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Sexual development of wild and mass-reared male Queensland fruit flies in response to natural food sources

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Abstract

Diet has a profound influence on the fitness of adult tephritid flies. Mass-reared flies are provided yeast hydrolysate as a rich source of nutrition that supports rapid sexual development and mating success. In contrast, wild tephritid flies often live in environments where food may be hard to find, and these are the conditions that sexually immature mass-reared sterile males encounter when released into the field during sterile insect technique campaigns. The effect of natural food sources (bat guano, bird droppings, citrus pollen, and wheat pollen) on the sexual development of adult mass-reared fertile, mass-reared sterile, and wild male Queensland fruit flies, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), was determined by measuring ejaculatory apodeme size. Inclusion of yeast hydrolysate in the adult diet was associated with faster growth of the ejaculatory apodeme in comparison with all other diets. Effects of diet were far less pronounced in mass-reared males, which may indicate reduced nutritional requirements, whereas the ejaculatory apodeme of wild males fed on natural sources of food or sucrose alone did not increase in size over the first 20 days of adult life.

Introduction

Diet has a profound influence on the fitness of adult tephritid flies (Diptera: Tephritidae). Females are incapable of producing mature oocytes without having consumed proteinaceous food in the adult stage (Bateman, 1972; Drew, 1987; Fanson et al., 2009). Furthermore, diet is closely linked to male sexual performance and mating success, including sexual maturity (Perez-Staples et al., 2007, 2008; Prabhu et al., 2008; Weldon et al., 2008), competitive ability and persistence in mating aggregations (Yuval et al., 1998; Kaspi & Yuval, 2000; Kaspi et al., 2000; Aluja et al., 2001, 2008; Shelly et al., 2005), mating latencies and copula duration (Perez-Staples et al., 2007, 2008, 2009; Prabhu et al., 2008), numbers of sperm stored by females (Taylor & Yuval, 1999; Perez-Staples et al., 2008), and capacity to inhibit female remating (Blay & Yuval, 1997; Perez-Staples et al., 2008; Aluja et al., 2009; Gavriel et al., 2009).

Most studies investigating the influence of diet on adult tephritid flies have considered yeast hydrolysate as a nutritional supplement. Yeast hydrolysate is a rich source of amino acids, minerals, vitamins, and cholesterol, which is routinely included in the diet of adult tephritid flies in laboratory cultures and mass-rearing facilities. In comparison with laboratory-reared flies, wild tephritid flies tend to exist in environments where nutrients may be hard to find (Courtice & Drew, 1984; Hendrichs et al., 1993; Aluja et al., 2001). Diet components available in the field that have been suggested as sources for tephritid flies include bacteria, honeydew (anal secretions) from sap-sucking Hemiptera, pollen, and vertebrate droppings, although the sexual performance benefits of these materials for adult tephritids varies between species and are often absent (Hagen, 1958; Hendrichs et al., 1991, 1993; Aluja et al., 2001; McQuate et al., 2003; Yee, 2003; Manrakhan & Lux, 2006).

Natural food sources are an important consideration for the successful implementation of sterile insect

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technique (SIT) programmes that aim to suppress or eradicate wild tephritid populations. Sterile insect technique programmes rely on the ability of sterile mass-reared males to mate with wild females, induce reproductive sterility in their mates, and thereby reduce population levels in the next generation (Knipling, 1959). Because SIT requires vast numbers of sterile insects, it is necessary to mass rear insects under factory conditions that bear little resemblance to their natural environment (Calkins & Parker, 2005), including sucrose and yeast hydrolysate as an artificial diet for adults. Female Queensland fruit flies, *Bactrocera tryoni* (Froggatt), are known to adapt rapidly to the adult diet of sucrose and yeast hydrolysate that is provided in the laboratory; their consumption increases, they become sexually mature at younger ages, and they produce more eggs per milligram of diet (Meats et al., 2004). However, it is unknown how effective laboratory-adapted, mass-reared tephritids that are sterilised for use in SIT programmes are at utilising food sources that are available in the field. The relatively low nutrient content of natural sources may not be sufficient to support the rapid sexual development, sexual performance, and longevity of sterile mass-reared males that has been noted when they are provided with yeast hydrolysate. Conversely, it may be that wild *B. tryoni* are able to efficiently utilise natural food sources to survive and become sexually mature.

