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Methoprene modulates the effect of diet on male melon fly, *Bactrocera cucurbitae*, performance at mating aggregations

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Abstract

The effect of access to dietary protein (P) (hydrolyzed yeast) and/or treatment with a juvenile hormone analogue, methoprene (M), (in addition to sugar and water) on male aggregation (lekking) behaviour and mating success was studied in a laboratory strain of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Six-day-old males were treated with (1) protein and methoprene (M+P+), (2) only protein (M−P+), or (3) only methoprene (M+P−), and compared with 14-day-old sexually mature untreated males (M−P−). The lekking behaviour of the four groups of males when competing for virgin sexually mature females (14–16 days old) was observed in field cages. The following parameters were measured at male aggregations: lek initiation, lek participation, males calling, male–male interaction, female acceptance index, and mating success. For all these parameters, the M+P+ males significantly outperformed the other males. Moreover, for all parameters, there was a similar trend with M+P+ > M−P+ > M−P− > M+P−. More M+P+ males called and initiated and participated in lek activities than all other types of male, which resulted in higher mating success. They had also fewer unsuccessful copulation attempts than their counterparts. Whereas treatment with methoprene alone had a negative effect in young males with only access to sugar, access to dietary protein alone significantly improved young male sexual performance; moreover, the provision of methoprene together with protein had a synergistic effect, improving further male performance at leks. The results are of great relevance for enhancing the application of the sterile insect technique (SIT) against this pest species. The fact that access to dietary protein and treatment of sterile males with methoprene improves mating success means that SIT cost-effectiveness is increased, as more released males survive to sexual maturity.

Introduction

In non-resource-based mating systems, males provide females with no resource other than their gametes (Bradbury & Davies, 1987). Females visit aggregations of courting males or leks solely for the purpose of insemination but show a skewed mating preference, resulting in a distorted male mating pattern (Höglund & Alatalo, 1995; Shelly & Whittier, 1997) which has been described as the lek paradox (Borgia, 1979). Certain features such as male size, length of sexual displays, courtship intensity, and position within leks provide males with some advantages (Thornhill & Alcock, 1983; Shelly & Whittier, 1997). A meta-analysis by Fiske et al. (1998) across a wide variety of taxa has also concluded that male display activities, aggression rate, and lek attendance were positively correlated with male mating success.

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Males of many tephritid species aggregate in leks, display visual, acoustic, and chemical signals, and defend marked territories (Prokopy, 1980). The acoustic signals probably act as close-range signals, but pheromones may have both short and long-range effects (Sivinski & Burk, 1989). The role of pheromone signalling in copulatory success in the lek mating system has been demonstrated in tephritids such as *Ceratitis capitata* (Wiedemann) (Shelly, 2000), *Anastrepha suspensa* (Loew) (Pereira et al., 2009), and *Bactrocera dorsalis* (Hendel) (Shelly & Dewire, 1994). The production of pheromones is regulated by juvenile hormone (JH) (Rantala et al., 2003) and environmental differences may modify their production and female attractiveness. Application of a juvenile hormone analogue (methoprene) to *A. suspensa* can accelerate sexual maturity (Teal et al., 2000) and enhance copulatory success because of increased production of pheromones (Teal & Gomez-Simuta, 2002). In addition to enhanced pheromone production, the application of methoprene also modulates behavioural attributes in insects. Methoprene application promotes dominance behaviour in lobster cockroach [*Nauphoeta cinerea* (Olivier)] males (Kou et al., 2008) and increases lek initiation and lek participation ability in males of *A. suspensa* (Pereira et al., 2009).

The acceleration of sexual maturity may have a metabolic cost, which means that an additional source of nutrition is required, as the males have less time to accumulate resources. The production of pheromone also has a high energetic cost (Johansson et al., 2005), which suggests that male pheromones are likely to be 'honest' signals for females. This 'honesty' correlates with the quality of the signallers and females choose to mate with males having signals corresponding to large resources (Kokko, 1997; Maynard Smith & Harper, 2003).

Access to a diet including protein has been reported to improve male lek participation by enhancing their endurance and sexual signalling, resulting in higher copulatory success in Hawaiian *Drosophila* (Droney, 1996, 2001; Droney & Hock, 1998) A similar response was observed in the Mediterranean fruit fly, *C. capitata*, where mass-reared sterile males with access to a diet including protein were more likely to emit pheromones and more likely to copulate in the lek (Kaspi & Yuval, 2000). Access to protein in a diet also is reported to enhance copulatory success in *B. dorsalis* (Shelly et al., 2007) and *Bactrocera tryoni* (Froggat) (Pérez-Staples et al., 2007, 2008, 2009) and plays a critical role in reproductive development in *C. capitata* (Yuval et al., 1998) and *Phormia regina* (Meigen) (Stoffolano et al., 1995).

The melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), is an important economic pest of fruits and vegetables (White & Elson-Harris, 1992). It causes severe losses, directly by damaging the fruits and vegetables, and indirectly because its presence, because of its quarantine status, seriously interferes with the international marketing of fruits and vegetables. Control of tephritid pests by traditional chemicals (Roessler, 1989) results in some environmental concerns. The sterile insect technique (SIT), as a component of an integrated area-wide approach (Jang et al., 2008), is an efficient and well-established, environment-friendly technique for suppression, and in some situations also eradication, of melon fly as shown in Japan (Kakinohana et al., 1990). SIT is based on the fact that mating of wild females and released sterile males produces no offspring (Knipling, 1955). For the success of SIT, the mass-reared males should be able to compete with wild males for lek initiation, lek participation, courtship displays, and consequently mating and insemination success. Reducing the sexual maturation period may enhance the mating success and also enhance the cost-effectiveness of SIT by reducing the holding cost of mass-reared males in release facilities and increasing the number of released males surviving to sexual maturity. Methoprene application is known to accelerate sexual maturity in *A. suspensa* (Pereira, 2005) and *Anastrepha fraterculus* (Wiedemann) (Segura et al., 2009), but has no effect on *B. dorsalis* (Shelly et al., 2009). Thus, detailed mating behaviour studies are an important component of developing and improving SIT programmes (Hendrichs et al., 2002).

It is also known that *B. cucurbitae* has evolved a non-resource-based mating system involving the aggregation of males on the tree canopy at early dusk, during which the males show agonistic interactions with rivals, defend their marked territories by physical fighting or wing waving, display courtship behaviours, and emit pheromones to attract females for mating. The females visiting leks exert selection and then choose certain males (Kuba & Koyama, 1985; Iwahashi & Majima, 1986). In a previous study, Haq et al. (2010) reported that methoprene application accelerated sexual maturity and enhanced sexual competitiveness in *B. cucurbitae*. This study was designed to assess whether methoprene (M), a mimic of JH, and/or protein (P) added to the adult diet can be used to improve the performance of mass-reared males by improving their lek behaviour and hence their mating success. This study also provides information on the process of mate choice within leks and the role of male quality in female choice.

Materials and methods

Strain and rearing

The genetic sexing strain of the melon fly developed by USDA-ARS, Hawaii (McInnis et al., 2004) was used for all

experiments. The colony was maintained on wheat-based diet modified from standard diet (Hooper, 1987) at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. The flies were maintained under low stress conditions (four larvae per gram of diet and ca. 100 flies in 20 × 20 × 20 cm cages). Following emergence, the flies were sexed and maintained in the laboratory with 60 ± 5% r.h. and a photoperiod of L14:D10 at 24 ± 1 °C, synchronized with the natural photoperiod.

Treatments

Emerged adult males were exposed to one of the following four treatments:

1. Topical application of JH-analogue methoprene, and sugar and hydrolyzed yeast (protein source) (ICN Biomedical) as adult food (M+P+).
2. No methoprene application but sugar and hydrolyzed yeast (protein source) as adult food (M-P+).
3. Topical application of methoprene and only sugar as adult food (M+P−).
4. No methoprene application and only sugar as adult food (M-P-).

The methoprene was applied 3–4 h after adult emergence at a rate of 5 μg μl^{-1} acetone solution per male by immobilizing males in a net bag (standard marking technique) and then applying the solution via pipette through the net onto the dorsal surface of the thorax; cold anaesthesia was not used to immobilize the flies. Males from each treatment were maintained in separate 30 × 20-cm-diameter cylindrical screen cages with a maximum male density of 200 flies per cage and with the type of food assigned to each treatment. In treatments without protein feeding (P−), only water and sugar ad libitum were supplied to the flies. In the treatments with protein (P+), hydrolyzed yeast was added to the sugar in a proportion of 3:1 sugar:hydrolyzed yeast, and the flies were supplied with water ad libitum. This diet is considered a high quality diet for *Anastrepha* species (Pereira et al., 2009). Females used in all experiments were maintained in 30 × 20-cm-diameter cylindrical screen cages without exposure to males. They were provided with a diet of 3:1 sugar:hydrolyzed yeast, and ad libitum water.

Lek behaviour studies in field cages

A previous study using the same laboratory melon fly strain, Haq et al. (2010) showed that peak mating age for M−P− was ca. 14 days, which was thus used as a control age for this study. In addition, Haq et al. (2010) concluded that methoprene induced maturity in protein-fed males at 5–7 days of age. Therefore, for this study, the behaviour of 6-day-old M+P+, M−P+, and M+P− males and 14-day-old M−P− males was assessed when competing at aggregations or leks for females in field cages. Lek behaviour was studied on a potted *Citrus sinensis* (Osbeck) (Rutaceae) tree (1.7-m tall, with a canopy of approximately 1.5 m in diameter), a non-host for this species, in screened field cages in the greenhouse under natural light. Eight replications were run, one per day by the same observer, and new flies of the specified age were used in each replicate. Although a plant is required to study lek behaviour in field cages, it does not have to be a host plant (Kuba et al., 1984), because it is well known that melon fly mating occurs on diverse trees at the edge of melon fields (Iwahashi & Majima, 1986). A temperature of 25 ± 2 °C and r.h. of 65 ± 5% were maintained throughout the experiment. Light intensity (lux) was recorded every 30 min. Males from each treatment were marked on the thorax by different colours of water-based paint, 1 day before the test. The allocation of colour marking was rotated to avoid any colour preference by females.

