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Diet-dependent reproductive investment in gumleaf skeletonizer moths, *Uraba lugens*

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Abstract

Juvenile diet can profoundly affect subsequent adult development, morphology and reproductive investment. Yet, little is known about how juvenile diet affects adult investment into chemical-based sexual signalling, perhaps due to the historical assumption that pheromone production is not costly. We explored how juvenile diet influenced the reproductive investment of adults in the gumleaf skeletonizer moth, *Uraba lugens*. Juveniles were reared on different host plant species (*Eucalyptus camaldulensis* and *Eucalyptus moorei*) and on *E. moorei* host plants with different fertilizer treatments (fertilized and non-fertilized). These juvenile diets differ in foliage carbon and nitrogen content. Several adult life history traits were influenced by juvenile diet, including body size in females, and longevity in males. However, we found no evidence from Y-maze olfactometer assays that diet affected the attractiveness of female pheromones to males. Finally, host plant species affected male pre-copulatory investment: males reared on *E. moorei* had longer antennae, but less dense sensilla. Combined, our experiments suggest that the effects of juvenile diet on investment in reproductive traits, including those associated with signalling, differ between males and females. Females allocate nutrients to adult body size, which determines fecundity. In contrast, males allocate nutrients to adult longevity and antennae size, both of which improve mate search and mating success.

KEYWORDS
chemical signalling, male mate choice, mating strategies, reproductive investment, sex pheromone

INTRODUCTION

Communication plays a crucial role in the process of sexual reproduction, from revealing the location and receptivity of potential mates to allowing individuals to exercise a choice of mating partner. Sexual communication may involve several sensory modalities, but chemical signalling is arguably the earliest and taxonomically most widespread modality (Symonds & Elgar, 2008). Sex pheromones, typically released by members of one sex to signal their location to the other sex, may also reveal the species, sex and sexual receptivity of the signaller and the attractiveness of the signaller in mate choice (see Johansson & Jones, 2007). Communication using chemical signals occurs only when the odorant molecule produced by the signaller binds with specific odorant receptor proteins located on the sensory organ of the receiver (Elgar, 2019; Malnic et al., 1999). While this mechanism predicts minimal within-population variation in chemical signals, it may be more ‘forgiving’ than originally thought, as within-species variation in chemical signals has been documented for several insects (Palacio Cortés et al., 2010; Svensson et al., 1997).

Variation in host plant nutritional quality can have an impact on adult survival and reproductive output of phytophagous insects (Moreau et al., 2017). Diet can also affect chemical communication by either altering the nature of the signal (Henneken et al., 2015; Henneken & Jones, 2017) or the level of investment in signal reception organs (Elgar et al., 2018). The effects of diet on reproductive traits may differ between the sexes, reflecting different roles as adults and concomitant investment in signalling and signal perception. Typically, females produce nutrient-rich or numerous eggs (see Awmack & Leather, 2002; Bonoan et al., 2015), whereas males invest in larger testes or spermatophores: both can be costly but may differ in severity between the sexes (Delisle & Hardy, 1997; Dewsbury, 1982; McNamara et al., 2009; Svard & Wiklund, 1989; Wedell et al., 2002). Additionally, investment in signalling and signal perception is likely to differ between the sexes, with the nature and direction of that difference depending upon the relative importance of the different levels of reproductive communication (Elgar et al., 2018).

The effects of juvenile diet on sex pheromones have been documented in several insects (Reddy & Guerrero, 2004), including beetles (Rantala et al., 2003; Xue et al., 2016), cockroaches (Clark et al., 1997), moths (Conner et al., 1990) and butterflies (Darragh et al., 2019). Different species of host plant can comprise different blends of macronutrients (Mattson, 1980), which may influence the synthesis of sex pheromones of herbivorous insects (Conner et al., 1990; Hendry, 1976; Hendry et al., 1975; Landolt & Phillips, 1997; McNeil & Delisle, 1989; Merli et al., 2018; Reddy & Guerrero, 2004) and thus their mating preferences (Di et al., 2021; Geiselhardt et al., 2012). Diet may also affect investment in the chemosensory structures of adult insects, including the number and type of antennal chemoreceptors (Bernays & Chapman, 1998; Chapman & Lee, 1991; Elgar et al., 2018; Rogers & Simpson, 1997; Safonkin et al., 2004; Wool & Hales, 1997).

While it is widely understood that the ratio of different macronutrients is crucial in determining larval growth and development (Behmer, 2009; Simpson & Raubenheimer, 2012), experiments that fertilize the host plant reveal the effect of nitrogen on the reproductive traits of both sexes, including female fecundity (Bonoan et al., 2015) and male spermatophores (Lewis & South, 2012), testes (Carsten-Conner et al., 2010) and ejaculate traits (Macartney et al., 2019). The effects of juvenile diet on both male and female adult traits associated with chemical communication, including investment in pheromone signals and traits associated with pheromone detection, have not been explored In the Lepidoptera, despite the extensive investigations of pheromones in this taxon (Symonds & Elgar, 2008).

The gumleaf skeletonizer moth, *Uraba lugens*, is a capital breeding species, so adult reproductive life-history parameters are determined by the resources obtained during the juvenile period. *U. lugens* is phytophagous, feeding on over 103 myrtaceous species, of which the genus *Eucalyptus* dominates (Berndt & Allen, 2010). The suitability of eucalypt host plants for larval development (mortality and development time) appears to be correlated with measures associated with adult performance, including pupal weight and fecundity (Campbell, 1962). *U. lugens* is sexually dimorphic: females have filiform and males bipectinate antennae. Larval density influences investment in adult reproductive communication traits, including pheromone attractiveness in females (Phạm et al., 2021) and antennae and testes size in males (Johnson et al., 2017a, 2017b).

Here, we explore the consequences of different diets on the adult reproductive traits of *U. lugens*, by rearing juveniles on (a) one of two eucalypt host plant species that apparently differ in nutritional quality (Bell & William, 1997), and (b) a single host plant species that had or had not been treated with a commercial fertilizer. Specifically, we investigate the effects of these different diets on (a) adult female reproductive output and therefore her attractiveness to males and (b) adult male pre- and post-copulatory reproductive traits. First, we confirmed the two host plant species differed in foliage nutrient availability, reflected in the concentration of nitrogen and carbon. Second, we examined the effects of juvenile diet on adult body size and adult longevity. Third, we examined the functional impact of different juvenile diets on female pheromonal attractiveness by comparing male preferences for the odour of females reared on different diets. Finally, we compared the pre-copulatory (antennae length and sensilla density) and post-copulatory (testes size) reproductive traits of males reared on different diets.

MATERIALS AND METHODS

Insect culturing

Clusters of eggs of the Australian gumleaf skeletonizer *U. lugens* were collected from several locations in Melbourne, Victoria, and transferred to the laboratory where they were maintained in plastic containers (1 L, one egg cluster per container) in an incubator (15 L:9D light conditions at 22.5°C and approximately 70% humidity) until they hatched. The larvae were then housed in plastic containers (1 L), supplied with fresh, mature leaves of *Eucalyptus* spp. that were replaced every 2 days, until they reached third instar. Thus, all individuals used in the following experiments were ‘wild’ caught.

