food to avoid runoff into cage floor. Two brushes were used: one for the sugar–protein mixture and methoprene (treated) and the other for the pure sugar–protein mixture (control).

The experimental design was a nested randomized block design with cages as blocks and presence/absence of methoprene in food as treatments. Given that in the case of *A. ludens*, we had access to two different populations derived from different host plants, such a variable (i.e., origin) was also factored into the design and analysis. There were four replicates per treatment per species. As noted above, cages containing flies were placed on four shelves, and cages containing different fly species were purposefully placed on different shelves and rotated daily to minimize possible microclimatic effects.

2.4. Recorded variables and observation protocol

We recorded the following variables (there were always two observers working the same shifts):

(1) Days elapsed until the first males called or the first female oviposited an egg into fruit. Male calling is very apparent as they vigorously fan their wings to emit courtship songs and disperse a sexual pheromone released through the anal gland (Sivinski et al., 2000). Since cages were systematically monitored by two observers, it was easy to determine when calling activity started (i.e., age of first calling). An effective oviposition can be ascertained by the fact that females

invariably deposit a host marking pheromone by dragging the tip of their aculeus on the surface of a fruit after they have laid an egg (Aluja and Díaz-Fleischer, 2006). Aculeus dragging is not observed if a female only probed (i.e., aculeus insertion without oviposition). Days to first oviposition was then used as the starting point for recording all the other variables for a total period of 10 days.

(2) Number of calling males. We followed a scan sampling scheme (Martin and Bateson, 1993), with each cage observed every 15 min over the entire observation period.

(3) Copula duration (min) and total number of copulations. Since we scan-sampled all cages every fifteen minutes to identify any new mating pair, it was possible to measure copula duration (time it started–time it ended) with an acceptable degree of accuracy. With few exceptions, mating pairs stand still while they are copulating, facilitating data collection. Once a pair formed, we placed a tag on the outside cage wall exactly over the mating pair to identify it. On the tag we recorded when the copulation had started and also when it ended. If the mating pair walked to a new location and we witnessed the move, we just moved the tag and placed it in the new location. In those cases where pairs moved without us witnessing the move or in case of doubt, data were discarded. We note that while all mating pairs were recorded, we only measured copula duration in 20 randomly selected individuals (five per replicate for each species).

(4) Total number of clutches, eggs within a clutch and, as a result, total number of eggs and mean clutch size.

(5) Egg hatch. To determine this, while dissecting the agar spheres, we set aside a sample of 20 eggs/sphere. These eggs were then placed in Petri dishes with a black piece of cloth in the bottom part. Closed Petri dishes were kept in a room at 30 °C and 70% humidity. After 4 days, incubation chambers were checked under a stereoscopic microscope (Stemi, Zeiss®) and hatched eggs counted.

Observations were carried out beginning with day 1 (i.e., day when flies were released into cages shortly after having emerged). Given that each species under study is known to exhibit different diel mating activity patterns (details in Table 1), the observation schedule was as follows: *A. ludens* from 15:00 to 20:00 h, *A. serpentina* from 11:00 to 19:00 h; *A. obliqua* from 8:00 to 18:00 h; and *A. striata* from 10:00 to 19:00 h (Aluja et al., 2000a). As noted above, we scan-sampled every cage every 15 min. Also, every 15 min we recorded temperature and relative air humidity with a hygrothermograph (Oakton® Minidrum Hygrothermographs, Cole-Parmer, Chicago, IL, USA) and light intensity with a lightmeter (Traceable® Light Meter, Control Company, Friendswood, TX, USA).

2.5. Statistical analyses

To meet parametric assumptions, count data (i.e., days until first male calling or female oviposition event, number of males calling and copulations, number of clutches and eggs) were transformed to $\sqrt{(X + 3/8}$ [according to Zar (1999) this transformation has better stabilizing qualities than $X = \sqrt{(X + 0.5)}$], mean clutch size and copulation duration to $\log_{10}(X + 1)$, and proportion of eggs that eclosed to arcsin of $\sqrt{X}$ prior to analyses (Zar, 1999), but untransformed data are shown in figures. Given that the response variables were correlated to each other, and to reduce the risk of a Type I error, we first applied a MANOVA followed by univariate analyses (i.e., same design as in MANOVA, but each response variable analyzed separately). Considering that only *A. ludens* had populations derived from two different hosts (i.e., *C. greggii* and *C. paradisi*), we ran the analyses comparing the four fly species with data from both populations separately. The MANOVA included the following six response variables: number of males calling, copulations, number of clutches, mean clutch size, total number of eggs, and proportion of eggs that eclosed. As noted earlier, each cage was considered a block (four replicates per treatment that for the purposes of analyses we then nested within treatments (i.e., protein with or without methoprene), host (only in the case of *A. ludens*) and fly species. By nesting blocks we dealt with the potential effect of environmental variables, and the possible effect of variation in fly mortality over time (different numbers of flies died in each cage as the study progressed) and the fact that in the case of *A. striata*, cages contained fewer individuals. Consequently, the effect of the principal factors (i.e., treatment, host, fly species) was tested by calculating the corresponding statistic (i.e., ratio between mean squares of the particular factor and block mean squares). The interaction effects of treatment × host and treatment × species were tested against the mean squares of the error.

To examine the effect of treatment and the other two factors (i.e., host and fly species) on days elapsed until the first male called or the first female oviposited and also for copulation duration, we ran two-way factorial univariate ANOVA's, due to the restrictions imposed by lack of sufficient degrees of freedom that hindered us from running a full analysis.

When the interactions among factors were significant, we carried out post hoc mean contrasts (*t*-tests) to examine differences between specific levels of each factor (i.e., between control and hormone treatment for each host in *A. ludens* and for each fruit fly species). Post hoc tests on differences among species were run via multiple comparisons by means of a Tukey HSD procedure. All analyses were run in Statistica 7.1 (StatSoft, 2005) by means of General linear models.

3. Results

We first present the results of the MANOVA analyses, considering first the comparison between hosts in the case of *A. ludens* (*C. greggii* vs. *C. paradisi*) and then the multiple species comparison. Secondly, we present the results of the univariate ANOVAS (further details in Table 2). We note that in the case of the multiple-species-comparison-univariate-ANOVAS, we performed the analyses with both the *C. greggii* and *C. paradisi A. ludens* populations (details in Tables 3 and 4) but chose the *C. greggii* population for result description and data presentation as this is the native host of *A. ludens* (all other species in the comparison also derived from native hosts). We felt this was justified as overall trends were quite similar. Of the 24 possible results (four sources of variation: cage, treatment, species, treatment × species interaction; and the six response variables), we detected differences in only three cases when comparing the *C. greggii* and *C. paradisi A. ludens* populations (Tables 3 and 4).

In the case of the two *A. ludens* populations, the MANOVA revealed a non-significant treatment (methoprene) effect (Pillai's trace = 0.552, $F_{(6,11)} = 2.258$, $P = 0.115$) but a highly significant treatment by host interaction effect (Pillai's trace = 0.210, $F_{(6,138)} = 6.125$, $P < 0.001$). In the case of the multiple species comparison considering the *A. ludens* population derived from *C. greggi*, the MANOVA revealed significant methoprene (Pillai's trace = 0.092, $F_{(6,278)} = 4.71$, $P < 0.001$), species (Pillai's trace = 2.15, $F_{(18,840)} = 118.5$, $P \sim 0$), and treatment by species effects (Pillai's trace = 0.34, $F_{(18,840)} = 5.98$, $P \sim 0$), despite a significant among block variance (Pillai's trace = 0.88, $F_{(144,1698)} = 2.026$, $P \sim 0$). When the same multiple species comparison was run considering the *A. ludens* population derived from *C. paradisi*, the treatment (i.e., methoprene) effect showed the same direction as the analysis with *C. edulis* (treatment: Pillai's trace = 0.071, $F_{(6,279)} = 3.54$, $P < 0.005$; species: Pillai's trace = 2.091, $F_{(18,843)} = 107.7$, $P \sim 0$; and treatment by

Table 2
Univariate ANOVAs that followed a MANOVA (results in text) analyzing the effect of methoprene on two *A. ludens* populations that derived from two different host plants (*Casimiroa greggii* (ancestral host) and *C. paradisi* (exotic host).