Twenty males from each treatment were released in one field cage 90 min before sunset. For the first 45 min, male behaviour was studied in the absence of females and then 20 sexually mature virgin females (14–16 days old) were released and male behaviour in leks was observed until the males terminated their sexual activity at complete darkness. The following parameters were measured:

1. Lek initiation: The first male started to emit pheromone (wing vibration and anus beating) (Kuba & Sokei, 1988) in a certain area of plant canopy, resulting in initiation of aggregations.
2. Lek participation: Number of males that joined the first male to create an aggregation, calling or not. Males that landed within 15 cm of other calling males were considered to be lek participants (Iwahashi & Majima, 1986).
3. Males calling: Number of males calling (emitting pheromones) in a lek.
4. Male–male interaction: Number of male–male interactions in a lek. Male–male interaction occurred with two males on the same leaf, when an intruder arrived and tried to displace the fly already present. The antagonistic encounters involved wing waving, which lasted for several seconds, and physical fighting, and resulted in the loser leaving the leaf while the winners stayed on.
5. Male–male mounting: Number of male–male mounting attempts ('escalated fighting behaviour' according to Iwahashi & Majima, 1986).
6. Mating success: Number of males that achieved copulation.
7. Female acceptance index: Number of successful matings divided by the number of males attempting to mate.

Statistical analysis

The data were analyzed using a general linear model (ANOVA) to detect the impact of methoprene, protein, and their interaction. Complementary pair-wise comparisons of means (Tukey's test) were performed (Ott & Longneaker, 2001). The probability of mating success was tested by multiple regressions. The significance value used in tests was 95% ($\alpha = 0.05$). Data were analyzed by STATISTICA software (StatSoft, 2000).

Results

Lek formation and male calling

After release, the males moved from the interior of the plant canopy to the periphery, initiated wing vibration, started to emit pheromones, and initiated some aggregations. These aggregations or leks were scattered throughout the canopy of the tree. At dusk, when light intensity was decreasing, males moved to the upper part of the plant canopy and the largest aggregation was observed in this part of the canopy. Mean (± SE) number of leks per replicate was 7.2 ± 0.49 (range: 5–9), mean number of males per aggregation was 7.5 ± 0.44 (4–14). The percentages of melon fly males of the four treatments that participated in lek initiation, lek participation, and calling are shown in Figure 1. In general, M+P− males performed least well, M−P+ and M−P− males performed slightly better but equally well, and M+P+ males performed best. Statistical analysis revealed that the hydrolyzed yeast diet significantly improved lek initiation ($F_{1,31} = 20.48$, $P<0.001$) and lek participation ($F_{1,31} = 61.36$, $P<0.001$). Methoprene application alone had no significant effect on lek initiation ($F_{1,31} = 0.65$, $P = 0.42$) or lek participation ($F_{1,31} = 0.16$, $P = 0.19$). The treatment with methoprene and access to diet containing hydrolyzed yeast significantly enhanced lek initiation and lek participation ($F_{1,31} = 15.26$, $P<0.001$ and $F_{1,31} = 36.31$, $P<0.001$, respectively). Tukey's comparison of means showed that M−P+ and M−P− males were equal in their lek initiation and lek participation, but significantly better than M+P− males for lek initiation and lek participation. Exposure to methoprene and hydrolyzed yeast diet significantly improved lek initiation and lek participation of treated males. A similar pattern was observed for the effect of methoprene and hydrolyzed yeast on number of males calling in a lek (Figure 1). The diet containing hydrolyzed yeast significantly increased the number of males calling in leks ($F_{1,31} = 64.25$, $P<0.001$). Methoprene application alone had no effect ($F_{1,31} = 1.53$, $P = 0.22$) but its interaction with access to hydrolyzed yeast significantly increased the number of males calling in leks ($F_{1,31} = 40.49$, $P<0.001$).

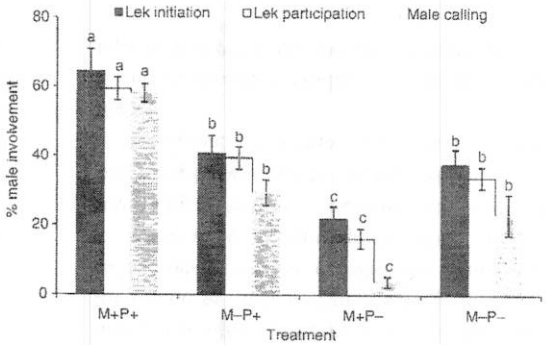

Figure 1 Performance in leks (mean ± SE) in terms of male involvement in lek initiation, lek participation, and calling by 6-day-old male *Bactrocera cucurbitae* treated with methoprene and/or protein (hydrolyzed yeast) competing with untreated 14-day-old mature males. Males were treated with methoprene and protein (M+P+), no methoprene and protein (M−P+), methoprene and only sugar (M+P−), or no methoprene and only sugar (M−P−). For each parameter, bars with the same letter are not significantly different (Tukey's test: P>0.05).

Male–male interactions

In 10 of 182 encounters recorded between residents and intruders, both males fell down from the leaf. In 172 cases, the contests were won by residents. M+P+, M−P+, and M−P− males were similar in their ability to defeat intruders (73–86% wins), but M+P− males won only 43% of contests with intruders (Table 1). In addition to male–male interactions in defence of positions within a lek, the males also attempted mounting other males. Among treatments, the highest percentage of males involved in such attempts was by M+P+ males (23%; n = 37), a significantly higher percentage ($F_{1,31} = 22.9$, $P<0.001$) than for all other types of male and the fewest mounting attempts (5.6%; n = 9) occurred with M+P− males. However, there was no significant difference between M−P−, M+P−, and M−P+ for male–male mountings (Figure 2).