Host plant stock

We obtained two species of host plants, *Eucalyptus camaldulensis* and *Eucalyptus moorei* from Kuranga Native Nursery, Victoria, which were maintained in 110 L plastic pots (Reko) in a large glass house facility (School of BioSciences, The University of Melbourne). The choice of species reflected their reported differences in nutritional quality (Bell & William, 1997) and commercial availability. Individual plants were watered once or twice per week and fertilized once per 6 months with a commercial slow-release fertilizer. We conducted two separate experiments (see below) with plants that were less than 2 years old: we used 15 plants of *E. camaldulensis* and 47 plants of *E. moorei* (no plants were used in both experiments).

Host plant species effects

In the first experiment, second and third instar larvae from the same egg cluster were allocated evenly between the potted plants of

E. moorei ($N = 15$ plants) and *E. camaldulensis* ($N = 15$ plants), which were watered every week. Analysis of the foliage nutrients of the two species (see Supplementary Material) revealed that the nitrogen content (per mg of leaf dry mass) in the leaf samples did not differ between the two species, but the carbon content was significantly higher in the leaves of *E. moorei* than in those of *E. camaldulensis* (Figure S1).

Larvae that reached the 7th instar (identified by the presence of three head-capsules) were then transferred to small plastic containers (1 L, 10 individuals per container) and maintained in the incubator as above. These larvae were fed with the leaves of their treatment host plant species until pupation. The pupae were transferred to individual vials (40 × 60 mm, 120 mL), where the adult moths eclosed.

Fertilizer treatment effects

In a second, separate experiment, second and third instar larvae were raised on potted plants of *E. moorei* that had been allocated to one of two treatments: non-fertilized (water only, $N = 17$ plants) or fertilized fortnightly with a commercial Aquasol soluble fertilizer (with a N:P:K ratio of 23:3.95:14), with 9 g in 4.5 litres of water ($N = 15$ plants). Analysis of the foliage nutrients of fertilized and non-fertilized plants revealed that the nitrogen content present in the leaf sample was significantly higher in fertilized than in non-fertilized leaves (Figure S2), but the carbon content was not affected by the fertilizer treatment.

Larvae that reached the 7th instar were transferred to small plastic containers and maintained in incubators as above. The larvae were fed with the leaves of their treatment host plant until pupation. Pupae were then transferred to individual vials (40 × 60 mm, 120 mL), where the adult moths eclosed.

Juvenile diet and adult longevity and morphology

We assessed the impact of juvenile diet on body size and longevity, by collecting a male and female representative of each experimental treatment host plant on the day they eclosed. Sample sizes for the host plant treatments were 30 males and 37 females from *E. moorei*; 25 males and 29 females from *E. camaldulensis*; and for the fertilizer treatments, 30 males and 56 females from the fertilized treatment; and 32 males and 19 females from the non-fertilized treatment. Adults were housed individually in a vial (40 × 60 mm, 120 mL) and observed daily until they died. Deceased individuals were preserved in ethanol (70%). Wing length was used as an index of body size: the left forewing of each individual was cut as close as possible to the base and dipped in bleach for 30 s to remove the scales. Wings were laid flat between two coverslips and digital images were obtained at 2400 dpi resolution using an Epson Perfection V800 scanner. The length of the second-most posterior vein was measured from its point of insertion with the thorax to its termination at the distal edge of the forewing, using ImageJ software (Schneider et al., 2012).

Juvenile diet and adult female attractiveness

We assessed whether juvenile diet affects the attractiveness of adult female pheromones to males by using a Y-maze olfactometer. This allowed us to compare the male responses to the pheromones produced by females reared on the different paired diets: different host plant species (*E. camaldulensis* vs. *E. moorei*) and different fertilized treated host plant (non-fertilized and fertilized *E. moorei*). The pairs of 'calling' females were each ≤48 h post-eclosion and were matched for body weight (mean ± standard error body weight difference $= 4.34 \pm 0.67$ mg in different host plant species and 5.35 ± 0.43 mg in different fertilizer treatments).

We used a Y-maze olfactometer to evaluate male preferences for females raised on different diets. The Y-maze of the olfactometer comprised a 2 cm diameter Y-tube with 10 cm arms and base. The arms were connected by a narrow tube to a container with a calling female, and a standardized, continuous air flow passed through each of the containers with a calling female and on to the arms of the Y-maze and hence to the base, where the male is released (Phạm et al., 2020, 2021, 2022). A single female raised on one diet was placed in one container and a female from the complementary diet was placed in the other container, and both were left to acclimate for 1 h. The containers were separated from the arms of the Y-maze with a fine mesh, preventing any visual and tactile communication. Once both females had commenced calling, a virgin male from the stock population (≤ 36 h post-eclosion, reared at an intermediate population density of 5–10 larvae/container with various Eucalyptus leaves collected from parks) was introduced at the base of the Y-maze. The male was deemed to have responded when he either walked or flew towards the airflow, travelling at least 5 cm into one of the arms of the Y-maze and remaining there for more than 1 min. Trials in which the male failed to move within 30 min or moved up one of the arms in less than 10 s after being introduced were discarded and a new trial initiated with another male. The trial lasted 60 min. Each male was used once only, and pairs of females were used for two trials only (with females never occupying the same arm of the olfactometer twice). The olfactometer apparatus was washed with soap and water and dried after each trial. The position of the low- and high-density females was alternated each trial to remove positional effects. The trials were conducted during the middle of the scotophase, when moths are most active (Phạm et al., 2021).

Juvenile diet and male reproductive investment

We examined the effect of juvenile diet on male pre- and post-copulatory reproductive investment by measuring both antennal morphology and testes size. Adult males from each treatment were killed two-days following adult eclosion (when the testes are fully developed) and stored in 70% ethanol until required.

The left antenna of each male ($N = 15$ raised on *E. camaldulensis* and $N = 16$ raised on *E. moorei*) was removed using micro-scissors and gently placed on a two-sided carbon sticky table (12.0 mm diameter, Proscitech) and mounted on an electron microscopy stub (12.6 mm diameter, Proscitech). The antennae were imaged with low-vacuum uncoated scanning electron microscopy (SEM) on a FEI Quanta 200F scanning electron microscope at the Bio21 Advanced Microscopy Facility (Bio21 Institute, The University of Melbourne, Australia). For all specimens, a spot size of 2.0 and pressure of 0.80 mbar were used, with a high voltage of 10.0 kV. These SEM images were used to measure sensilla density, the length of the antennae (from tip to scape) and the total number of flagellomeres. These measurements were obtained using ImageJ software (Schneider et al., 2012).