Dependent variable	Source of variation	d.f.	Sum of squares	Mean squares	*F*	*P* value
Days elapsed until first male called	Cage (treatment, host)	1	115.86	115.86	11960.05	<**0.001**
	Treatment	1	0.60	0.60	62.07	<**0.001**
	Host	1	0.08	0.08	8.25	**0.014**
	Treatment × host	1	0.34	0.34	35.28	<**0.001**
	Residual	12	0.12	0.01		
Number of calling males	Cage (treatment, host)	12	30.06	2.51	1.21	0.284
	Treatment	1	0.77	0.77	0.31	0.589
	Host	1	120.14	120.14	47.96	<**0.001**
	Treatment × host	1	5.62	5.62	2.71	0.102
	Residual	144	298.52	2.07		
Number of copulations	Cage (treatment, host)	12	2.13	0.18	0.66	0.789
	Treatment	1	2.30	2.30	6.55	**0.025**
	Host	1	1.16	1.16	12.94	**0.004**
	Treatment × host	1	0.30	0.30	1.13	0.290
	Residual	144	38.87	0.27		
Days elapsed until first female oviposited	Cage (treatment, host)	1	196.59	196.59	12462.67	<**0.001**
	Treatment	1	0.06	0.06	3.91	0.071
	Host	1	0.15	0.15	9.41	**0.009**
	Treatment × host	1	0.01	0.01	0.68	0.427
	Residual	12	0.19	0.02		
Total number of clutches	Cage (treatment, host)	12	103.58	8.63	1.18	0.300
	Treatment	1	6.49	6.49	1.03	0.331
	Host	1	8.87	8.87	0.75	0.403
	Treatment × host	1	0.66	0.66	0.09	0.764
	Residual	144	1049.48	7.29		
Mean clutch size	Cage (treatment, host)	12	0.77	0.06	1.54	0.117
	Treatment	1	2.96	0.15	2.35	0.151
	Host	1	0.15	2.96	46.37	<**0.001**
	Treatment × host	1	0.91	0.91	21.95	<**0.001**
	Residual	144	5.98	0.04		
Total number of eggs	Cage (treatment, host)	12	278.59	23.22	0.86	0.586
	Treatment	1	509.20	509.20	2.92	0.113
	Host	1	67.87	67.87	21.93	<**0.001**
	Treatment × host	1	41.47	41.47	1.54	0.217
	Residual	144	3874.87	26.91		
Egg hatch	Cage (treatment, host)	12	1.59	0.13	2.60	**0.004**
	Treatment	1	0.13	0.13	0.98	0.342
	Host	1	0.001	0.001	0.004	0.948
	Treatment × host	1	0.09	0.09	1.85	0.176
	Residual	143	7.29	0.05		

Bold values denote significant effect of the source of variation.

species: Pillai's trace = 0.178, $F_{(18,843)} = 2.95$, $P < 0.0001$; among block variance: Pillai's trace = 0.97, $F_{(144,1704)} = 2.28$, $P \sim 0$).

3.1. Days elapsed until the first males called and total number of calling males

When comparing the two *A. ludens* populations, the factorial ANOVA revealed that methoprene significantly influenced the number of days until the first calling event and that there were also significant host and treatment by host interaction effects (*F* and *P* values in Table 2). Post hoc contrasts showed that methoprene application in *A. ludens* derived from *C. greggii* significantly reduced the time it takes males to exhibit calling behavior, but that such an effect is not detected in populations derived from *C. paradisi* (Fig. 1A).

In the case of the among species comparison, the factorial ANOVA revealed significant treatment (methoprene), species and treatment by species interaction effects (*F* and *P* values in Table 3). Post hoc contrasts showed that in the case of *A. ludens* (*C. greggii* population) the number of days until the first males starts calling was significantly reduced, but that such an effect was not discernible in the other three *Anastrepha* species (Figs. 1A and B).

With respect to the number of calling males in the case of the two *A. ludens* populations, the two-way factorial univariate ANOVA revealed a significant host effect, but no treatment (methoprene) and treatment by host interaction effects were detected (*F* and *P* values in Table 2). *A. ludens* males derived from *C. paradisi* called almost twice as many times as males derived from *C. greggii* (mean ± S.E. = 31.51 ± 1.45 [*n* = 80] and 14.88 ± 1.45 [*n* = 80], respectively) (Fig. 2A).

The interspecific comparison among *Anastrepha* species in terms of number of calling males revealed that the overall effect of methoprene was negligible (*F* and *P* values in Table 3; Fig. 2B) but importantly, there was a significant effect of the interaction between treatment (methoprene) and species; Table 3; Fig. 2B). In the case of *A. serpentina*, treated males called almost three times as often when compared to untreated males (Fig. 2B).

3.2. Number and duration of copulations

In the case of number of copulations in *A. ludens*, significant treatment (methoprene) and host effects were detected, but the interaction was not significant. *A. ludens* adults under the effect of methoprene mated a mean (±S.E.) of 2.79 ± 0.19 times per day per

Table 3
Univariate ANOVAs that followed a MANOVA (results in text) analyzing the effect of methoprene on four *Anastrepha* species: *A. ludens* (derived from *C. greggii*), *A. obliqua*, *A. serpentina* and *A. striata*.

Dependent variable	Source of variation	d.f.	Sum of squares	Mean squares	*F*	*P* value
Days elapsed until first male called	Cage (treatment, species)	1	4163.28	4163.28	2337.28	<**0.001**
	Treatment	1	30.03	30.03	16.86	<**0.001**
	Species	3	378.09	126.03	70.75	<**0.001**
	Treatment × species	3	8.84	2.95	1.66	**0.036**
	Residual	24	42.75	1.78		
Number of calling males	Cage (treatment, species)	24	151.48	6.31	3.19	<**0.001**
	Treatment	1	17.02	17.02	2.70	0.114
	Species	3	46.12	15.37	2.44	0.089
	Treatment × species	3	45.54	15.18	7.68	<**0.001**
	Residual	288	569.39	1.98		
Number of copulations	Cage (treatment, species)	24	1.61	0.07	0.36	0.998
	Treatment	1	1.13	1.13	16.83	<**0.001**
	Species	3	20.30	6.77	101.03	<**0.001**
	Treatment × species	3	0.52	0.17	0.92	0.432
	Residual	288	53.87	0.19		
Days elapsed until first female oviposited	Cage (treatment, species)	1	11742.78	11742.78	7774.53	<**0.001**
	Treatment	1	1.53	1.53	1.01	0.324
	Species	3	780.34	260.11	172.21	<**0.001**
	Treatment × species	3	6.09	2.03	1.35	0.283
	Residual	24	36.25	1.51		
Total number of clutches	Cage (treatment, species)	24	207.16	8.63	1.64	**0.033**
	Treatment	1	16.59	16.59	1.92	0.178
	Species	3	1624.78	541.59	62.75	<**0.001**
	Treatment × species	3	7.32	2.44	0.46	0.709
	Residual	288	1519.97	5.28		
Mean clutch size	Cage (treatment, species)	24	1.60	0.07	3.02	<**0.001**
	Treatment	1	0.09	0.09	1.33	0.260
	Species	3	44.44	14.82	222.45	<**0.001**
	Treatment × species	3	0.93	0.31	14.09	<**0.001**
	Residual	288	6.36	0.02		
Total number of eggs	Cage (treatment, species)	24	264.81	11.03	1.25	0.197
	Treatment	1	62.37	62.37	5.65	**0.026**
	Species	3	3701.62	1233.87	111.83	<**0.001**
	Treatment × species	3	61.98	20.66	2.34	0.073
	Residual	288	2538.39	8.81		
Egg hatch	Cage (treatment, species)	24	1.69	0.07	2.49	<**0.001**
	Treatment	1	0.001	0.001	0.01	0.923
	Species	3	4.79	1.60	22.66	<**0.001**
	Treatment × species	3	0.36	0.12	4.28	**0.006**
	Residual	283	8.01	0.03		