This study investigates the effect of natural food sources on the sexual development of adult mass-reared fertile, mass-reared sterile, and wild male *B. tryoni*. Bacteria were not included in this study. The contribution of bacteria to the fitness of wild and mass-reared *B. tryoni* has been explored in numerous studies (Drew et al., 1983; Drew, 1987; Drew & Lloyd, 1987; Prokopy et al., 1991; Murphy et al., 1994; Meats et al., 2009), whereas the role of other prospective sources of nutrition remains to be determined.

Materials and methods

Preparation of diets

Adult male *B. tryoni* were provided with four diets that included material that has been suggested as a potential source of nutrition in the field and could be available in a citrus orchard (citrus pollen, wheat pollen, bird droppings) or in host plants found in the endemic rainforest habitat of *B. tryoni* (bat guano, bird droppings). Pollen can have a protein content of 2.5–61% (by dry weight) (Roulston et al., 2000), starch content of 0–22%, and lipid content of 1–20% (Roulston & Cane, 2000). Bird droppings have high nitrogen content principally in the form of uric acid, but low levels of unabsorbed carbohydrates and lipids may also be available (e.g., see Wolf et al., 2007). Similarly, bat guano contains unabsorbed nutrients from the gut, but the ratio of carbohydrates, lipids, and nitrogenous compounds is dependent on the feeding guild of bat species (Emerson & Roark, 2007). A further group of males was given access to yeast hydrolysate (yeast hydrolysate enzymatic; MP Biomedicals, Aurora, OH, USA). A control group was provided with granulated sucrose (cane sugar) only. Granulated sucrose was provided in a separate dish in all treatments as a source of carbohydrate.

Citrus pollen was obtained from citrus flowers [*Citrus* × *limon* (L.) Burm.f. 'Eureka', *Citrus* × *meyeri* (Rutaceae)] in orchards located at Macquarie University (33°46′S, 151°6′E) and the Gosford Primary Industries Institute (33°23′S, 151°20′E), Narara, NSW, Australia. Citrus pollen is sticky, which made it difficult to harvest large quantities of pure pollen, so citrus pollen used in this experiment was a mix of both citrus anthers and pollen. To harvest the citrus pollen, elongated flower buds that were soon to open were removed from plants and left overnight in Petri dishes lined with filter paper. The following day, the petals were removed before the dehiscent anthers were pulled from the stamens using jeweller's forceps and transferred to a centrifuge tube. Wheat, *Triticum aestivum* L. (Poaceae), pollen was purchased from Allergon AB (90% purity; Välingevägen, Ängelholm, Sweden). Conditions imposed by the Australian Quarantine and Inspection Service (under IP09008644) required that the wheat pollen be treated with 25 kGy of gamma radiation before being released for in vivo use with *B. tryoni*. On collection and receipt of citrus and wheat pollen, respectively, samples were stored at −20 °C. It has been reported that 23.1% of the dry weight of wheat pollen is protein (Roulston et al., 2000), and the protein content of *Citrus* × *limon* 'Lisbon' pollen is 18.8% of air-dried weight (Gilliam et al., 1980).

Bat guano was collected from the leaf surfaces of vegetation located beneath a grey-headed flying fox, *Pteropus poliocephalus* (Temminck), roosting site in Parramatta Park, Parramatta, NSW, Australia (33°48′S, 151°00′E). Analysis of the guano from a related species of frugivorous flying fox, *Pteropus rodricensis* Dobson, revealed that it had a lipid and nitrogen content of 9.6 and 1.9% of dry weight, respectively (Emerson & Roark, 2007). Bird droppings were collected from the leaf surfaces of citrus trees in orchards located at Macquarie University and the Gosford Primary Industries Institute. Bat guano and bird droppings were homogenised with a small amount of water, dried at 80 °C for 4 h, ground to a powder with a mortar and pestle, and stored dry in a Petri dish sealed with laboratory film (Parafilm 'M'; Pechiney Plastic Packaging, Chicago, IL, USA) at −20 °C.