Following Hansson (1995), six types of sensilla were detected on the antennae of the males, based on their external morphology, and included sensilla trichodea, basiconica, coeloconica, auriculate, chaetica and styloconica (Figure 1). Sensilla trichodea is the dominant type of sensilla in all flagellomeres of the male antenna and was the only type found in the antennal branches. These sensilla are biologically significant for our experiments: sensilla trichodea have numerous pores and their main sensory channel is thought to be olfactory, and they have been implicated in mate search in tortricid moths (Triseleva & Safonkin, 2006). Accordingly, we measured the density of trichodea only, and for convenience, refer to this as sensilla density. The average sensilla density (μm^2) for each male, based on the density of trichodea sensilla of three flagellomeres (1st, 15th and 35th from the antennal tip), was calculated by dividing the total number of trichodea sensilla observed in those three flagellomeres by the total visible area of those flagellomeres.

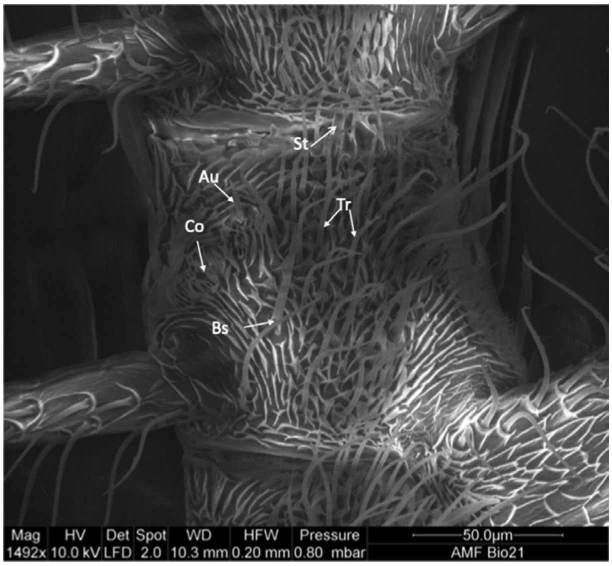

FIGURE 1 Representative SEM image highlighting different types of sensilla located on the ventral side of one segment: the long sensilla, trichodea sensilla (Tr), were the dominant types of sensilla; basiconica sensilla (Bs) are similar to Tr, but larger and much longer; auricillica sensilla (Au) have a flat shape and are wide at the base; coeloconia sensilla (Co) have a grooved peg surrounded by 12–16 spines; and styloconica sensilla (St) are peg-like, set on top of a conical cuticular style found at the distal margin of each flagellomere. Chaetica sensilla (not visible in the image) are similar to Tr but the basal socket is different.

Each male was dissected to obtain a measure of testes size. The testes comprise a fused spherical organ located in the 6th–9th abdominal segment (Johnson et al., 2017a). The testes were removed with micro-scissors and placed on a slide. The testes were then imaged immediately (to avoid desiccation) with a Sony camera (ILCE-QX1) under an Olympus microscope (SZX16) at × 5 magnification. The area of the testes was measured using the outline tool in ImageJ (Schneider et al., 2012). Wing length was used as an index of body size and measured as above.

Statistical analysis

All statistical analyses were conducted in R and R studio, version 3.5.2 (R_Core_Team, 2018). We explored the impact of juvenile diet on male and female body size and longevity using general linear models, with a diet by sex interaction term. For the analysis of supplementary fertilizer on longevity, there were two male outliers. These were more than four standard deviations from the mean, and once removed, the data could be transformed such that the model residuals were normally distributed.

We investigated the impact of juvenile diet on the reproductive investment of adult males (antennae length, number of flagellomeres, density of sensilla and testes size) with general linear models that included wing length and a diet by wing length interaction term. For analysis of host plant species, we used relative male wing length (calculated as mean treatment wing length/standard deviation treatment wing length), as male wing length was significantly affected by host plant nutrition, and therefore colinear with diet treatment.

For all models containing interaction terms, non-significant interactions were removed from the final models (Engqvist, 2005). For all general linear models, we optimally power transformed all dependent variables to maximize normality of model residuals, and the exponents used were noted with every analysis. Tukey HSD post-hoc tests were used to test for statistical differences between treatment levels.

RESULTS

Juvenile diet, adult morphology and longevity

Eucalypt host plant species

The variation in adult wing length (raised to the exponent 2.08) was explained by the host plant species by adult sex interaction term ($F_{1,117} = 11.27$, $p = 0.001$; Figure 2a). Post-hoc tests revealed that females were larger than males, and individuals raised on *E. moorei* were significantly larger than those raised on *E. camaldulensis*. The interaction term reflects the greater effect of diet on wing length for females than for males. The variation in adult longevity (raised to the

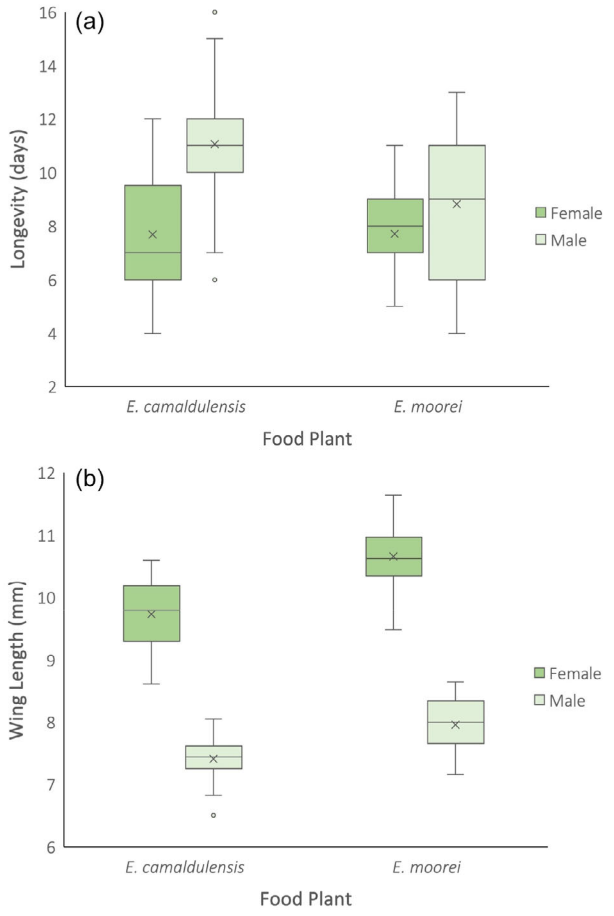

FIGURE 2 (a) Longevity (days) and (b) wing length (mm) of male and females reared on *Eucalyptus camaldulensis* and *Eucalyptus moorei* host plants. The box contains the interquartile range, with the median as a single line and x indicates the mean.