Mating success and female acceptance

Not only did access to diet containing hydrolyzed yeast improve other parameters of male performance at aggregations but it also increased the probability of male mating success significantly ($\chi^2 = 19.68$, d.f. = 1, $P<0.001$) over males deprived of hydrolyzed yeast (Figure 3). The application of methoprene to males fed on hydrolyzed yeast further increased their mating success ($\chi^2 = 8.05$, d.f. = 1, $P<0.001$), while methoprene alone did not improve mating success and M+P− males did not succeed in mating at all (Figure 3). In general, unsuccessful mating attempts were common; 187 out of 299 male–female interactions ended without copulation. On aver-

Table 1 Percentages of resident 6-day-old *Bactrocera cucurbitae* males in leks treated with methoprene and/or protein (hydrolyzed yeast) that won contests against intruders when also competing with untreated 14-day-old mature males

Resident	Intruder M+P+	M−P+	M+P−	M−P−	Total resident wins
M+P+	72.22 (18)	82.35 (34)	87.50 (16)	100 (22)	85.55 (90) a
M−P+	75 (12)	80 (15)	88.88 (9)	83.33 (6)	80.95 (42) a
M+P−	100 (1)	40 (5)	50 (4)	25 (4)	42.85 (14) b
M−P−	83.33 (6)	62.5 (8)	80 (5)	71.40 (7)	73.07 (26) a

Totals with the same letter are not significantly different (Tukey's test: P>0.05).
Number of interactions is in parentheses.
Males were treated with methoprene and protein (M+P+), no methoprene and protein (M−P+), methoprene and only sugar (M+P−), or no methoprene and only sugar (M−P−)

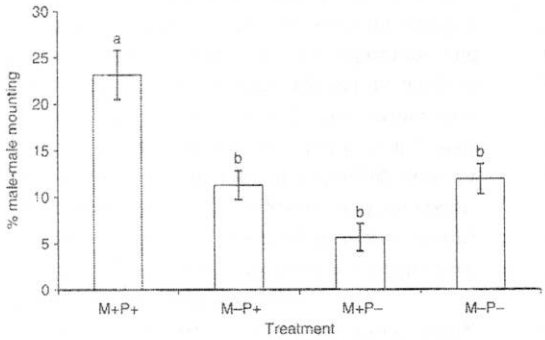

Figure 2 Mean (± SE) percentage of 6-day-old male *Bactrocera cucurbitae* treated with methoprene and/or protein (hydrolyzed yeast) and untreated 14-day-old mature males involved in male–male mounting attempts. Males were treated with methoprene and protein (M+P+), no methoprene and protein (M−P+), methoprene and only sugar (M+P−), or no methoprene and only sugar (M−P−). Bars with the same letter are not significantly different (Tukey's test: P>0.05).

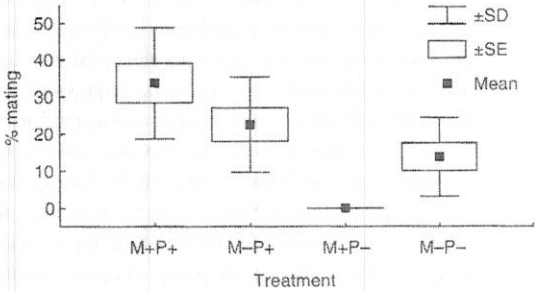

Figure 3 Mating success by 6-day-old male *Bactrocera cucurbitae* treated with methoprene and/or protein (hydrolyzed yeast) competing with untreated 14-day-old mature males for 14- to 16-day-old virgin females. Males were treated with methoprene and protein (M+P+), no methoprene and protein (M−P+), methoprene and only sugar (M+P−), or no methoprene and only sugar (M−P−).

age, in all treatments, males succeeded in mating after 2.6 ± 0.4 male–female interactions. M+P+ males were able to mate after 1.43 ± 0.26 interactions, whereas M−P+ and M−P− males succeeded to mate only after 2.62 ± 0.43 and 3.06 ± 0.63 interactions, respectively. ANOVA showed that M+P+ males had a significantly higher ($F_{1,31}$ = 56.55, P<0.001) female acceptance index as compared with M−P+ and M+P− males (Figure 4).

Discussion

The results obtained in this study showed the strong effect of nutritional status on male lekking behaviour. Males fed on hydrolyzed yeast engaged vigorously in sexual calling, far more than hydrolyzed yeast-deprived males, and they started pheromone calling earlier. This behavioural response to a diet containing protein is similar to that in the Mediterranean fruit fly (Kaspi et al., 2000) and other tephritid fruit flies (Drew & Yuval, 2000). A balanced sugar-hydrolyzed yeast diet also improved the ability of males to initiate and participate in leks.