Bold values denote significant effect of the source of variation.

cage, while flies fed on protein without methoprene did so 2.31 ± 0.19 times ($n = 80$) (Fig. 3A). Furthermore, flies derived from *C. paradisi* mated on average almost one time more often per day than those derived from *C. greggii* (2.99 ± 0.19 vs. 2.11 ± 0.19, respectively) (Fig. 3A). With respect to copulation duration, there were neither significant treatment (methoprene) ($F_{(1,76)} = 0.06$, $P = 0.81$), nor host ($F_{(1,76)} = 0.43$, $P = 0.51$), nor treatment by host interaction effects ($F_{(1,76)} = 0.12$, $P = 0.74$).

In the case of the among species comparison of mean number of copulations, the ANOVA detected significant treatment (methoprene) and species effects, but the treatment by species interaction was not significant (details in Table 3). Overall (all species considered), individuals fed on protein mixed with methoprene mated 1.28 ± 0.1 (mean ± S.E.) times per day, whereas those fed on protein and sugar only did so 1.01 ± 0.1 times per day. *A. ludens* males mated significantly more often (mean of 2.11 ± 0.13 times per day) than did males of the other three species ($P < 0.05$) (Fig. 3B). *A. serpentina* and *A. obliqua* did not differ in this respect (mean of 0.94 ± 0.13 and 1.21 ± 0.13, respectively [$P > 0.05$]), but mating activity in the latter two species did differ from that exhibited by *A. striata* (mean of 0.34 ± 0.19 copulations per day, $P < 0.05$) (Fig. 3B). A very similar pattern was observed with respect to copulation duration: non-significant treatment (methoprene) ($F_{(1,146)} = 0.638$, $P = 0.426$), significant species ($F_{(3,146)} = 30.188$, $P \sim 0$) and non-significant treatment by species interaction effects ($F_{(3,146)} = 1.045$, $P = 0.374$). The pattern observed was the following: *A. ludens* (*C. greggii*) 64.85 ± 3.83 min, *A. obliqua* 57.3 ± 3.83 min, *A. serpentina* 31.13 ± 3.83 min and *A. striata* 21.32 ± 3.83 min. We note that the difference between *A. striata* and all the other species was significant ($P < 0.05$).

3.3. Days elapsed until the first female oviposited an egg

In the case of the two *A. ludens* populations, the effect of treatment (methoprene) was marginally insignificant ($F_{(1,12)} = 3.91$, $P = 0.071$; Fig. 6B), but the difference between hosts was highly significant ($F_{(1,12)} = 9.41$, $P = 0.0097$). The treatment by host interaction was not significant ($F_{(1,12)} = 0.68$, $P = 0.427$). It took *A. ludens* females derived from *C. greggii* almost a day longer to start laying eggs when compared with those originating from *C. paradisi* (mean ± S.E. = 12.63 ± 0.32 and 11.25 ± 0.32, respectively) (Fig. 4A).

In the case of the same comparison at the level of species, we found that there was neither a significant treatment ($F_{(1,24)} = 1.06$, $P = 0.313$) nor treatment by species interaction effect ($F_{(3,24)} = 1.48$,

Table 4
Univariate ANOVAs that followed a MANOVA (results in text) analyzing the effect of methoprene on four *Anastrepha* species: *A. ludens* (derived from *C. paradisi*), *A. obliqua*, *A. serpentina* and *A. striata*.

Dependent variable	Source of variation	d.f.	Sum of squares	Mean squares	*F*	*P* value
Days elapsed until first male called	Cage (treatment, species)	1	359.48	359.48	9962.80	<**0.001**
	Treatment	1	0.23	0.23	6.24	<**0.001**
	Species	3	9.41	3.14	86.94	<**0.001**
	Treatment × species	3	0.02	0.01	0.19	**0.036**
	Residual	24	0.87	0.04		
Number of calling males	Cage (treatment, species)	24	132.53	5.52	2.41	<**0.001**
	Treatment	1	6.00	6.00	1.09	0.308
	Species	3	139.50	46.50	8.42	<**0.001**
	Treatment × species	3	52.40	17.47	7.61	<**0.001**
	Residual	288	661.16	2.30		
Number of copulations	Cage (treatment, species)	24	1.91	0.08	0.37	0.998
	Treatment	1	0.45	0.45	5.67	**0.026**
	Species	3	38.09	12.70	159.39	<**0.001**
	Treatment × species	3	0.003	0.001	0.00	0.999
	Residual	288	62.38	0.22		
Days elapsed until first female oviposited	Cage (treatment, species)	1	600.70	600.70	39498.86	<**0.001**
	Treatment	1	0.01	0.01	0.39	0.536
	Species	3	12.87	4.29	282.07	<**0.001**
	Treatment × species	3	0.06	0.02	1.32	0.290
	Residual	24	0.37	0.02		
Total number of clutches	Cage (treatment, species)	24	209.33	8.72	1.64	**0.032**
	Treatment	1	12.25	12.25	1.40	0.248
	Species	3	1722.64	574.21	65.83	<**0.001**
	Treatment × species	3	6.84	2.28	0.43	0.732
	Residual	288	1528.10	5.31		
Mean clutch size	Cage (treatment, species)	24	0.29	0.01	4.36	<**0.001**
	Treatment	1	0.03	0.03	2.25	0.146
	Species	3	12.27	4.09	343.77	<**0.001**
	Treatment × species	3	0.03	0.01	3.22	**0.023**
	Residual	288	0.79	0.003		
Total number of eggs	Cage (treatment, species)	24	367.23	15.3	1.26	0.191
	Treatment	1	11.18	11.18	0.73	0.401
	Species	3	7353.23	2451.08	160.19	<**0.001**
	Treatment × species	3	7.07	2.36	0.19	0.901
	Residual	288	3499.42	12.15		
Egg hatch	Cage (treatment, species)	24	2.45	0.10	2.50	<**0.001**
	Treatment	1	0.06	0.06	0.58	0.456
	Species	3	4.94	1.65	16.16	<**0.001**
	Treatment × species	3	0.09	0.03	0.71	0.550
	Residual	283	11.60	0.04		

Bold values denote significant effect of the source of variation.

$P = 0.244$), but the effect of species was significant ($F_{(3,24)} = 160.03$, $P \sim 0$). While *A. ludens* females exhibited the shortest period to first egg-laying event (12.633 ± 0.44 d), *A. serpentina* (26 ± 0.44 d, $n = 8$; $P < 0.05$) exhibited the longest. The other two species fell in between (*A. obliqua* [17.0 ± 0.44] and *A. striata* [21 ± 0.44]; Fig. 4B).

3.4. Number of clutches, mean clutch size and total number of eggs

When comparing the two *A. ludens* populations, the ANOVA detected no significant differences with respect to the total number of clutches (Table 2, Fig. 5A). There were, however significant host and treatment by host interaction effects with respect to clutch size and a significant host effect in the case of the number of eggs. *A. ludens* females derived from *C. paradisi* laid significantly more eggs per clutch and total number of eggs (mean ± S.E. = 4.59 ± 0.11 and 323 ± 15.8, respectively) than females derived from *C. greggii* (3.32 ± 0.11 and 202.1 ± 15.8, respectively) (Figs. 6A and 7A).