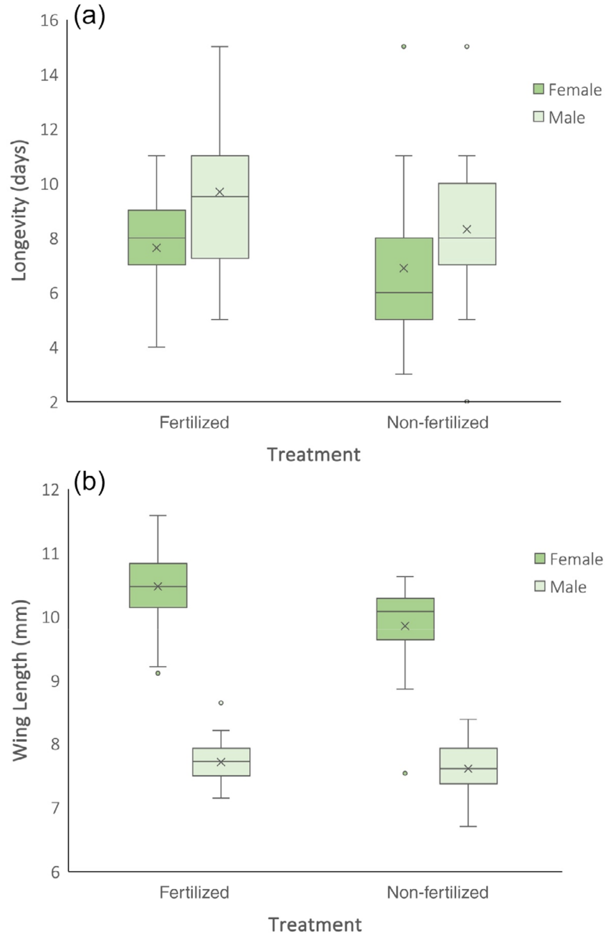

FIGURE 3 (a) Adult longevity (days) and (b) wing length (mm) of males and females reared on fertilized and non-fertilized treated *Eucalyptus moorei*.

exponent 1.2) was explained by the host plant species by adult sex interaction term ($F_{1,117} = 8.12$, $p = 0.005$; Figure 2b). Post-hoc tests revealed that host plant species did not affect female longevity, but that males lived longer than females and that males raised on *E. camaldulensis* lived longer than males raised on *E. moorei*.

Fertilizer treatment

The variation in adult wing length (raised to the exponent 1.12) was explained by a significant fertilizer treatment by adult sex interaction term ($F_{1,131} = 7.69$, $p = 0.006$; Figure 3a). Post-hoc tests revealed that females fed on fertilizer-treated plants were significantly larger than females fed on non-fertilizer-treated plants, but this was not the case for males. Adult longevity (raised to the exponent 0.64) was affected by fertilizer treatment ($F_{1,130} = 6.99$, $\beta = -0.34 \pm 0.13$, $p = 0.009$; Figure 3) and sex ($F_{1,130} = 18.60$, $\beta = 0.54 \pm 0.12$, $p < 0.001$; Figure 3b). Post-hoc tests revealed that individuals fed on fertilizer-treated plants lived significantly longer than individuals fed on non-fertilizer-treated plants, and those males lived longer than females. A non-significant interaction between fertilizer treatment and sex ($F_{1,129} = 0.33$, $p < 0.57$) was removed from the final model.

Juvenile diet and female attractiveness

The Y-maze olfactometer choice tests revealed that when males were given a choice between the odour from females reared on a diet of either *E. camaldulensis* or *E. moorei* leaves (mean ± standard error body weight difference = 4.34 ± 0.67 mg), 19 males (from 23 trials) made a valid choice, but there was no consistent preference for the pheromones produced by females raised on either diet (*E. camaldulensis* = 8; *E. moorei* = 11; $\chi^2 = 0.47$, $p = 0.49$). When males were given a choice between females reared on a diet of either fertilized or non-fertilized *E. moorei* plants (mean ± standard error body weight difference = 5.35 ± 0.43 mg), 23 males (from 28 trials) made a valid choice, but there was no consistent preference for the pheromones produced by females reared on either diet (fertilized plant = 15; non-fertilized plant = 8; $\chi^2 = 2.13$, $p = 0.14$).

Juvenile diet and male reproductive investment

Eucalypt host plant species

The length of antennae (raised to the exponent 0.04) of males raised on *E. moorei* was longer than that of males raised on *E. camaldulensis* ($F_{1,27} = 14.89$, $\beta = 4.27\text{e-}05 \pm 2.07\text{e-}05$, $p = 0.048$; Figure 4a), but was not affected by the relative wing length of the male ($F_{1,27} = 2.90$, $\beta = 6.13\text{e-}08 \pm 1.06\text{e-}05$, $p = 0.99$). A non-significant host plant species by relative wing length interaction term ($F_{1,26} = 0.02$, $p = 0.87$) was removed from the final model. The number of flagellomeres (raised to the exponent 3.44) was not affected by either host plant diet (mean ± standard error number of flagellomeres: *E. moorei* = 48.8 ± 0.6; *E. camaldulensis* = 49.0 ± 0.6; $F_{1,27} = 0.04$, $\beta = -11{,}081 \pm 37{,}296$, $p = 0.77$) or relative wing length ($F_{1,27} = 0.51$, $\beta = 15{,}127 \pm 19{,}260$, $p = 0.44$). A non-significant host plant species by relative wing length interaction term ($F_{1,26} = 0.17$, $p = 0.68$) was removed from the final model. The density of antennal sensilla (raised to the exponent 1.52) was greater for males raised on *E. camaldulensis* than for males raised on *E. moorei* ($F_{1,27} = 4.27$, $\beta = -4.28\text{e-}05 \pm 2.07\text{e-}05$, $p = 0.048$, Figure 4b), but was not affected by the relative wing length of the male ($F_{1,27} = 0.00$, $p = 0.99$). A non-significant host plant species by relative wing length interaction term ($F_{1,26} = 0.03$, $p = 0.87$) was removed from the final model.

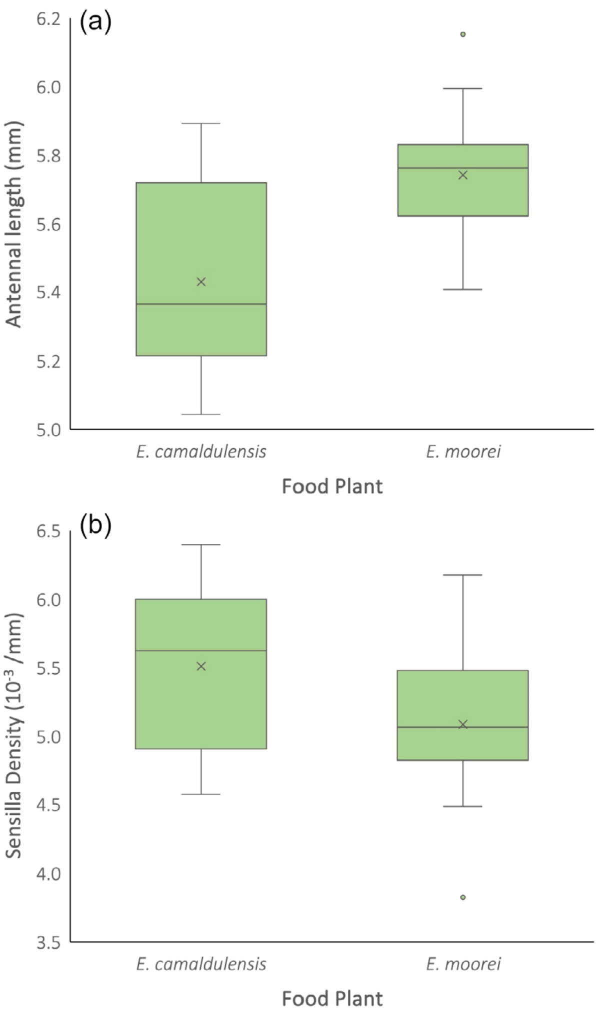

FIGURE 4 (a) Male antennal length and (b) sensilla density for males reared on *Eucalyptus camaldulensis* and *Eucalyptus moorei* host plants.