The disparity in ability to participate in a lek by differently fed males showed that participation has a high resource cost. Consequently, M−P+ males were much more likely to participate in a lek than M+P− males. Young M−P+ males have a similar ability to participate as hydrolyzed yeast-deprived, but sexually fully mature (14-days old) M−P− males. A similar impact of diet was observed for males calling, as similar fractions of M−P+ and M−P− but higher than M+P− males, engaged in pheromone calling within a lek. This provides strong evidence that lek participation and calling is dependent on energy reserves as M−P− males, although protein-

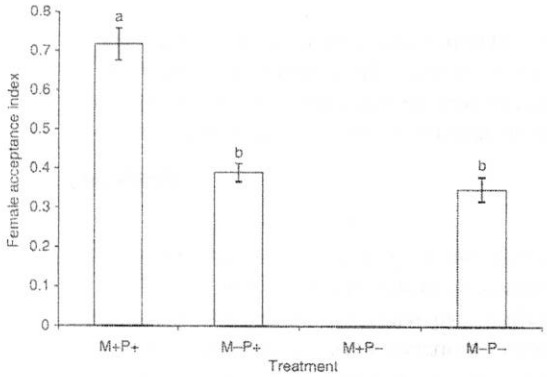

Figure 4 Female acceptance index for 6-day-old male *Bactrocera cucurbitae* treated with methoprene and/or protein (hydrolyzed yeast) competing with untreated 14-day-old mature males for 14- to 16-day-old virgin females. Males were treated with methoprene and protein (M+P+), no methoprene and protein (M−P+), methoprene and only sugar (M+P−), or no methoprene and only sugar (M−P−). Bars with the same letter are not significantly different (Tukey's test: P>0.05).

deprived, have accumulated some resources during their long adult maturation period. This diet effect on male performance in a lek was similar to previous studies with *C. capitata* (Yuval et al., 1998); moreover, the application of methoprene had an additive effect with protein which increased male lek participation and pheromone calling, leading in turn to higher mating success.

How have methoprene and hydrolyzed yeast influenced the male's behaviour in leks, resulting in higher mating success? Some possibilities include greater lekking endurance, calling for a longer period, or defeating rivals by using winning strategies in the lek (Fiske et al., 1998; Robacker et al., 1998; Droney, 2001). The results confirmed that more M+P+ males were engaged in calling in the aggregations. Thus, application of methoprene and protein may make a difference by increasing the number of males engaged in calling or enabling the males to produce more attractive pheromone per unit of time (Teal et al., 2000), which led to higher mating success. That more M+P+ males engaged in calling than any other type of males suggests that pheromone calling is not only dependent on energy reserves, but also on hormonal status. Pheromone production may also be linked to attaining a certain level of reproductive development and M+P+ males reach this level earlier than males not treated with methoprene. The application of methoprene seems to elevate the JH titre (Contreras-Garduño et al., 2009), which enables males to produce attractive pheromones. Pheromone production is reported to be governed by JH (Rantala et al., 2003) and this impact of methoprene and hydrolyzed yeast is similar to that in *A. suspensa* (Teal et al., 2000). Thus, the increased number of M+P+ males engaged in calling as compared with M+P- males may be due to the elevated JH level diverting the nutritional resources for pheromone production (Rantala et al., 2003) in M+P+ males, whereas the nutritional resources were a limiting factor for M+P− males.

The differential ability of M+P+ and M+P− males provides evidence that engagement in emitting pheromones may require significant energy reserves (Droney & Hock, 1998). Thus, pheromones are costly and condition-dependent, making them 'honest' signals for females evaluating displaying males at leks (Johansson et al., 2005). M+P+ males were able to allocate more resources for pheromone signalling than M−P+ males. The higher mating success by M+P+ males showed that females may perceive the nutritional and hormonal or developmental status of males from their pheromones and preferred males with a higher level of JH, perhaps for certain benefits such as insemination (Cusson & McNeil, 1989). Previous studies already showed that M+P+ males had a longer copulation duration than M−P+ males when aged 5–7 days, but subsequently there was no difference in copulation duration as the M−P+ males could also attain their reproductive developmental threshold (Haq et al., 2010).

Another possibility explaining the lower ability of 6-day-old M+P− males for pheromone calling and lek participation than 14-day-old M−P− males may be that as JH enhances protein metabolism, the males with a higher level of JH titre require additional protein, as in *P. regina* (Yin et al., 1999). Thus, young M+P− males, lacking sufficient nutritional resources for enhanced metabolism were not able to compete with other males in terms of pheromone calling and lek participation. An alternative possibility could be that methoprene is toxic to protein-deprived males but this has not been reported for melon fly (Haq et al., unpubl.) and any other tephritid males exposed to the same protocol for methoprene application (Teal et al., 2000, 2007; Pereira et al., 2009; Segura et al., 2009)

Higher mating success of the M+P+ males may also be attributed to their greater ability to defeat intruders. This may provide the opportunity to spend more time in aggregations and all other things being equal, be available longer to visiting receptive females. In addition, certain territories may be particularly valuable within the lek, because they serve as superior signalling sites and occupation of these sites may be indicator of male quality which lead to female preference for such males (Kaspi & Yuval, 1999; Field et al., 2002). A higher mating success of M+P+ males, corresponding to greater success in antagonistic male–male interactions, is substantiated by the findings of Robacker

et al. (1991) who reported that mating success is influenced by males' fighting ability in *A. ludens*.

In addition to fighting for certain territories, some males attempted to mount other males. It is reported that male–male mounting in other fruit flies is due to little or no ability of males to discriminate among sexes prior to contact (Morgante et al., 1983). But according to Iwahashi & Majima (1986), melon fly males can discriminate the sexes prior to physical contact; they interpreted mounting attempts as escalated fighting behaviour and reported that a large fraction of the observed wild males attempted to mount other males. Mass rearing can alter mating behaviour (Suzuki & Koyama, 1980) and may have stimulated the males to mount other males due to olfactory confusions in the mass-rearing environment (Gaskin et al., 2002). In another study, mass-reared *B. tryoni* males exhibited less discrimination between mating partners and showed more mounting attempts than wild males (Weldon, 2005). Nevertheless, all treatments of our experiment used laboratory-reared males, but only M+P+ males showed more mounting attempts than the other types of male. Thus it is difficult to conclude that enhanced male mounting attempts are due only to methoprene treatment or access to protein. Therefore, our results support the interpretation of Iwahashi & Majima (1986). However, a study comparing the lek behaviour of laboratory-reared with wild flies may explain enhanced male–male mounting behaviour in M+P+ males.