In the case of the multiple species comparison, the ANOVA detected a non-significant effect with respect to treatment (methoprene) but a significant species effect with respect to total number of clutches and mean clutch size. Importantly, there was a significant treatment by species interaction effect with respect to clutch size (Table 3; Figs. 5B and 6B). While methoprene reduced the mean number of eggs per clutch in *A. serpentina*, in the case of *A. ludens* (from *C. greggii*) it increased the number (Fig. 6B). Finally, methoprene had a significant effect on the total number of eggs (Table 3, Fig. 7B). There was also a highly significant effect of species (Table 3), but this was expected given the varying natural histories of the species being compared. *A. ludens* laid the most eggs (202.1 ± 7.25), followed by *A. obliqua*, *A. serpentina* and *A. striata* (97.05 ± 7.25, 47.39 ± 7.25 and 18.75 ± 7.25, respectively) (Fig. 7B).

3.5. Egg hatch

When comparing the two *A. ludens* populations, the ANOVA showed no significant treatment, host or treatment by host interaction effects. The only source of significant variation was the one caused by cages (blocks) (Table 2). In the case of the multiple species comparison, the ANOVA detected significant species and treatment by species interaction effects (Table 3). Significantly more eggs hatched if they had been laid by untreated *A. ludens* females (*C. greggii* population) (mean proportion ± S.E. = 0.73 ± 0.02, $P < 0.05$) (Fig. 8A and B). Egg hatch was statistically equal in the case of *A. striata*

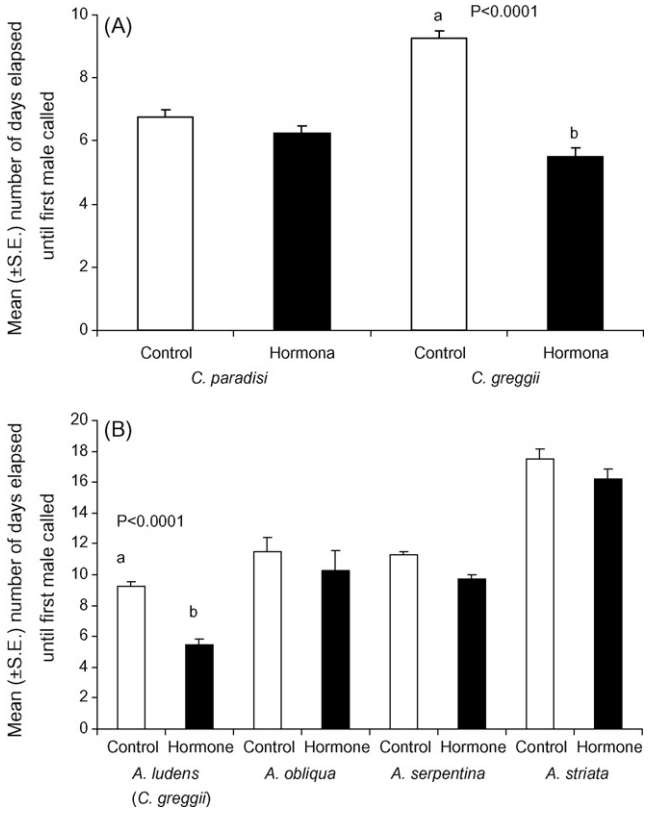

Fig. 1. Effect of methoprene on mean ± S.E. number of days elapsed until first male called. (A) Comparisons between control and hormone treatment for two *Anastrepha ludens* populations stemming from *Casimiroa greggii* (larvae feed on seeds) and *Citrus paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test.

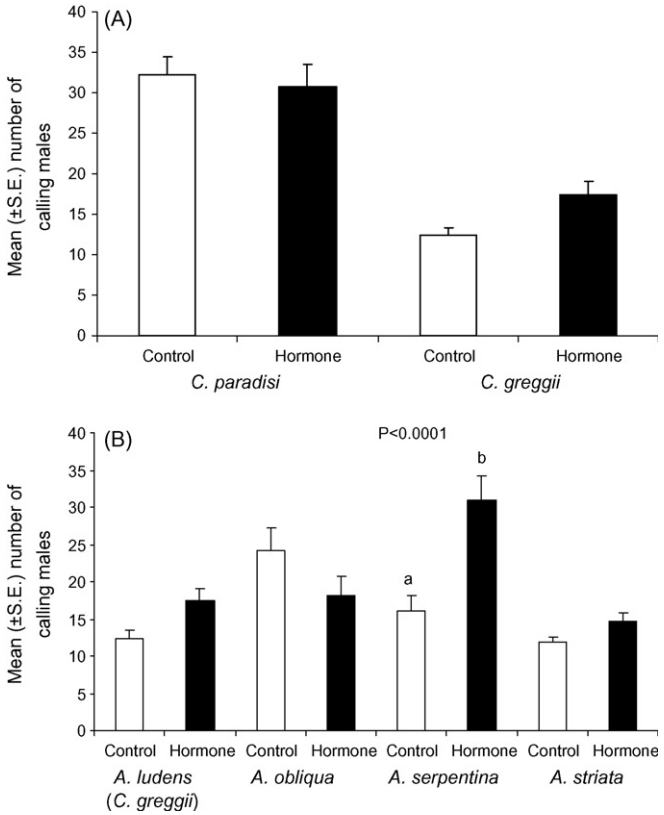

Fig. 2. Effect of methoprene on mean ± S.E. number of calling males. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test.

and *A. obliqua* (0.54 ± 0.02 and 0.55 ± 0.02, respectively), but both species differed significantly when compared to *A. serpentina,* the species exhibiting the lowest egg hatch (0.41 ± 0.02, $P < 0.05$) (Fig. 8B).

4. Discussion

In congruence with our two hypotheses, we found that the effects of methoprene, when they occurred, varied according to species and, in the case of *A. ludens,* according to larval host. Interestingly, the effect of methoprene was only apparent in the two species that can lay large clutches (*A. ludens* and *A. serpentina*) and totally absent in the species that consistently lay eggs singly (*A. obliqua*) or do so preferentially (*A. striata*). In the case of the two *A. ludens* populations, the effect of methoprene on first appearance of male calling behavior and number of copulations was only apparent in flies derived from *C. greggii*. In contrast, males derived from *C. paradisi* called and mated almost twice as often and females started to lay eggs almost 1 day earlier than individuals derived from *C. greggii*, but in this case there was no significant effect of treatment with methoprene, only a significant host effect. Given the varied life histories of the *Anastrepha* species examined, it is perhaps not surprising to find significant differences among them in many male and female reproductive characteristics. Such differences could be due to species specific physiologies that respond differently to JH (“physiology hypothesis”) or to similar physiologies confronted with different resource chemistry when developing in different fruit (“larval–host hypothesis”). We will focus our discussion on these two hypotheses and the related discoveries in our study. We also discuss some practical implications of our findings.