The variation in testes size (raised to the exponent 0.04) was not explained by the host plant diet (mean ± standard error testes size (mm^2): *E. moorei* = 0.25 ± 0.01; *E. camaldulensis* = 0.23 ± 0.01; $F_{1,27} = 2.14$, $\beta = 0.004 \pm 0.003$, $p = 0.15$), but increased with male relative wing length ($F_{1,27} = 4.55$, $p = 0.04$). A non-significant host plant species by relative wing length interaction term ($F_{1,26} = 1.95$, $p = 0.17$) was removed from the final model.

Fertilizer treatment

The length of antennae (raised to the exponent 0.04) was not affected by the host plant fertilizer treatment (mean ± standard error antennal length (mm): fertilized = 5.5 ± 0.1; non-fertilized = 5.5 ± 0.1; $F_{1,34} = 0.01$, $\beta = 6.33\text{e-}05 \pm 5.79\text{e-}04$, $p = 0.91$), but was positively correlated with wing length ($F_{1,34} = 16.34$, $\beta = 3.65\text{e-}03 \pm 9.02\text{e-}04$, $p = 0.0002$). A non-significant fertilizer status by wing length interaction term ($F_{1,33} = 1.74$, $p = 0.19$) was removed from the final model.

Similarly, the number of flagellomeres (raised to the exponent 0.04) was not affected by the host plant fertilizer treatment (mean ± standard error number of flagellomeres: fertilized = 44.2 ± 0.7; non-fertilized = 44.3 ± 0.5; $F_{1,34} = 0.01$, $\beta = 1.20\text{e-}04 \pm 9.80\text{e-}04$, $p = 0.90$) or wing length ($F_{1,34} = 0.12$, $\beta = -5.45\text{e-}04 \pm 1.54\text{e-}03$, $p = 0.72$). A non-significant fertilizer status by wing length interaction term ($F_{1,33} = 1.00$, $p = 0.32$) was removed from the final model.

The density of antennal sensilla (raised to the exponent 0.04) was not affected by the host plant fertilizer treatment ($F_{1,34} = 0.92$, $\beta = -1.32\text{e-}03 \pm 1.37\text{e-}03$, $p = 0.34$) or the relative male wing length ($F_{1,34} = 0.19$, $\beta = 9.35\text{–}04 \pm 2.13\text{–}03$, $p = 0.66$). A non-significant host plant species × wing length interaction term ($F_{1,33} = 0.00$, $p = 0.97$) was removed from the final model.

Finally, testes size (raised to the exponent 0.04) was not affected by the host plant fertilizer treatment (mean ± standard error testes size (mm^2): fertilized = 0.27 ± 0.01; non-fertilized = 0.30 ± 0.02; $F_{1,33} = 0.62$, $\beta = 2.91\text{e-}03 \pm 3.68\text{e-}03$, $p = 0.43$) or relative male wing length ($F_{1,33} = 0.19$, $\beta = -2.62\text{e-}03 \pm 5.99\text{e-}03$, $p = 0.66$). A non-significant fertilizer status by wing length interaction term ($F_{1,32} = 0.00$, $p = 0.94$) was removed from the final model.

DISCUSSION

Adult life-history and reproductive traits in the capital breeding, gum-leaf skeletonizer moth, *U. lugens*, are affected by juvenile diet. Our experiments reveal how differences in larval diet, reflected by feeding on different species of host plants or experimentally fertilized host plants, have different consequences for adult males and females. In general, diet influenced female fecundity through differences in adult size, while diet influenced male longevity and thus their capacity to detect and locate reproductive partners. Data from our foliage analysis suggest that adult females were larger if they had been reared on host plants with greater nitrogen and carbon content, but we found little evidence that this translated into differences in pheromone attractiveness. Foliage analysis also indicates that male traits were influenced by host plant species, but not by enhancing nitrogen content: the host plant species with a greater level of carbon content resulted in a shorter lifespan, but longer antennal length. While juvenile diet can have profound effects on male testes size in insects (Svard & Wiklund, 1989), and in some cases, creates a trade-off between pre-and post-copulatory traits (Durrant et al., 2016; Simmons & Emlen, 2006), the testes size in *U. lugens* did not differ between host plant species or between host plants that varied in nitrogen content.

While the foliage analyses revealed variation in N leaf content, this may not necessarily translate into differences in the quantity of nitrogen the foraging insect can ingest, especially in those plants that are rich in tannins, such as *Eucalyptus* (Wallis et al., 2010). We were unable to measure tannins, and thus reveal their role in causing differences in the life history traits of *U. lugens* raised on different host plant species, although we note that inter-specific studies across eucalypt genera reveal that measures of available nitrogen are correlated with measures of total nitrogen (Wallis et al., 2010).

The influence of juvenile diet on the adult reproductive traits of female *U. lugens* is broadly consistent with previous studies revealing how juvenile diet influences female size and thus fecundity (Awmack & Leather, 2002; Bonoan et al., 2015; Garcia-Barros, 2000). For example, female tortricid moths, *Choristoneura rosaceana*, reared on a nutritious diet were larger and hence laid more eggs (Carrière, 1992), while female larvae of the Melissa blue butterfly, *Lycaeides melissa*, that fed on a poor-quality host plant eclosed as smaller adults and were neglected by males (Forister & Scholl, 2012). In contrast, diet did not have a consistent influence on adult male traits in *U. lugens*: antennal size, sensilla density and longevity differed for individuals raised on different host plant species, but host plant species did not affect post-copulatory investment. The latter is not consistent with other species, where males may allocate more resources towards testes or ejaculates. For example, males of the European grapevine moth, *Lobesia botrana* fed on a high-quality host plant were larger and transferred larger spermatophores (Muller et al., 2015). Similarly, walnut flies *Rhagoletis juglandis* reared on a rich nutrient diet had larger testes and larger ejaculates (Carsten-Conner et al., 2010). Interestingly, the absence of a treatment effect on male testes size in this study is consistent with the absence of a change in male testes investment in response to resource stressors, via an immune challenge (Phạm et al., 2022).