Beyond the physiological mechanism of dietary protein and methoprene, their impact on mating behaviour has enabled a better understanding to be gained of the lek system and sexual selection for the melon fly within such aggregations. It is difficult to determine which factor alone is responsible for the higher mating success of M+P+ males at leks. Nevertheless, their significantly higher female acceptance index and fewer unsuccessful mating attempts show the female preference for quality of males, which seems to be an important reason for the skewed mating success. Differences in lek performance and reproductive success by differently treated males showed that certain treatments make the males more attractive in a lek (i.e., hotshots). M+P+ males indicated to visiting females their attractiveness by emitting more attractive signals, by remaining in the lek, and by dominating their rivals there. These successful behaviours are attributed to the elevated level of hormone and the nutritional status, of which nutrition appears to be the more critical. M+P– males may have a higher level of hormone but lack nutritional reserves to perform well in a lek. M+P– males tried to participate in leks and presented themselves to receptive females visiting leks, but were unable to secure copulations. Clearly, such males did not have the adequate pheromone profile or were not energetic enough to succeed within a lek, but adopted the strategy of joining the more successful males and trying to intercept visiting females (Beehler & Foster, 1988). Obviously, these results support the 'hotshot lek strategy' (Bradbury et al., 1986), however, with the present data, it is difficult to rule out the hotspot strategy (Bradbury & Gibson, 1983): lek sites may function as a hotspot and M+P+ males may be more competent in locating and defending the hotspots, resulting in higher mating success. Previous studies reported that melon fly males compete with other males for occupying their calling sites within leks and females visiting these leks exert a choice for mating (Iwahashi & Majima, 1986). Similarly, the present results showed that sexual success in this species is governed by a combination of intra-sexual male competition and female choice for a certain quality of males. The higher female acceptance index for M+P+ males supports a female choice for quality males. Such a selection for quality males may be due to the package of advantages offered to the female, such as direct fecundity benefits or indirect, genetic benefits via the production of superior offspring (see Droney, 1996).

In fruit flies with a long adult maturation period, such as melon fly, there is considerable sterile male mortality in the field in the period between the release of the sterile males (ca. 3–4 days old) and their age of peak sexual maturation (ca. 14-days old). This represents a major economic loss for programmes that incorporate SIT as part of integrated fruit fly management. We have shown that treating males with methoprene before release at the sterile fly emergence and release facilities, and giving them access to hydrolyzed yeast, can effectively accelerate their sexual maturation by several days. Holding sterile males in the emergence and release facilities until they are sexually mature at the time of release is not cost-effective (Koyama et al., 2004). However, in view of the possibility to significantly shorten the maturation period, such an option is now more viable, and even when the release age is not changed, significantly more sterile males are likely to survive in the field to reach sexual maturity. In addition, a significant increase in the ability of sterile males to participate and perform in leks, resulting in higher mating success because of methoprene exposure and access to hydrolyzed yeast, further supports their application to mass-reared males. Larger-scale studies on the effect and cost-effectiveness of exposing sterile males to methoprene and hydrolyzed yeast need to be done to support their incorporation into operational SIT programmes.

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References

Beehler BM & Foster MS (1988) Hotshots, hotspots, and female preference in the organization of lek mating systems. American Naturalist 138: 203–219.

Borgia G (1979) Sexual selection and the evolution of mating systems. Sexual Selection and Reproductive Competition in Insects (ed. by MS Blum & NA Blum), pp. 19–80. Academic Press, New York, NY, USA.

Bradbury JW & Davies NB (1987) Relative roles of intra- and intersexual selection. Sexual Selection: Testing the Alternatives (ed. by JW Bradbury & MB Andersson), pp. 143–164. John Wiley, Chichester, UK.

Bradbury J & Gibson R (1983) Leks and mate choice. Mate Choice (ed. by P Bateson), pp. 109–138. Cambridge University Press, Cambridge, UK.

Bradbury J, Gibson R & Tsai IM (1986) Hotspots and the dispersion of leks. Animal Behaviour 34: 1694–1709.

Contreras-Garduño J, Córdoba-Aguilar A, Lanz-Mendoza H & Rivera AC (2009) Territorial behaviour and immunity are mediated by juvenile hormone: the physiological basis of honest signalling? Functional Ecology 23: 157–163.

Cusson M & McNeil JN (1989) Involvement of juvenile hormone in the regulation of pheromone release activities in a moth. Science 243: 210–212.

Drew RAI & Yuval B (2000) The evolution of fruit fly feeding behavior. Fruit Flies (Tephritidae) Phylogeny and Evolution of Behavior (ed. by M Aluja & AL Norrbom), pp. 731–749. CRC Press, Boca Raton, FL, USA.

Droney DC (1996) Environmental influences on male courtship and implications for female choice in a lekking Hawaiian *Drosophila*. Animal Behaviour 51: 821–830.

