Journal of Insect Physiology 55 (2009) 231–242

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

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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ARTICLE INFO

Article history:
Received 8 May 2008
Received in revised form 21 November 2008
Accepted 24 November 2008

Keywords:
Methoprene
Juvenile hormone analog
Mating behavior
Oviposition behavior
Anastrepha
Tephritidae

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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doi:10.1016/j.jinsphys.2008.11.013

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).