There is increasing evidence that sex pheromones are affected both qualitatively and quantitatively by larval diet, and thus can act as 'honest' signals to attract mates (Henneken et al., 2015; Henneken et al., 2017; Henneken & Jones, 2017). We anticipated that females reared on a diet rich in nitrogen or carbon would emit more attractive pheromones: for example, a male may benefit from mating with females reared on a high-quality, nitrogen-rich diet because they may produce more, and better nourished eggs (Xie et al., 2015). Surprisingly, our experiments provided no support for this prediction.

There are several explanations why males showed no preference between females raised on different *Eucalyptus* host plant species, despite the inter-specific differences in carbon content. First, the sex pheromones emitted by females reared on these two species of *Eucalyptus* host plant do not differ, despite the difference in carbon content affecting female body size. Perhaps the leaves of both *E. camaldulensis* and *E. moorei* have sufficient carbon content to ensure the larvae can sequester the specific precursors to synthesize a similarly attractive pheromone. Previous studies indicate that both species of *Eucalyptus* are high-quality host plants for *U. lugens*, with relatively low mortality rates, short development times and heavier pupae (Cobbinah, 1983; Cobbinah, 1985; Cobbinah et al., 1982). It would be interesting to compare the attractiveness of pheromones produced by females reared on host plants of more substantial differences in quality. Finally, the sex pheromones produced by females reared on these different host plant species may differ, but there may be no clear benefits to males that prefer females reared on one host-plant species over another: larvae reared on *E. moorei* develop into larger adults with a shorter lifespan, while larvae reared on *E. camaldulensis* develop into smaller adults with a longer lifespan. It is possible that males can distinguish between the pheromones produced by females reared on different host plants, but their preferences for one over the other are context dependent.

Juvenile diet affects male longevity, and thus time spent mate-searching, but not consistently across the different diets. The positive correlation between nitrogen content and longevity is not consistent with studies of other insects that report negative effects of nitrogen-rich diets (Kurze et al., 2018; Zhu et al., 2019). The geometric framework for nutrition (Simpson & Raubenheimer, 2012) may help explain the negative correlation between adult male longevity and carbon content in the juvenile diet. This framework assumes that animals require a particular mixture of nutrients during their life, and an imbalance between an individual's intake target and the nutrient content of its food may not necessarily result in improved performance (Behmer, 2009; Raubenheimer & Simpson, 1993): in this case, the diet incorporated a higher carbon content without a compensating higher nitrogen content. Further, high nutrient content diets may reduce insect performance by affecting specific physiological processes, such as increasing the metabolic costs of storing and excreting excess nutrients (Anderson et al., 2005; Boersma & Elser, 2006). Thus, 'digestive efficiency' may decline with increasing nutrient content (Raubenheimer & Simpson, 1993).

There is accumulating evidence that both natural and sexual selection pressures may act on antennae size and the number of sensilla that are important for chemical communication (Chapman, 1982; Elgar et al., 2018; Elgar et al., 2019). Sexual selection is thought to favour males with highly efficient chemical receptor organs, such as longer or more complex antennae that support more sensilla, as this will improve their chances of securing matings (Elgar et al., 2019). Field experiments revealed that male *U. lugens* with longer antennae were better able to locate solitary, younger females (Johnson et al., 2017b), who may have greater reproductive value (Foster &

Howard, 1999; Xu & Wang, 2009). Laboratory experiments revealed that male neriid flies, *Telostylinus angusticollis*, that have relatively longer antennae (similar body size) were more likely to win male–male competitive interactions and achieved matings more quickly (Fricke et al., 2015). Studies of several insects have also demonstrated the effects of host plants on the number and density of antennal sensilla (Bernays & Chapman, 1998; Chapman, 2002; Chapman & Lee, 1991; Rogers & Simpson, 1997). Males of *U. lugens* that were reared on carbon-rich *E. moorei* had longer antennae but lower sensilla density than males reared on *E. camaldulensis*. This suggests that while a diet with a higher carbon content allows males to increase the size of their antennae, the costs of the underlying neural networks (Niven & Laughlin, 2008; Stöckl et al., 2016) may prevent investment in larger numbers of sensilla. Whether antennal length per se or the number of sensilla are more important in determining male chemical detection capacity remains unknown.

Our experiments reveal that juvenile diet has sex-specific effects on adult traits associated with reproduction in *U. lugens*. Investment in adult male, but not female, sensory organs, including the length of antennae and density of olfactory sensilla, was affected by host plant species, although these traits did not differ in the host plant fertilization experiment. We found no evidence that juvenile diet affected the attractiveness of female sex pheromones, but there were sex-specific effects on adult body size and longevity. These differences likely arise through the importance of these adult traits for reproductive success and the consequential sex differences in nutritional requirements. Further experiments are required to tease apart these effects.

AUTHOR CONTRIBUTIONS

Hiếu Thị Phạm: Conceptualization; methodology; investigation; formal analysis; data curation; writing – original draft. **Kathryn B. McNamara:** Conceptualization; methodology; formal analysis; supervision; data curation; writing – review and editing; resources. **Mark A. Elgar:** Conceptualization; methodology; supervision; writing – review and editing; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare https://doi.org/10.6084/m9.figshare.25333480. R code is provided in the electronic supplementary material.

ETHICS STATEMENT

None.

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REFERENCES

Anderson, T.R., Hessen, D.O., Elser, J.J. & Urabe, J. (2005) Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. *American Naturalist*, 165, 1–15.

Awmack, C. & Leather, S. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, 47, 817–844.

Behmer, S.T. (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology*, 54, 165–187.

Bell, D.T. & William, J.E. (1997) Eucalypt ecophysiology. In: Williams, J.E. & Woinarski, J.C.Z. (Eds.) *Eucalypt ecology: individual to ecosystems*. Cambridge: Cambridge University Press, pp. 168–196.

Bernays, E.A. & Chapman, R.F. (1998) Phenotypic plasticity in numbers of antennal chemoreceptors in a grasshopper: effects of food. *Journal of Comparative Physiology A*, 183, 69–76.

Berndt, L.A. & Allen, G.R. (2010) Biology and pest status of *Uraba lugens* Walker (Lepidoptera: Nolidae) in Australia and New Zealand. *Australian Journal of Entomology*, 49, 268–277.

Boersma, M. & Elser, J.J. (2006) Too much of a good thing: on stoichiometrically balanced diets and maximal growth. *Ecology*, 87, 1325–1330.

Bonoan, R.E., Al-Wathiqui, N. & Lewis, S. (2015) Linking larval nutrition to adult reproductive traits in the European corn borer *Ostrinia nubilalis*. *Physiological Entomology*, 40, 309–316.

Campbell, K.G. (1962) The biology of *Roeselia lugens* (walk.), the gum-leaf skeletonizer moth, with particular reference to the *Eucalyptus camaldulensis* Dehn. (river red gum) forests of the Murray Valley region. *Proceedings of the Linnean Society of New South Wales*, 87, 316–338.

Carrière, Y. (1992) Host plant exploitation within a population of a generalist herbivore, *Choristoneura rosaceana*. *Entomologia Experimentalis et Applicata*, 65, 1–10.