Droney DC (2001) Male lekking duration and mating benefits in a lek-forming Hawaiian *Drosophila*. Journal of Insect Behavior 14: 621–635.

Droney DC & Hock MB (1998) Male sexual signals and female choice in *Drosophila grimshawi* (Diptera: Drosophilidae). Journal of Insect Behavior 11: 59–71.

Field SA, Kaspi R & Yuval B (2002) Why do calling medflies (Diptera: Tephritidae) cluster? Assessing the empirical evidence for models of medfly lek evolution. Florida Entomologist 85: 63–72.

Fiske P, Rintamaki PT & Karvonen E (1998) Mating success in lekking males: a meta-analysis. Behavioral Ecology 9: 328–338.

Gaskin T, Futerman P & Chapman T (2002) Increased density and male-male interactions reduce male longevity in the medfly, *Ceratitis capitata*. Animal Behaviour 63: 121–129.

Haq I, Cáceres C, Hendrichs J, Teal PEA, Wornoayporn V et al. (2010) Effects of the juvenile hormone analogue methoprene and dietary protein on male melon fly *Bactrocera cucurbitae* (Diptera: Tephritidae) mating success. Journal of Insect Physiology, doi:10.1016/j.jinsphys.2010.04.018.

Hendrichs J, Robinson AS, Cayol JP & Enkerlin W (2002) Medfly areawide Sterile Insect Technique programmes for prevention, suppression or eradication: The importance of mating behavior studies. Florida Entomologist 85: 1–13.

Höglund J & Alatalo RV (1995) Leks. Princeton University Press, Princeton, NJ, USA.

Hooper GHS (1987) Application of quality control procedures to large scale rearing of the Mediterranean fruit fly. Entomologia Experimentalis et Applicata 44: 161–167.

Iwahashi O & Majima T (1986) Lek formation and male-male competition in the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Applied Entomology and Zoology 21: 70–75.

Jang EB, McQuate GT, Mcinnis DO, Harris EJ, Vargas RI et al. (2008) Targeted trapping, bait spray, sanitation, sterile-male, and parasitoid releases in an areawide integrated melon fly (Diptera: Tephritidae) control program in Hawaii. American Entomologist 54: 240–250.

Johansson BG, Jones TM & Widemo F (2005) Cost of pheromone production in a lekking *Drosophila*. Animal Behaviour 69: 851–858.

Kakinohana H, Kuba H, Yamagishi M, Kohama T, Kiniyo K et al. (1990) The eradication of the melon fly from the Okinawa Islands, Japan. II. Actual control program. Proceedings of the International Symposium on Fruit Flies of Economic Importance (ed. by M Aluja & P Liedo), pp. 465–469. Springer, New York, NY, USA.

Kaspi R & Yuval B (1999) Mediterranean fruit fly leks: factors affecting male location. Functional Ecology 13: 539–545.

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 PW & Yuval B (2000) Diet and size influence sexual advertisement and copulatory success of males Mediterranean fruit fly leks. Ecological Entomology 25: 279–284.

Knipling EF (1955) Possibilities of insect control or eradication through the use of sexual sterile males. Journal of Economic Entomology 48: 459–462.

Kokko H (1997) Evolutionarily stable strategies of age-dependent sexual advertisement. Behavioral Ecology and Sociobiology 41: 99–107.

Kou R, Chou S-Y, Huang ZY & Yang R-L (2008) Juvenile hormone levels are increased in winners of cockroach fights. Hormones and Behavior 54: 521–527.

Koyama J, Kakinohana H & Miyatake T (2004) Eradication of the melon fly, *Bactrocera cucurbitae*, in Japan: Importance of behaviour, ecology, genetics, and evolution. Annual Review of Entomology 49: 331–349.

Kuba H & Koyama J (1985) Mating behavior of wild melon flies, *Dacus cucurbitae* Coquillett. (Diptera: Tephritidae) in a field

cage: courtship behavior. Applied Entomology and Zoology 20: 365–372.

Kuba H & Sokei Y (1988) The production of pheromone clouds by spraying in the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Journal of Ethology 6: 105–110.

Kuba H, Koyama J & Prokopy RJ (1984) Mating behavior of wild melon flies, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae) in a field cage: distribution and behavior of flies. Applied Entomology and Zoology 19: 367–373.

Maynard Smith J & Harper D (2003) Animal Signals. Oxford University Press, Oxford, UK.

McInnis DO, Tam S, Lim R, Komatsu J, Kurashima R & Albrecht C (2004) Development of a pupal color-based genetic sexing strain of the melon fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae). Annals of the Entomological Society of America 97: 1026–1033.

Morgante JS, Malavasi A & Prokopy RJ (1983) Mating behavior of wild *Anastrepha fraterculus* (Diptera: Tephritidae) on a caged host tree. Florida Entomologist 66: 234–241.

Ott RL & Longneaker M (2001) An Introduction of Statistics Methods and Data Analyses. 5th edn. Duxbury Publishers, Pacific Grove, CA, USA.

Pereira R (2005) Influence of a Juvenile Hormone Analog and Dietary Protein on Male Caribbean Fruit Fly, *Anastrepha suspensa* (Diptera: Tephritidae), Sexual Behaviour. PhD dissertation, University of Florida, Gainesville, FL, USA.

Pereira R, Sivinski J & Teal PEA (2009) Influence of methoprene and dietary protein on male *Anastrepha suspensa* (Diptera: Tephritidae) mating aggregations. Journal of Insect Physiology 55: 328–335.