Origin of flies

Wild flies used in this study were the offspring of adults reared from infested fruit that had been collected from trees in Bathurst (33°26′S, 149°34′E; apples and pears) and Tomakin (35°50′S, 150°11′E; nectarines), New South Wales, Australia. Wild adult flies reared from these fruits were housed in a large plastic and nylon mesh cage (47.5 × 47.5 × 47.5 cm, BugDorm 4090 Insect Rearing Cage; MegaView Science, Taichung, Taiwan) with ad libitum access to water, sucrose, and yeast hydrolysate. The cage was placed in a controlled environment room at 24–26 °C and 60–70% r.h., with an L14:D10-h light cycle. The lights were on full intensity for 12 h, and flies also experienced simulated dawn and dusk as the lights stepped on and off in four stages over the course of 1 h. After flies had matured sexually (males were observed to call at dusk), they were provided with artificial fruits (ca. 30 mm diameter) of carrot-based larval medium (based on the recipe of Bateman, 1967) wrapped in laboratory film, into which females could oviposit. Artificial fruits were removed after 3–4 days and placed on a 20-mm-deep bed of vermiculite (Grade 1, Ausperl; Orica Australia, Banksmeadow, NSW, Australia) in plastic trays. The vermiculite was sieved gently after 14 days to remove pupae. Pupae emerged into a 5-l, ventilated, plastic cage (24 × 15 × 15 cm) in the controlled environment room.

Fertile mass-reared *B. tryoni* were obtained as pupae from a mass-rearing facility at Elizabeth Macarthur Agricultural Institute (NSW Department of Primary Industries). Adults emerging from irradiated pupae produced by this facility (approximately 5 million per week) are released routinely in SIT programmes to control *B. tryoni* outbreaks (Dominiak et al., 2008). The mass-reared colony was housed in two or three adult cages with approximately 200 000 adults in each cage (Jessup, 1999) and had been maintained in the facility for over 20 generations at the time of the present study. Sterile mass-reared flies were produced by exposing hypoxic pupae to gamma radiation (70–75 Gy) from a cobalt-60 source (Gamma Technology Research Irradiator) operated by the Australian Nuclear Science and Technology Organization (for details, see Collins et al., 2009). Fricke dosimeters confirmed that all irradiation treatments fell within the target range. Fertile and sterile mass-reared pupae were allowed to emerge into 5-l cages in the controlled environment room.

Male sexual development

To determine the effects of natural sources of food on sexual development of male *B. tryoni*, it was necessary to sample individuals over a range of ages. On the day of adult emergence, males of the same age and type were transferred into 5-l cages that were furnished with a water reservoir and two plastic dishes that contained sucrose and one of the six diet treatments. Cages that housed fertile and sterile mass-reared males contained up to 50 males, whereas cages that housed wild males held only 25 flies because of the difficulty in obtaining high emergence of wild flies on the same day. The 5-l cages are used routinely to house up to 200 flies, so the mass-reared and wild flies were unlikely to suffer from any effects of crowding on longevity and performance. At ages of 0, 4, 8, 12, and 20 days after adult emergence, up to five males were taken from each cage that contained fertile or sterile mass-reared males and immediately killed by placing them in individual plastic centrifuge tubes that contained absolute ethanol. Wild *B. tryoni* were taken from cages at 0, 8, and 20 days after adult emergence because they are known to have a slower rate of sexual development in comparison with mass-reared flies (Meats et al., 2004; Meats & Kelly, 2008). Each tube was labelled with the age, diet, and type before being stored for later dissection.