In the case of *A. suspensa* reared on artificial diet, Teal et al. (2000) reported that mean age to first male sexual signal emission was reduced almost by half when comparing control vs. methoprene-treated males. We found a similar trend in the *A. ludens* individuals arising from *C. greggii*, albeit less pronounced in *A. ludens* derived from *C. paradisi*, but highlight the fact that in our study flies ingested the methoprene and that in the Teal et al. (2000) study, methoprene was applied topically to flies. Notably, in none of the other three *Anastrepha* species was there any indication that methoprene accelerated sexual maturation. But in the case of *A. serpentina*, exposure to methoprene did have a highly significant effect on the number of calling males (and as discussed below, clutch size). Such variable responses to methoprene may be explained by interspecific differences in life histories that in turn favored selection for varying adult physiologies. For example, in the cases of *A. ludens* and *A. serpentina*, the only two species were methoprene treatments showed significant effects, the treatment effect followed opposite directions with respect to clutch size: positive in the case of *A. ludens* and negative in the case of *A. serpentina.* As noted earlier, females of both species can lay large clutches of eggs. This suggests possible differences in physiology governing oogenesis, that in turn might influence the overall effect of JH. In the case of *A. obliqua*, a single egg layer, we had noted earlier that females need to quickly mature eggs after emergence as they usually attack fruit with highly ephemeral ripening schedules (Aluja and Birke, 1993; Díaz-Fleischer and Aluja, 2003a). When compared to *A. ludens*, *A. obliqua* females have more than double the amount of mature oocytes at age 15 days and such a

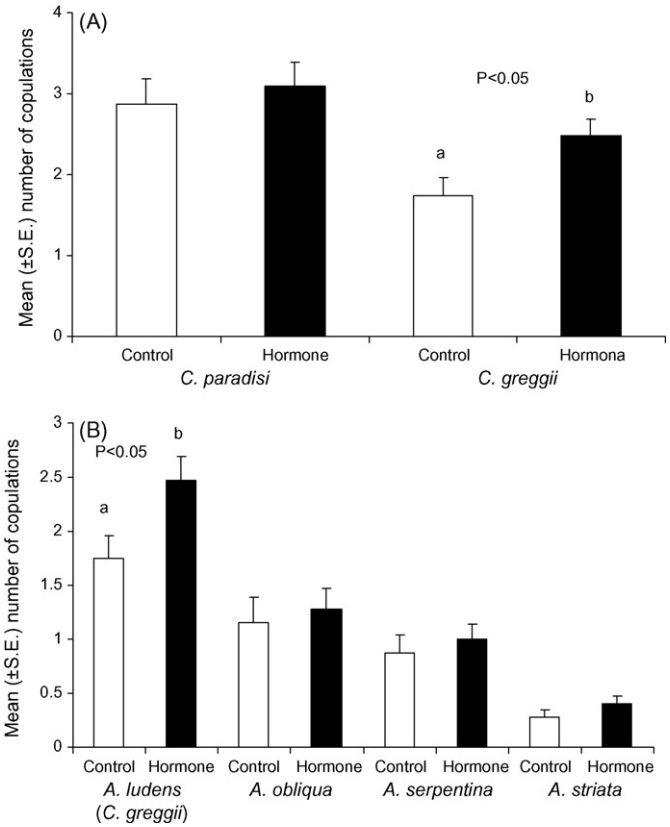

Fig. 3. Effect of methoprene on mean ± S.E. number of copulations. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test.

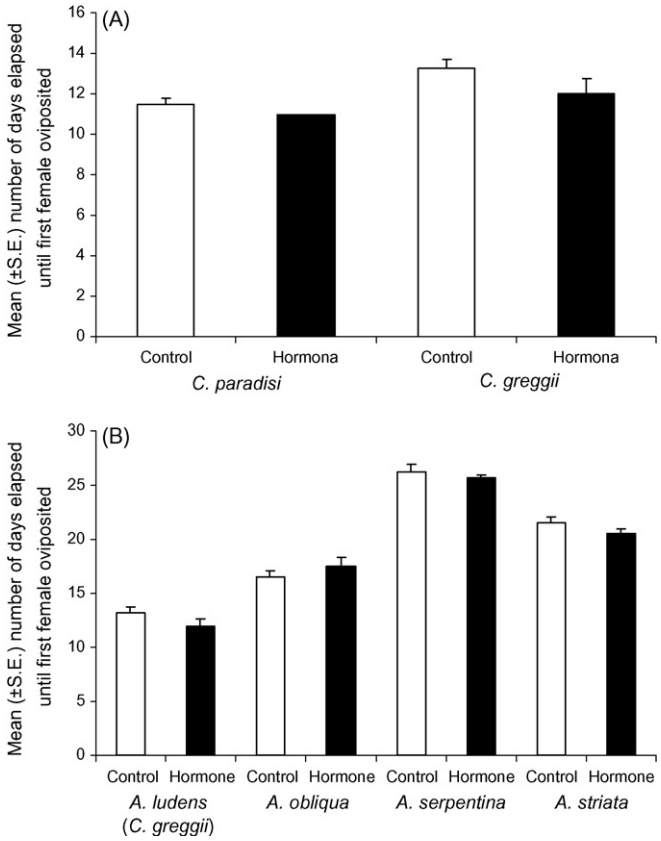

Fig. 4. Effect of methoprene on mean ± S.E. number of days elapsed until first female oviposited an egg into a fruit. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test. We note that in one case (*C. paradisi*, hormone bar), the S.E. was basically nonexistent (<0.000).

difference is maintained until they reach 45 days (Aluja et al., 2001a). *A. obliqua* females also produce many more eggs than *A. ludens* when exposed to host stimuli (Aluja et al., 2001a). In the case of males, they have large reserves of sperm and are able to mate up to nine times in a day without exhibiting sperm depletion (Pérez Staples and Aluja, 2006). All the latter could partially explain why methoprene had little effect on the rate of maturation in the shorter-lived species *A. obliqua*, but a significant effect in the longer-lived *A. ludens* (Table 1).

Before continuing, we would like to clarify an apparent incongruity in our argument. That is, while we argue that a pre-reproductive feeding period is particularly critical for *A. ludens*, its males maturated significantly faster than males of all other species, particularly *A. obliqua*, which we represent as relying heavily on resources obtained as larvae for rapid development. As shown in Fig. 1, methoprene treated *A. ludens* males started calling at ca. 5 days of age while untreated ones did so in ca. 9 days. In comparison, treated *A. obliqua*, *A. serpentina* and *A. striata* males started calling at ca. 10, 11 and 18 days, respectively. Most likely this is due to our study being performed in an area that is ideal for *A. ludens* (mean temperature during the study was 22.9 °C). All other species live normally in areas were ambient temperature is higher. For example in the case of *A. obliqua*, Aluja and Birke (1993), reported a mean temperature of 32.1 °C during their study on habitat use by adults of this species in an orchard surrounded by tropical, sub-deciduous forests. As sexual development is tightly correlated with temperature in fruit flies (Baker et al., 1944), it is not surprising to have observed lower rates of development in adults of three species adapted to warmer climates.

An accelerated adult maturation rate influenced by JH consumption might limit an important resource-gathering period that could potentially lower early reproductive performance. Here, *A. striata* males took the longest to start calling (e.g., >17 days in untreated males compared to ca. 9 days in untreated *A. ludens* males) and we detected no significant effect of methoprene on this parameter. In this species, males offer nuptial gifts to females and it has been shown that females that mate with virgin males live longer (Pérez-Staples and Aluja, 2004). Aluja et al. (2008) also recently showed that *A. striata* females discriminated strongly against males that had fed on a low quality diet (sucrose offered every third day) as opposed to those fed a high quality one (mixture of sucrose and protein offered *ad libitum*). This supports our prediction that males in this species should be resilient to environmental stimuli accelerating sexual maturation as that would render them less competitive if they need to accrue resources over a minimum period to be able to offer high quality nuptial gifts.