Pérez-Staples D, Prabhu V & Taylor PW (2007) Post-teneral protein feeding enhances sexual performance of Queensland fruit flies. Physiological Entomology 32: 225–232.

Pérez-Staples D, Harmer AMT, Collins SR & Taylor PW (2008) Potential for pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies. Agricultural and Forest Entomology 10: 255–262.

Pérez-Staples D, Weldon CW, Smallridge CJ & Taylor PW (2009) Pre-release feeding on yeast hydrolysate enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. Entomologia Experimentalis et Applicata 131: 159–166.

Prokopy RJ (1980) Mating behavior of frugivorous Tephritidae in nature. Symposium on Fruit Fly Problems, pp. 37–46. XVI International Congress of Entomology, Kyoto, Japan.

Rantala MJ, Vainikka A & Kortet R (2003) The role of juvenile hormone in immune function and pheromone production trade-offs: a test of the immunocompetence handicap principle. Proceedings of the Royal Society of London B: Biological Sciences 270: 2257–2261.

Robacker DC, Mangan RL, Moreno DS & Tarshis-Moreno AM (1991) Mating behavior and male mating success in wild *Anastrepha ludens* (Diptera: Tephritidae) on a field caged host tree. Journal of Insect Behavior 4: 471–481.

Robacker DC, Martinez AJ, Garcia JA & Bartelt RJ (1998) Volatiles attractive to the Mexican fruit fly (Diptera: Tephritidae) from eleven bacteria taxa. Florida Entomologist 81: 497–508.

Roessler Y (1989) Insecticidal bait and cover sprays. Fruit Flies, Their Biology, Natural Enemies and Control, World Crop Pests 3B (ed. by AS Robinson & G Hooper), pp. 329–336. Elsevier, Amsterdam, The Netherlands.

Segura DF, Cáceres C, Vera TM, Wornoayporn V, Islam A et al. (2009) Enhancing mating performance after juvenile hormone treatment in *Anastrepha fraterculus*: A differential response in males and females acts as a physiological sexing system. Entomologia Experimentalis et Applicata 131: 75–84.

Shelly TE (2000) Male signalling and lek attractiveness in the Mediterranean fruit fly. Animal Behaviour 60: 245–251.

Shelly TE & Dewire AM (1994) Chemically mediated mating success in male Oriental fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America 87: 375–382.

Shelly TE & Whittier TS (1997) Lek behavior of insects. The Evolution of Mating Systems in Insects and Arachnids (ed. by JC Choe & BJ Crespi), pp. 273–293. Cambridge University Press, Cambridge, UK.

Shelly TE, Edu J & Pahio E (2007) Condition-dependent mating success in male fruit flies: ingestion of a pheromone precursor compensates for a low-quality diet. Journal of Insect Behavior 20: 347–365.

Shelly TE, Nishimoto JI & Edu J (2009) Influence of methoprene on the age-related mating propensity of males of the Oriental fruit fly and the Mediterranean fruit fly (Diptera: Tephritidae). Florida Entomologist 92: 193–198.

Sivinski J & Burk T (1989) Reproductive and mating behaviour. Fruit Fies, Their Biology, Natural Enemies and Control, World Crop Pests 3A (ed. by AS Robinson & G Hooper), pp. 343–351. Elsevier, Amsterdam, The Netherlands.

StatSoft (2000) STATISTICA for windows (Computer Program Manual). Statsoft, Tulsa, OK, USA.

Stoffolano JG, Jr, Tobin EN, Wilson J & Yin C-M (1995) Diet affects insemination and sexual activity in male *Phormia regina* (Diptera: Caliphoridae). Annals of the Entomological Society of America 88: 240–246.

Suzuki Y & Koyama J (1980) Temporal aspects of mating behavior of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae): a comparison between laboratory and wild strains. Applied Entomology and Zoology 15: 215–224.

Teal PEA & Gomez-Simuta Y (2002) Juvenile hormone: action in regulation of sexual maturity in Caribbean fruit flies and potential use in improving efficacy of sterile insect control technique for tephritid fruit flies. IOBC/WPRS Bulletin 25: 167–180.

Teal PEA, Gomez-Simuta Y & Proveaux AT (2000) Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. Proceedings of the National Academy of Sciences of the USA 97: 3708–3712.

Teal PEA, Gomez-Simuta Y, Dueben BD, Holler TC & 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. Area-wide Control of Insect Pests. From Research to Field Implementation (ed. by MJB Vreysen, AS Robinson & J Hendrichs), pp. 163–173. Springer, Dordrecht, The Netherlands.

Thornhill R & Alcock J (1983) The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, MA, USA.

Weldon CW (2005) Mass-rearing and sterilisation alter mating behaviour of Queensland fruit fly, *Bactrocera tryoni* (Frogatt) (Diptera: Tephritidae). Australian Journal of Entomology 55: 158–163.

White IM & Elson-Harris MM (1992) Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International, London, UK.

Yin C-M, Qin W-H & Stoffolano JG, Jr (1999) Regulation of mating behaviour by nutrition and the corpus allatum in both male and female *Phormia regina* (Meigen). Journal of Insect Physiology 45: 815–822.

Yuval B, Kaspi R, Shloush S & Warburg MS (1998) Nutritional reserves regulate male participation in Mediterranean fruit fly leks. Ecological Entomology 23: 211–215.