The width and area of the ejaculatory apodeme were measured to assess the sexual development of preserved male *B. tryoni*. The ejaculatory apodeme is a pumping organ that forces seminal fluid through the ejaculatory duct to the tip of the aedeagus during copulation (Drew, 1969). The growth of the ejaculatory apodeme after adult emergence has been linked with the attainment of male reproductive maturity in *B. tryoni* (Drew, 1969) and *Bactrocera cacuminata* (Hering) (Raghu et al., 2003). The males that were preserved in alcohol were rinsed in water before being dissected in a drop of water under a stereomicroscope (SZX12; Olympus, Tokyo, Japan). The ejaculatory apodeme was removed and photographed using a 3-megapixel digital camera (ProgRes C10, Jenoptik Laser; Optik Systeme, Jena, Germany) through the phototube of the microscope at 50× magnification. Following the same methodology as Radhakrishnan & Taylor (2008), the width and area of the ejaculatory apodeme on the images were then measured using IMAGEJ software, version 1.37 (US National Institutes of Health, Bethesda, MD, USA).

Data analysis

The relation of ejaculatory apodeme area to ejaculatory apodeme width was determined using linear regression analysis for each type. The effect of providing male *B. tryoni* with access to natural food sources of dietary nutrition on ejaculatory apodeme width and area was analysed using general linear models with diet, type, and age as fixed effects. It was necessary to exclude data from mass-reared fertile and sterile flies at 4 and 12 days after adult emergence, because data for ejaculatory apodeme width and area of wild flies were not available for those ages. Post-hoc multiple comparisons of sexual development group means

were performed using Tukey–Kramer honestly significant difference tests. All analyses were performed using JMP Version 5 (SAS Institute, Cary, NC, USA).

Results

Male *B. tryoni* held in groups for measurement of ejaculatory apodeme size were observed during periodic inspections to feed on all of the provided natural food sources, as well as yeast hydrolysate and sucrose. This behaviour, which involved pressing the sponge-like mouthparts against the food, was noted primarily within the 4 days after adult emergence.

There was a strong positive correlation between ejaculatory apodeme width and area in mass-reared fertile ($\rho = 0.932$, $n = 164$), mass-reared sterile ($\rho = 0.925$, $n = 178$), and wild ($\rho = 0.827$, $n = 113$) adult male *B. tryoni*. Owing to this, there was a similar pattern of growth between ejaculatory apodeme area and width (Figure 1) in response to diet, age and type. Ejaculatory apodeme area and width were significantly affected by the interaction of diet, type, and age (area: $F_{20,401} = 2.532$, $P = 0.0003$; width: $F_{20,401} = 2.774$, $P<0.0001$). On the day of adult emergence, the area and width of the ejaculatory apodeme from mass-reared fertile, mass-reared sterile, and wild males did not differ significantly (Figure 1). Area and width of the ejaculatory apodeme from fertile and sterile mass-reared males increased with age before reaching an asymptote (Figure 1). Maximum ejaculatory apodeme area and width was attained by 8 days after adult emergence by fertile and sterile mass-reared males that had been provided with access to yeast hydrolysate. Development of the ejaculatory apodeme from fertile and sterile mass-reared males was slower when provided with access to bat guano, bird droppings, citrus pollen, wheat pollen, and sucrose only. Regardless of type or age, there was no significant difference between mass-reared fertile and sterile males in ejaculatory apodeme area or width when provided these diets.

In wild males, there was no significant change in area or width of the ejaculatory apodeme with age when they were provided with access to bat guano, bird droppings, citrus pollen, wheat pollen, or sucrose only (Figure 1). In contrast, the ejaculatory apodeme area and width of wild males that had been provided yeast hydrolysate increased with age. The change in area or width of the ejaculatory apodeme in wild males provided yeast hydrolysate did not differ significantly from those of fertile or sterile mass-reared males that were provided natural food sources or sucrose only. Similarly, by 20 days after adult emergence, there was no significant difference in the area or width of the ejaculatory apodeme from wild and fertile and sterile mass-reared males that were provided yeast hydrolysate (Figure 1).