Carsten-Conner, L.D., Papaj, D.R. & O'Brien, D.M. (2010) Resource allocation to testes in walnut flies and implications for reproductive strategy. *Journal of Insect Physiology*, 56, 1523–1529.

Chapman, R.F. (1982) Chemoreception: the significance of receptor numbers. *Advances in Insect Physiology*, 16, 247–356.

Chapman, R.F. (2002) Development of phenotypic differences in sensillum populations on the antennae of a grasshopper, *Schistocerca americana*. *Journal of Morphology*, 254, 186–194.

Chapman, R.F. & Lee, J.C. (1991) Environmental effects on numbers of peripheral chemoreceptors on the antennae of a grasshopper. *Chemical Senses*, 16, 607–616.

Clark, D.C., Debano, S.J. & Moore, A.J. (1997) The influence of environmental quality on sexual selection in *Nauphoeta cinerea* (Dictyoptera: Blaberidae). *Behavioral Ecology*, 8, 46–53.

Cobbinah, J.R. (1983) Suitability of various *Eucaluptus* for growth of the larvae of *Uraba lugens* (walk). *Forest Ecology and Management*, 6, 361–366.

Cobbinah, J.R. (1985) The gum leaf skeletonizer, *Uraba lugens*, and its hosts. Possible selection of strains of insects that are able to feed on resistant trees. *Insect Science and its Application*, 6, 291–299.

Cobbinah, J.R., Morgan, F.D. & Douglas, T.J. (1982) Feeding responses of the gum leaf skeletoniser *Uraba lugens* Walker to sugars, amino acids, lipids, sterols, salts, vitamins and certain extracts of *Eucalyptus* leaves. *Journal of the Australian Entomological Society*, 21, 225–236.

Conner, W.E., Roach, B., Benedict, E., Meinwald, J. & Eisner, T. (1990) Courtship pheromone production and body size as correlates of larval diet in males of the arctiid moth, *Utetheisa ornatrix*. *Journal of Chemical Ecology*, 16, 543–552.

Darragh, K., Byers, K., Merrill, R.M., Mcmillan, W.O., Schulz, S. & Jiggins, C.D. (2019) Male pheromone composition depends on larval but not adult diet in *Heliconius melpomene*. *Ecological Entomology*, 44, 397–405.

DeLisle, J. & Hardy, M. (1997) Male larval nutrition influences the reproductive success of both sexes of the spruce budworm, *Choristoneura fumiferana* (lepidoptera: Tortricidae). *Functional Ecology*, 11, 451–463.

Dewsbury, D.A. (1982) Ejaculate cost and male choice. *American Naturalist*, 119, 601–610.

Di, X.-Y., Yan, B., Wu, C.-X., Yu, X.-F., Liu, J.-F. & Yang, M.-F. (2021) Does larval rearing diet lead to premating isolation in *Spodoptera litura* (Fabricius) (lepidoptera: Noctuidae)? *Insects*, 12, 203.

Durrant, K.L., Skicko, I.M., Sturrock, C. & Mowles, S.L. (2016) Comparative morphological trade-offs between pre- and post-copulatory sexual selection in giant hissing cockroaches (Tribe: Gromphadorhini). *Scientific Reports*, 6, 7.

Elgar, M.A. (2019) Chemical signaling: air, water, and on the substrate. In: Choe, J.C. (Ed.) *Encyclopedia of animal behavior*, Vol. 1, 2nd edition. Academic Press: Elsevier, pp. 462–473.

Elgar, M.A., Johnson, T.L. & Symonds, M.R.E. (2019) Sexual selection and organs of sense: Darwin's neglected insight. *Animal Biology*, 69, 63–82.

Elgar, M.A., Zhang, D., Wang, Q., Wittwer, B., Phạm, H.T., Johnson, T.L. et al. (2018) Insect antennal morphology: the evolution of diverse solutions to odorant perception. *Yale Journal of Biology and Medicine*, 91, 457–469.

Engqvist, L. (2005) The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, 70, 967–971.

Forister, M.L. & Scholl, C.F. (2012) Use of an exotic host plant affects mate choice in an insect herbivore. *American Naturalist*, 179, 805–810.

Foster, S.P. & Howard, A.J. (1999) The effects of mating, age at mating, and plant stimuli, on the lifetime fecundity and fertility of the generalist herbivore *Epiphyas postvittana*. *Entomologia Experimentalis et Applicata*, 91, 287–295.

Fricke, C., Adler, M.I., Brooks, R.C. & Bonduriansky, R. (2015) The complexity of male reproductive success: effects of nutrition, morphology, and experience. *Behavioral Ecology*, 26, 617–624.

Garcia-Barros, E. (2000) Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biological Journal of the Linnean Society*, 70, 251–284.

Geiselhardt, S., Otte, T. & Hilker, M. (2012) Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. *Ecology Letters*, 15, 971–977.

Hansson, B.S. (1995) Olfaction in lepidoptera. *Experientia*, 51, 1003–1027.

Hendry, L.B. (1976) Insect pheromones: diet related? *Science*, 192, 143–145.

Hendry, L.B., Wichmann, J.K., Hindenlang, D.M., Mumma, R.O. & Anderson, M.E. (1975) Evidence for origin of insect sex pheromone: presence in food plants. *Science*, 188, 59–63.

Henneken, J., Goodger, J.Q.D., Jones, T.M. & Elgar, M.A. (2017) Diet-mediated pheromones and signature mixtures can enforce signal reliability. *Frontiers in Ecology and Evolution*, 4, 145.

Henneken, J. & Jones, T.M. (2017) Pheromones-based sexual selection in a rapidly changing world. *Current Opinion in Insect Science*, 24, 84–88.

Henneken, J., Jones, T.M., Goodger, J.Q.D., Dias, D.A., Walter, A. & Elgar, M.A. (2015) Diet influences female signal reliability for male mate choice. *Animal Behaviour*, 108, 215–221.

Johansson, B.G. & Jones, T.M. (2007) The role of chemical communication in mate choice. *Biological Reviews of the Cambridge Philosophical Society*, 82, 265–289.

Johnson, T.L., Symonds, M.R.E. & Elgar, M.A. (2017a) Anticipatory flexibility: larval population density in moths determines male investment in antennae, wings and testes. *Proceedings of the Royal Society B*, 284, 20172087.

Johnson, T.L., Symonds, M.R.E. & Elgar, M.A. (2017b) Sexual selection on receptor organ traits: younger females attract males with longer antennae. *Science of Nature*, 104, 44.

Kurze, S., Heinken, T. & Fartmann, T. (2018) Nitrogen enrichment in host plants increases the mortality of common lepidoptera species. *Oecologia*, 188, 1227–1237.

Landolt, P.J. & Phillips, T.W. (1997) Host plant influences on sex pheromone behaviour of phytophagous insects. *Annual Review of Entomology*, 42, 371–391.