In this study we were also able to identify different responses to methoprene by *A. ludens* adults based on host origin (the native host *C. greggii* versus the exotic *C. paradisi*). For example, there was a highly significant treatment by host interaction effect in the parameters "days elapsed until first male called" and "mean clutch size". In other instances (e.g., "number of calling males" and "total number of eggs"), the treatment effect was not significant but the host effect was. That is, host origin can apparently have a mitigating effect on a compound otherwise able to accelerate

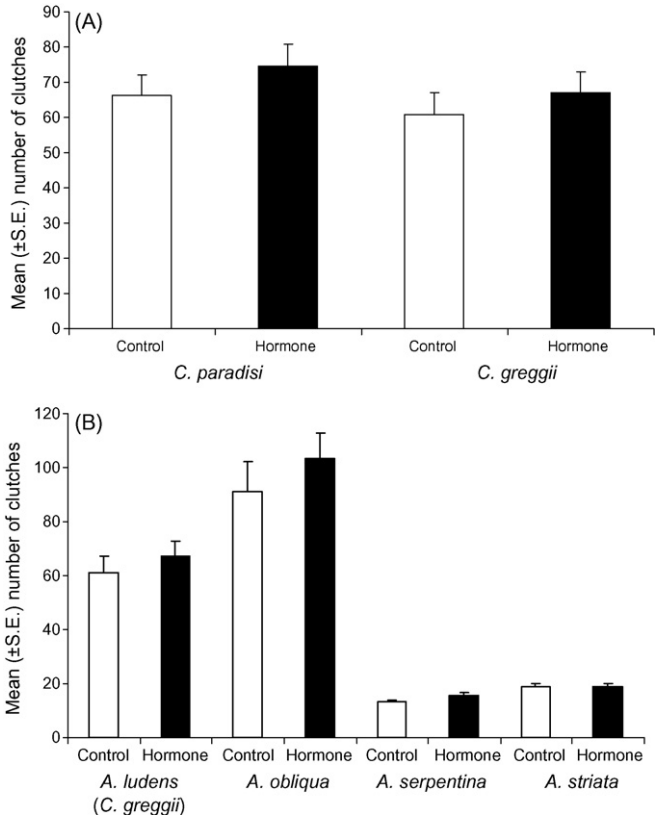

Fig. 5. Effect of methoprene on mean ± S.E. number of clutches. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test.

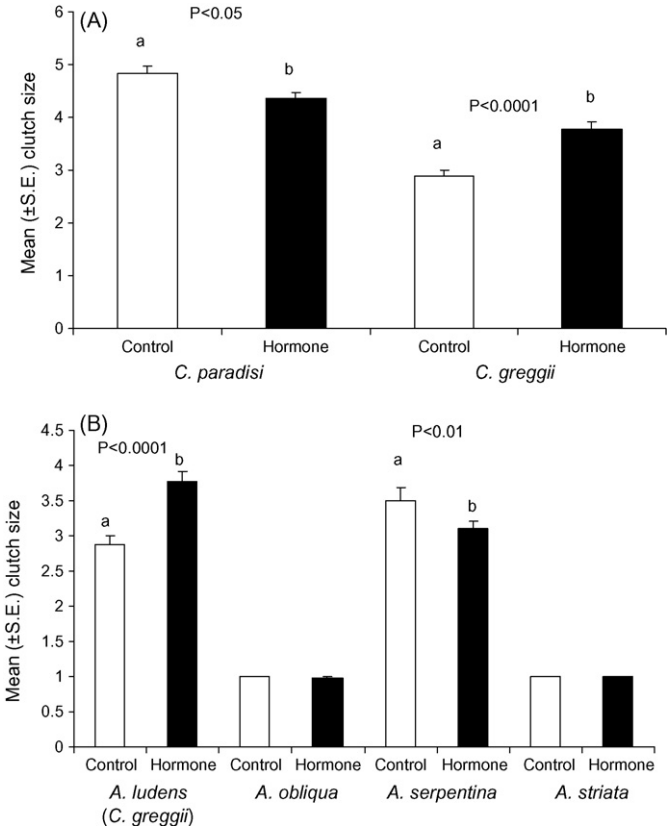

Fig. 6. Effect of methoprene on mean ± S.E. clutch size. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test. We note that in one case (*A. obliqua*, control bar bar), the S.E. was basically nonexistent (<0.000).

reproductive development. Recently, working with *A. obliqua*, Pérez-Staples et al. (2008) also reported an effect of larval host on one of the parameters studied here: copulation duration. Males derived from the native host, *S. mombin* exhibited the shortest copulations whereas those reared in the exotic host *M. indica*, exhibited the longest. These two findings (i.e., ours here and the one by Pérez-Staples et al., 2008), lend support to our "larval–host hypothesis". It also highlights the importance of female decisions while searching for hosts in areas were several hosts are simultaneously available, as progeny might face severe handicaps in their adult phase if the larvae they derived from developed in a lower quality host. In addition and as recently discussed by Bossdorf et al. (2008), these types of findings help us better understand sources of variability in the field, underscoring the need to study possible epigenetic changes induced by environmental variability (in our case, use of different hosts by highly polyphagous species such as *A. ludens*).

While it is difficult to extrapolate from the single case of the long-lived *A. ludens*' two disparate populations that larval diet plays the major role in explaining all the species differences in susceptibility to methoprene, we believe the larval–food hypothesis to be well worth pursuing for its potential insights into fruit fly physiology. One approach might be based on the number of highly consistent male-sexual responses to dermal applications of methoprene in *A. suspensa* reared on an artificial diet (Pereira, 2005). Would these responses be different if the larvae developed in the ancestral host guava? Would any changes resemble those exhibited by *A. striata* (e.g., failure of JH to accelerate sexual maturation), another fly that develops in guava? If so, does this mean that fruit are inferior sources of nutrients compared to formulated diets and so do not allow accelerated maturation regardless of exaggerated hormonal cues? Or as noted above, does the necessity of sequestering nutrients for trophallaxis preclude early development in *A. striata* alone? Further, could it be that the expression of certain proteins is reduced by the presence of some chemicals in the larval host and that this in turn could influence the effect of JH on the rate of sexual maturation in adult flies reared on those hosts? In this sense, a comparison of the effect of methoprene on *A. ludens* reared from a greater range of host fruits would be useful.

We would like to finish by discussing the practical implications of our findings. The four species under study here are either currently mass reared in the MoscaFrut mass-rearing facility in Metapa de Domínguez, Chiapas, Mexico or have/are being adapted to mass rearing conditions in the same facility (Gutierrez-Samperio et al., 1993; Rull et al., 1996; Reyes et al., 2000). As recently shown by Aluja et al. (2008) working with wild *A. ludens*, adult diet, more than size, influenced male sexual performance over a continued 4-day observation period in this species. Furthermore, females that copulated with low-quality fed males, exhibited significantly shorter maximum longevities, when compared to those that mated with males fed a high-quality diet. This supports our argument that males need to accrue important resources to guarantee optimal sexual performance. If mass-rearing facility managers opt for methoprene treatments in mass-reared *A. ludens*, then offering adults protein prior to being released becomes essential to increase the chances that these handicapped males (see Rull et al., 2007; Rull and Barreda-Landa, 2007 for

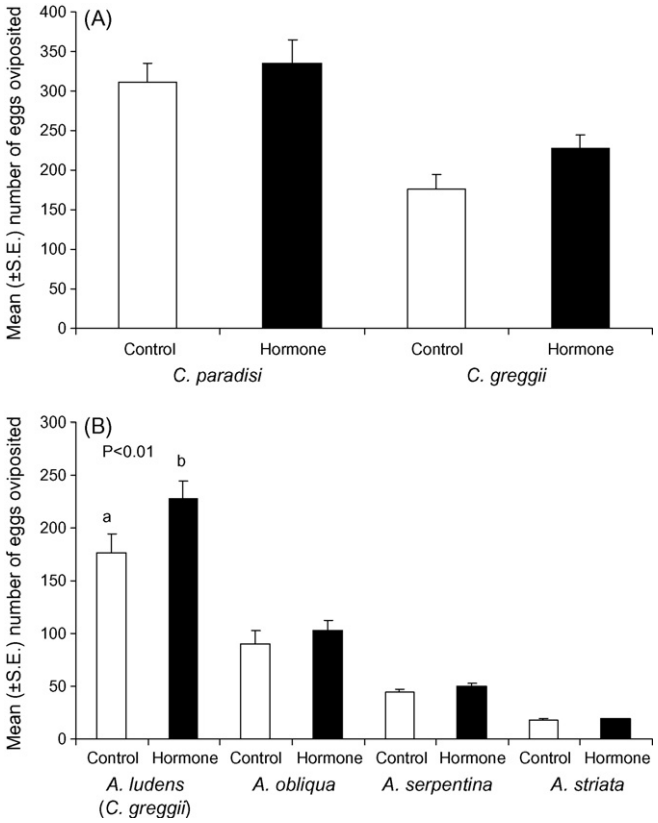

Fig. 7. Effect of methoprene on mean ± S.E. number of eggs oviposited. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test.