Discussion

Growth of the ejaculatory apodeme was slowed in fertile and sterile mass-reared male *B. tryoni* fed on sucrose alone or provided with access to natural food sources when compared with males that received yeast hydrolysate. However, by 20 days after adult emergence, there was no significant difference in the ejaculatory apodeme size of mass-reared fertile or sterile males, regardless of their adult diet. The results of this study for mass-reared fertile and sterile male *B. tryoni* are in general agreement with earlier studies on the effects of diet on sexual maturation and performance in this species. Mass-reared male *B. tryoni* that were provided with a diet that included yeast hydrolysate have been reported to exhibit earlier mating and response to a parapheromone (cue-lure), higher mating propensity, longer duration of copulations, higher sperm storage by females, and were more successful at inhibiting female remating (Perez-Staples et al., 2007, 2008, 2009; Prabhu et al., 2008; Weldon et al., 2008). These increases in sexual maturation and performance were observed even when males were provided yeast hydrolysate for a period of only 48 h immediately after adult emergence (Perez-Staples et al., 2008, 2009; Weldon et al., 2008). However, growth of the ejaculatory apodeme in fertile and sterile mass-reared male *B. tryoni* in the current study is also consistent with the assertion of Drew (1987) that male *B. tryoni* do not require a diet that is high in protein to attain sexual maturity per se.

In contrast, this study demonstrated that wild male *B. tryoni* were not able to obtain sufficient nutrients from natural food sources to facilitate development of the ejaculatory apodeme. Ejaculatory apodeme size of wild males fed yeast hydrolysate increased with age, whereas the ejaculatory apodeme of wild males provided natural food sources or sucrose alone did not significantly increase in size throughout the duration of the experiment. This is counterintuitive as one might expect that wild flies would be better adapted to nutrient-poor environments. The onset of sexual behaviour occurs at around the same age as the maximum ejaculatory apodeme size is reached in yeast hydrolysate-fed laboratory-adapted male *B. tryoni* (Drew, 1969; Vijaysegaran et al., 2002), and a similar pattern has been reported in male *B. cacuminata* from a laboratory colony that received regular injection of wild stock (Raghu et al., 2003). Therefore, it is likely that males with poorly developed ejaculatory apodemes were not sexually mature. The pronounced effect of a diet including yeast hydrolysate on the size of the ejaculatory apodeme of wild male *B. tryoni* has not been recorded previously, but wild *Anastrepha*