Lewis, L, & South, A. (2012) The evolution of animal nuptial gifts. *Advances in the Study of Behavior*, 44, 53–97.

Macartney, E.L., Crean, A.J., Nakagawa, S. & Bonduriansky, R. (2019) Effects of nutrient limitation on sperm and seminal fluid: a systematic review and meta-analysis. *Biological Reviews*, 94, 1722–1739.

Malnic, B., Hirono, J., Sato, T. & Buck, L.B. (1999) Combinatorial receptor codes for odors. *Cell*, 96, 713–723.

Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, 11, 119–161.

McNamara, K.B., Elgar, M.A. & Jones, T.M. (2009) Larger spermatophores reduce female receptivity and increase male paternity success in the almond moth, *Cadra cautella*. *Animal Behaviour*, 77, 931–936.

McNeil, J.N. & Delisle, J. (1989) Are host plants important in pheromone-mediated mating systems of lepidoptera? *Experientia*, 45, 236–240.

Merli, D., Mannucci, B., Bassetti, F., Corana, F., Falchetto, M., Malacrida, A.R. et al. (2018) Larval diet affects male pheromone blend in a laboratory strain of the medfly, *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Chemical Ecology*, 44, 339–353.

Moreau, J., Desouhant, E., Louapre, P., Goubault, M., Rajon, E., Jarrige, A. et al. (2017) How host plant and fluctuating environments affect insect reproductive strategies? In: Sauvion, N., Thiery, D. & Calatayud, P.A. (Eds.) *Insect-plant interactions in a crop protection perspective*. London: Elsevier, pp. 259–288.

Muller, K., Thiery, D., Moret, Y. & Moreau, J. (2015) Male larval nutrition affects adult reproductive success in wild European grapevine moth (*Lobesia botrana*). *Behavioral Ecology and Sociobiology*, 69, 39–47.

Niven, J.E. & Laughlin, S.B. (2008) Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology*, 211, 1792–1804.

Palacio Cortés, A.M., Zarbin, P.H.G., Takiya, D.M., Bento, J.M.S., Guidolin, A.S. & Consoli Fernando, L. (2010) Geographic variation of sex pheromone and mitochondrial DNA in *Diatraea saccharalis* (fab., 1794) (lepidoptera: Crambidae). *Journal of Insect Physiology*, 56, 1624–1630.

Phạm, H.T., Elgar, M.A., Van Lieshout, E. & McNamara, K.B. (2022) Experimental immune challenges reduce the quality of male antennae and female pheromone output. *Scientific Reports*, 12, 3578.

Phạm, H.T., McNamara, K.B. & Elgar, M.A. (2020) Socially cued anticipatory adjustment of female signalling effort in a moth. *Biology Letters*, 16, 20200614.

Phạm, H.T., McNamara, K.B. & Elgar, M.A. (2021) Age-dependent chemical signalling and its consequences for mate attraction in the gumleaf skeletonizer moth *Uraba lugens* Walker. *Animal Behaviour*, 173, 207–213.

R_CORE_TEAM. (2018) *R: a language and environment for statistical computing [online]*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Rantala, M.J., Kortet, R., Kotiaho, J.S., Vainikka, A. & Suhonen, J. (2003) Condition dependence of pheromones and immune function in the grain beetle *Tenebrio molitor*. *Functional Ecology*, 17, 534–540.

Raubenheimer, D. & Simpson, S.J. (1993) The geometry of compensatory feeding in the locust. *Animal Behaviour*, 45, 953–964.

Reddy, G.V.P. & Guerrero, A. (2004) Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Science*, 9, 253–261.

Rogers, S.M. & Simpson, S.J. (1997) Experience-dependent changes in the number of chemosensory sensilla on the mouthparts and antennae of *Locusta migratoria*. *Journal of Experimental Biology*, 200, 2313–2321.

Safonkin, A.F., Triseleva, T.A. & Kryuchkova, E.E. (2004) Influence of feeding plant upon the number of antenna sensillae in the apple surface eating tortricid, *Archips podana* (lepidoptera, Tortricidae). *Zoologichesky Zhurnal*, 83, 575–579.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675.

Simmons, L.W. & Emlen, D.J. (2006) Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences, USA*, 103, 16346–16351.

Simpson, S.J. & Raubenheimer, D. (2012) *The nature of nutrition: a unifying framework from animal adaptation to human obesity*. N J, USA, Princeton University Press: Princeton.

Stöckl, A., Heinze, S., Alice Charalabidis, A., el Jundi, B., Warrant, E. & Kelber, A. (2016) Differential investment in visual and olfactory brain areas reflects behavioural choices in hawk moths. *Scientific Reports*, 6, 26041.

Svard, L. & Wiklund, C. (1989) Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behavioral Ecology and Sociobiology*, 24, 395–402.

Svensson, M.G.E., Bengtsson, M. & Löfqvist, J. (1997) Individual variation and repeatability of sex pheromone emission of female turnip moths *Agrotis segetum*. *Journal of Chemical Ecology*, 23, 1833–1850.

Symonds, M.R.E. & Elgar, M.A. (2008) The evolution of pheromone diversity. *Trends in Ecology & Evolution*, 23, 220–228.

Triseleva, T.A. & Safonkin, A.F. (2006) Variation in antennal sensory system in different phenotypes of large fruit-tree tortrix *Archips podana* Scop. (Lepidoptera: Tortricidae). *Biology Bulletin*, 33, 568–572.

Wallis, I.R., Nicolle, D. & Foley, W.J. (2010) Available and not total nitrogen in leaves explains key chemical differences between the eucalypt subgenera. *Forest Ecology and Management*, 260, 814–821.

Wedell, N., Gage, M.J.G. & Parker, G.A. (2002) Sperm competition, male prudence and sperm-limited females. *Trends in Ecology & Evolution*, 17, 313–320.

Wool, D. & Hales, D.F. (1997) Phenotypic plasticity in Australian cotton aphid (Homoptera: Aphididae): host plant effects on morphological variation. *Annals of the Entomological Society of America*, 90, 316–328.

Xie, J.Q., De Clercq, P., Zhang, Y.H., Wu, H.S., Pan, C. & Pang, H. (2015) Nutrition-dependent phenotypes affect sexual selection in a ladybird. *Scientific Reports*, 5, 12.

Xu, J. & Wang, Q. (2009) Male moths undertake both pre- and in-copulation mate choice based on female age and weight. *Behavioral Ecology and Sociobiology*, 63, 801–808.

Xue, H.J., Wei, J.N., Magalhaes, S., Zhang, B., Song, K.Q., Liu, J. et al. (2016) Contact pheromones of 2 sympatric beetles are modified by the host plant and affect mate choice. *Behavioral Ecology*, 27, 895–902.

Zhu, Y., Zhong, Z.W., Pages, J.F., Finke, D., Wang, D., Ma, Q.H. et al. (2019) Negative effects of vertebrate on invertebrate herbivores mediated by enhanced plant nitrogen content. *Journal of Ecology*, 107, 901–912.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting information.

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