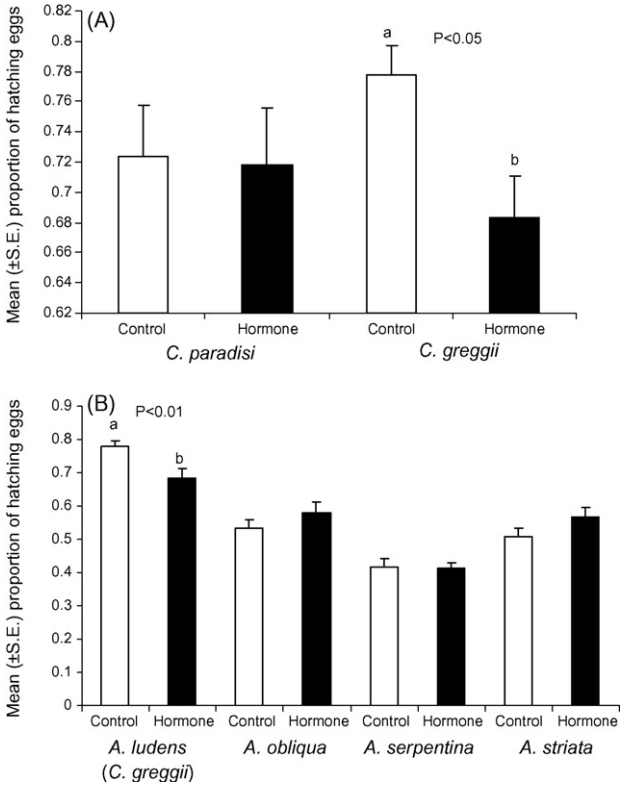

Fig. 8. Effect of methoprene on mean ± S.E. proportion of eggs that eclosed. (A) Comparisons between control and hormone treatment for two *A. ludens* populations stemming from *C. greggii* (larvae feed on seeds) and *C. paradisi* (larvae feed on pulp). (B) Comparisons between control and hormone treatments for different species. Different letters indicate significant difference between control and hormone treatments within each host, according to mean contrasts test.

details on this) will be able to compete for mates with wild males. In addition, it becomes clear from our results here, that methoprene treatments could not be effective in the cases of two important pestiferous species slatted for Sterile Insect Technique programs (Reyes et al., 2000). We are aware that additional research is needed to determine if the lack of significant effects detected here in *A. obliqua* or *A. striata* had to do with among other factors, methoprene dosage or dispensing method (i.e., topical applications vs. ingestion). Finally, it could be possible that methoprene treatments are most effective with flies reared on artificial diets, and that also needs to be determined in the future.

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References

Aluja, M., Birke, A., 1993. Habitat use by *Anastrepha obliqua* flies (Diptera:Tephritidae) in a mixed mango and tropical plum orchard. Annals of the Entomological Society of America 86, 799–812.

Aluja, M., Jácome, I., Birke, A., Lozada, N., Quintero, G., 1993. Basic patterns of behavior in wild *Anastrepha striata* Schiner (Diptera:Tephritidae) flies under field-cage conditions. Annals of the Entomological Society of America 86, 776–793.

Aluja, M., Piñero, J., Jácome, I., Díaz-Fleischer, F., Sivinski, J., 2000a. Behavior of flies in the genus *Anastrepha* (Trypetinae:Toxotrypanini). In: Aluja, M., Norrbom, A. (Eds.), Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, pp. 375–406.

Aluja, M., Piñero, J., López, M., Ruiz, C., Zúñiga, A., Piedra, E., Díaz-Fleischer, F., Sivinski, J., 2000b. New host plant and distribution records in Mexico for *Anastrepha* spp., *Toxotrypana curvicauda* Gerstacker, *Rhagoletis zoqui* Bush, *Rhagoletis* sp., and *Hexachaeta* sp. (Diptera:Tephritidae). Proceedings of the Entomological Society of Washington 102, 802–815.

Aluja, M., Díaz-Fleischer, F., Papaj, D.R., Lagunas, G., Sivinski, J., 2001a. Effects of age, diet, female density and the host resource on egg load in *Anastrepha ludens* and *Anastrepha obliqua* (Diptera:Tephritidae). Journal of Insect Physiology 47, 975–988.

Aluja, M., Jácome, I., Macías-Ordóñez, R., 2001b. Effect of adult nutrition on male sexual performance in four neotropical fruit fly species of the genus *Anastrepha* (Diptera:Tephritidae). Journal of Insect Behavior 14, 759–775.

Aluja, M., Díaz-Fleischer, F., 2006. Foraging behavior of *Anastrepha ludens*, *A. obliqua* and *A. serpentina* in response to feces extracts containing host marking pheromone. Journal of Chemical Ecology 32, 367–389.

Aluja, M., Rull, J., Pérez-Staples, D., Díaz-Fleischer, F., Sivinski, J., 2009. Random mating among *Anastrepha ludens* (Diptera:Tephritidae) adults of geographically distant and ecologically distinct populations in Mexico. Bulletin of Entomological Research, doi:10.1017/S0007485308006299.

Aluja, M., Pérez-Staples, D., Sivinski, J., Sánchez, A., Piñero, J., 2008. Effects of male condition on fitness in two tropical tephritid flies with contrasting life histories. Animal Behaviour 76, 1997–2009.

Baker, A.C., Stone, W.E., Plummer, C.C., McPhail, M., 1944. A review of studies on the Mexican fruit fly and related Mexican species. U.S. Dep. Agric. Misc. Publ. No. 531, pp. 155.

Birke, A., Aluja, M., Greany, P., Bigurra, E., Pérez-Staples, D., McDonald, R., 2006. Long aculeus and behavior of *Anastrepha ludens* renders gibberellic acid ineffective as an agent to reduce 'Ruby Red' grapefruit susceptibility to the attack of this pestiferous fruit fly in commercial citrus orchards. Journal of Economic Entomology 99, 1184–1193.

Blay, S., Yuval, B., 1997. Nutritional correlates to reproductive success of male Mediterranean fruit flies. Animal Behaviour 54, 59–66.

Bossdorf, O., Richards, C.L., Pigliucci, M., 2008. Epigenetics for ecologists. Ecology Letters 11, 106–115.

Bressan, S., da Costa Teles, M., 1991. Longevidade e curva de sobrevivencia de tres espécies do género *Anastrepha Schiner*, 1868 (Diptera, Tephritidae) em laboratorio. Revista Brasileira de Entomologia 35, 685–690.

Cusson, M., McNeil, J.N., 1989a. Involvement of juvenile hormone in the regulation of pheromone release activities in a moth. Science 243, 210–212.

Cusson, M., McNeil, J.N., 1989b. Ovarian development in female armyworm moths. *Pseudaletia unipuncta*: its relationship with pheromone release activities. Canadian Journal of Zoology 67, 1380–1385.

Cusson, M., Yu, C.G., Carruthers, K., Wyatt, G.R., Tobe, S.S., McNeil, J.N., 1994. Regulation of vitellogenin production in armyworm moths, *Pseudaletia unipuncta*. Journal of Insect Physiology 40, 129–136.