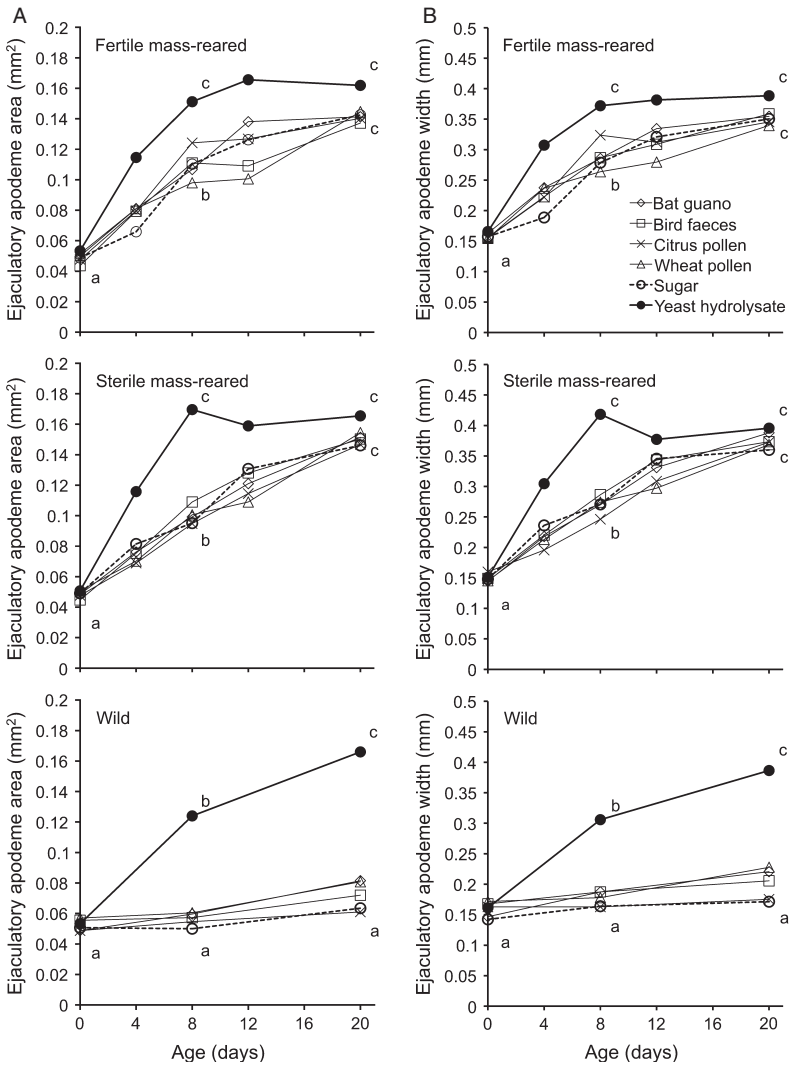

Figure 1 Mean ejaculatory apodeme (A) area and (B) width in fertile mass-reared, sterile mass-reared, and wild male *Bactrocera tryoni* of different ages that were provided with access to natural sources of food in the laboratory. Natural sources of food were bat guano, bird droppings, citrus pollen, and wheat pollen. A further group of males was given access to yeast hydrolysate, and a control group was provided with granulated sucrose only. Granulated sucrose was provided in a separate dish in all treatments as a source of carbohydrate. Points not connected by the same letter are significantly different (Tukey–Kramer HSD test: $P<0.05$); letters in the upper portion of each graph denote the significance level of males that were fed yeast hydrolysate, whereas letters in the lower portion of each graph denote the significance level of males that were fed sucrose only. There was no significant difference in ejaculatory apodeme area between males provided with access to natural sources of food and sucrose only within any male type or at any age.

obliqua (Macquart), *Anastrepha serpentina* (Wiedemann), and *Anastrepha striata* Schiner have been reported to exhibit significantly improved sexual performance when provided a diet that includes yeast hydrolysate, despite the artificial nature of the diet (Aluja et al., 2001). The cause for the stark contrast between the growth of the ejaculatory apodeme from wild and mass-reared flies that were not provided yeast hydrolysate can only be speculated at this time. Laboratory adaptation in female *B. tryoni* is characterised by increased efficiency in the conversion of dietary intake to eggs (Meats et al., 2004). Changes in the efficiency of dietary resource uptake and assimilation may also explain the difference in development of the ejaculatory apodeme in wild and mass-reared *B. tryoni*. It may also be that the intense selection pressure imposed by the mass-rearing environment (Cayol, 2000) leads to changes in the levels of nutrients retained by emerging adult male *B. tryoni* that can then permit more rapid sexual development. Newly emerged, mass-reared

adult *Ceratitis capitata* (Wiedemann) contained similar lipid and protein contents regardless of the quantity of these nutrients that were accumulated during the larval phase and present in the pupa, and it has been suggested that nutrient reserves in the emerging adult are determined genetically (Nestel et al., 2004). However, no studies have compared the energy reserves of newly emerged wild and mass-reared tephritids.

Male *B. tryoni*, whether wild or mass-reared, fed on the natural foods provided to them in this study, which raises the question of why there was no effect of these natural food sources on sexual development. All diets were presented in dry form, but this does not pose a particular problem for this species because dry, sticky or solid foods can be utilised by liquefying them in regurgitated drops of fluid that are then reingested using their sponge-like mouthparts (Vijaysegaran et al., 1997). Similarly, although the mouthparts of *B. tryoni* preclude the ingestion of particles larger than 0.5 μm, nutrients from droppings and ruptured pollen grains may be imbibed when suspended in solution (Vijaysegaran et al., 1997). However, the structure of pollen grains makes them very resistant to damage and digestion (Roulston & Cane, 2000), so despite the high nutrient content of pollen (Roulston et al., 2000) the amount of nutrition available from pollen in this study may have been considerably restricted. In line with this scenario, it may be that each natural diet component provided a source of some nutrients, but the quantity from each was insufficient on its own for marked improvements in sexual development.

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