Dávila, A.B., 1995. Estudio de algunos parámetros demográficos de la Mosca Mexicana de la Fruta (*Anastrepha ludens* [Loew]) y su relación con diferentes dietas. Thesis, Universidad Veracruzana, Xalapa, México.

Díaz-Fleischer, F., Aluja, M., 2003a. Behavioral plasticity in relation to egg and time limitation: the case of two fly species in the genus *Anastrepha* (Diptera:Tephritidae). Oikos 100, 125–133.

Díaz-Fleischer, F., Aluja, M., 2003b. Clutch size in frugivorous insects as a function of host firmness: the case of the tephritid fly *Anastrepha ludens*. Ecological Entomology 28, 268–277.

Gadenne, C., 1993. Effects of fenoxycarb, juvenile hormone mimetic, on female sexual behaviour of the black cutworm, *Agrotis ipsilon* (Lepidoptera: noctuidae). Journal of Insect Physiology 39, 25–29.

Gutierrez-Samperio, J., Reyes, J., Villaseñor, A., 1993. National plan against fruit flies in Mexico. In: Aluja, M., Liedo, P. (Eds.), Fruit Flies: Biology and Management. Springer, New York, NY, pp. 419–423.

Jácome, I., Aluja, M., Liedo, P., 1999. Impact of adult diet on demographic and population parameters in the tropical fruit fly *Anastrepha serpentina* (Dipter-a:Tephritidae). Bulletin of Entomological Research 89, 165–175.

Kaspi, R., Yuval, B., 2000. Post-teneral protein feeding improves sexual competitiveness but reduces longevity of mass reared sterile male Mediterranean fruit flies (Diptera:Tephritidae). Annals of the Entomological Society of America 93, 949–955.

Kaspi, R., Taylor, P.W., Yuval, B., 2000. Diet and size influence sexual advertisement and copuatory success of males in Mediterranean fruit fly leks. Ecological Entomology 25, 279–284.

Martin, P., Bateson, P., 1993. Measuring Behaviour: An Introductory Guide, 2nd edn. Cambridge University Press, Cambridge.

Norrbom, A.L., Zucchi, R.A., Hernández-Ortiz, V., 2000. Phylogeny of the genera *Anastrepha* and *Toxotrypana* (Trypetinae:Toxotrypanini) based on morphology. In: Aluja, M., Norrbom, A.L. (Eds.), Fruit Flies (Diptera:Tephritidae): Phylogeny and Evolution of Behaviour. CRC Press, Boca Raton, FL, pp. 375–406.

Norrbom, A.L., 2004. The Diptera site. http://www.sel.barc.usda.gov/Diptera/tephriti/tephriti.htm.

Papadopoulos, N.T., Katsoyannos, B.I., Kouloussis, N.A., Economopoulos, A.P., Carey, J.R., 1998. Effect of adult age, food, and time of the day on sexual calling incidence of wild and mass reared *Ceratitis capitata* males. Entomologia Experimentalis et Applicata 89, 175–182.

Pereira, R., 2005. Influence of a juvenile hormone analog and dietary protein on male Caribbean fruit fly, *Anasterpha suspensa* (Diptera:Tephritidae), sexual behaviour. Dissertation, University of Florida, Gainesville.

Pereira, R., Teal, P.E.A., Sivinski, J., Dueben, B.D., 2006. Influence of male presence on sexual maturation in female Caribbean fruit fly, *Anastrepha suspensa* (Dipter-a:Tephritidae). Journal of Insect Behavior 19, 31–43.

Pérez-Staples, D., Aluja, M., 2004. *Anastrepha striata* (Diptera:Tephritidae) females that mate with virgin males live longer. Annals of the Entomological Society of America 97, 1336–1341.

Pérez Staples, D., Aluja, M., 2006. Sperm allocation and cost of mating in a tropical tephritid fruit fly. Journal of Insect Physiology 52, 839–845.

Pérez-Staples, D., Aluja, M., Macías-Ordóñez, R., Sivinski, J., 2008. Reproductive trade-offs from mating with a successful male: the case of the tephritid fly *Anastrepha obliqua*. Behavioral Ecology & Sociobiology 62, 1333–1340.

Picimbon, J.F., Gadenne, C., Becard, J.M., Sreng, L., Clement, J.L., 1994. Polymorphism in sex pheromone blend in two populations of the black cutworm, *Agrotis ipsilon* (Lep., Noctuidae). In: Proceedings of the International Congress of Chemical Ecology, Syracuse, NY, June 6–8.

Reyes, J., Santiago, G., Hernández, P., 2000. The Mexican fruit fly eradication programme. In: Tan, K.H. (Ed.), Area-wide Control of Fruit Flies and Other Insect Pests. Penerbit Universiti Sains Malaysia, Penang, Malaysia, pp. 377–380.

Rull, J., Reyes, J., Enkerlin, W., 1996. The Mexican fruit fly eradication campaign: largest fruit fly industrial complex in the world. In: McPheron, B.A., Steck, G. (Eds.), Fruit Fly Pests: A World Assessment of their Biology and Management. St. Lucie Press, Delray Beach, FL, pp. 561–563.

Rull, J., Barreda-Landa, A., 2007. Colonization of a hybrid strain to restore male *Anastrepha ludens* (Diptera:Tephritidae) mating competitiveness for sterile insect technique programs. Journal of Economic Entomology 100, 752–758.

Rull, J., Díaz-Fleischer, F., Arredondo, J., 2007. Irradiation of *Anastrepha ludens* (Diptera:Tephritidae) revisited: optimizing sterility induction. Journal of Economic Entomology 100, 1153–1159.

Schal, C., Gu, X., Burns, E.L., Blomquist, G.J., 1994. Patterns of biosynthesis and accumulation of hydrocarbons and contact sex pheromone in the female German cockroach, *Blattella germanica*. Archives of Insect Biochemistry and Physiology 25, 375–391.

Sivinski, J., Aluja, M., Dodson, G., Freidberg, A., Headrick, D., Kaneshiro, K., Landolt, P., 2000. Topics in the evolution of sexual behavior in the tephritidae. In: Aluja, M., Norrbom, A. (Eds.), Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, pp. 751–792.

StatSoft, Inc., 2005. STATISTICA (Data Analysis Software System), Version 7.1. www.statsoft.com.

Teal, P.E.A., Gómez-Simuta, Y., Proveaux, A.T., 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. Proceedings of the National Academy of Sciences 97, 3708–3712.

Teal, P.E.A., Gomez-Simuta, Y., Dueben, B.D., Holler, T.C., Olson, S., 2007. Improving the efficacy of the sterile insect technique for fruit flies by incorporation of hormone and dietary supplements into adult holding protocols. In: Vreysen, M.J.B., Robinson, A.S., Hendrichs, J. (Eds.), Area-wide Control of Insect Pests: From Research to Field Implementation. Springer, Dordrecht, pp. 163–173.

Thomas, D.B., 2003. Reproductive phenology of the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera:Tephritidae) in the Sierra Madre Oriental, Northern Mexico. Neotropical Entomology 32, 385–397.

Thomas, D.B., 2004. Hot peppers as a host for the Mexican fruit fly *Anastrepha ludens* (Diptera:Tephritidae). Florida Entomologist 87, 603–608.

Warburg, M.S., Yuval, B., 1997. Effects of energetic reserves on behavioral patterns of Mediterranean fruit flies. Oecologia 113, 314–319.

Yuval, B., Kaspi, R., Field, S.A., Blay, S., Taylor, P., 2002. Effects of post-teneral nutrition on reproductive success of male Mediterranean fruit flies (Diptera:-Tephritidae). Florida Entomologist 85, 165–170.

Zar, J., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.