

RESEARCH ARTICLE

Hormone-mediated dispersal and sexual maturation in males of the social paper wasp *Polistes lanio*

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ABSTRACT

Sex-biased dispersal is common in social species, but the dispersing sex may delay emigration if associated benefits are not immediately attainable. In the social Hymenoptera (ants, some bees and wasps), newly emerged males typically disperse from the natal nest whilst most females remain as philopatric helpers. However, little information exists on the mechanisms regulating male dispersal. Furthermore, the conservation of such mechanisms across the Hymenoptera and any role of sexual maturation are also relatively unknown. Through field observations and mark–recapture, we observed that males of the social paper wasp *Polistes lanio* emerge from pupation sexually immature, and delay dispersal from their natal nest for up to 7 days whilst undergoing sexual maturation. Delayed dispersal may benefit males by allowing them to mature in the safety of the nest and thus be more competitive in mating. We also demonstrate that both male dispersal and maturation are associated with juvenile hormone (JH), a key regulator of insect reproductive physiology and behaviour, which also has derived functions regulating social organisation in female Hymenoptera. Males treated with methoprene (a JH analogue) dispersed earlier and possessed significantly larger accessory glands than their age-matched controls. These results highlight the wide role of JH in social hymenopteran behaviour, with parallel ancestral functions in males and females, and raise new questions on the nature of selection for sex-biased dispersal.

KEY WORDS: Life history, Dispersal, Social insect, Sexual maturation, Juvenile hormone, *Polistes lanio*

INTRODUCTION

Patterns of dispersal vary considerably within and between species in response to internal physiological or external environmental changes (Bowler and Benton, 2005; Clobert et al., 2009). Dispersal from the natal birth site allows for gene flow across populations and reduces the risk of inbreeding whilst facilitating the acquisition of alternative breeding or resource opportunities (Keller and Waller, 2002; Ronce, 2007; Matthysen, 2012; Hansson and Åkesson, 2014). Natal dispersal is often sex biased within species or populations, arising from different selection pressures on the two sexes and inbreeding avoidance (Pusey, 1987; Lawson Handley and Perrin, 2007; Gros et al., 2008). Sex-biased dispersal may also have consequences on social evolution, as philopatry promotes social

interactions among members of the non-dispersing sex (Lehmann and Boesch, 2009; Johnstone et al., 2012; Nagy and Knörnschild, 2016). In the social Hymenoptera (ants, some bees and wasps), dispersal from the natal nest is typically male biased (Hamilton, 1972; Johnstone et al., 2012). Despite the limited role of newly emerged males within natal nests and social groups, male dispersal is often delayed (Cameron, 1986; Poidatz et al., 2018). What regulates male dispersal in social Hymenoptera, and what function delayed dispersal plays, is poorly understood because research on these organisms is almost exclusively focused on females. We address this knowledge gap by examining male dispersal from the natal nest in a social wasp, by explaining natal dispersal patterns in terms of reproductive physiology and regulatory processes.

Social Hymenoptera lineages have ancestrally solitary life histories (Hunt, 1999; Hughes et al., 2008). Philopatry to a nest or area in female offspring was likely a key evolutionary innovation for sociality, allowing females to remain with relatives and help raise related brood via developed traits such as adult–larval trophallaxis (Hunt, 1999). Ancestrally, and in many extant species, female helpers retain the ability to reproduce and so may inherit the position of reproductive in the natal nest or disperse to found new nests (Tibbetts, 2007). Therefore, dispersal patterns in female social Hymenoptera reflect diverse reproductive strategies, allowing for both indirect and direct fitness, and have been studied extensively (Queller and Strassmann, 1998). By contrast, male behaviour in hymenopteran societies is poorly studied. Male Hymenoptera are thought to be evolutionarily limited to direct fitness investment and, with the exception of some complex ant societies where males have restricted dispersal, typically depart from the natal nest soon after emergence in search of mating opportunities (Hamilton, 1964, 1972; Johnstone et al., 2012; Heinze, 2016; Hakala et al., 2019). Males of social Hymenoptera therefore appear to have mostly retained ancestral behaviours and life histories (e.g. male sex-biased dispersal, rarity of brood care and selection towards increased mating success), which are likely to be regulated by similar mechanisms and processes as those of solitary ancestors.

Research on male dispersal and reproductive strategies has been neglected because males are often regarded as little more than ‘flying sperm’ within Hymenoptera societies (Wilson, 1971; O’Donnell, 1999; Beani et al., 2014). Males have limited direct contributions to the natal group, possessing relatively fast-paced life-history characteristics in comparison to females (Heinze, 2016). However, studies indicate that male life histories are not so simple, and that newly emerged males may delay dispersal from the natal nest whilst sexually maturing or to synchronize with nuptial flights (Hamilton, 1972; Moors et al., 2009; Poidatz et al., 2018; Hakala et al., 2019). Of benefit to males, after eclosion, post-pupation sexual maturation on the natal nest is likely to enhance fitness if the added protection and nutritional resources of the society allows sexual maturity to reach optimal reproductive potential (Litte, 1977; Hunt et al., 1982; O’Donnell and Jeanne, 1992; Leatemia et al.,

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1995; Yuval et al., 2002; Costamagna and Landis, 2004; Hunt, 2007). Males that remain in the natal nest may, however, be detrimental to the society as they drain resources and contribute little or nothing in terms of resource gathering, brood care or nest-defence behaviour (though see Cameron, 1986; Southon, 2018). Female nestmates may tolerate males on the natal nest for a short period of time, if it boosts their indirect fitness via the mating success of their brothers. Thus, selection for delayed dispersal by males may occur if it is coupled with maximising male mating success.

If delayed male dispersal is associated with sexual maturation, a robust mechanism for triggering departure would be one that co-regulates reproductive physiology and behaviour. One such candidate mechanism is the family of juvenile hormones (JHs). JHs play an important role in a wide range of developmental and behavioural processes in insects, with pleiotropic functions that regulate metamorphosis, diapause, polyphenism and reproduction (Dingle and Winchell, 1997; Hartfelder, 2000; Zera and Cisper, 2001). In both sexes after emergence, JH regulates sexual characteristics, such as inducing the propensity to disperse and mate (Wyatt and Davey, 1996; Goodman and Cusson, 2012). JH (specifically JH3) is strongly associated with the onset of dispersal flight behaviour in hymenopteran females, although it has also been co-opted to regulate nonreproductive behaviour in sterile female castes (Hartfelder, 2000).

The ancestral function of JH in pre-social Hymenoptera lineages was probably a regulator of female reproductive physiology and associated behaviours (Giray et al., 2005). This ancestral function is conserved in many social Hymenoptera, in which upregulation of JH in females (or application of a JH analogue) promotes dominance behaviour, ovarian activation and sexual receptivity (Bloch et al., 2000; Giray et al., 2005; Tibbetts and Izzo, 2009; Smith et al., 2013; Walton et al., 2020). For other social Hymenoptera, the ancestral once-reproductive role of JH has been modified. In the honeybee *Apis mellifera*, JH regulates reproductive caste determination during development; in adult females, JH serves a limited function in reproductive queen physiology but is involved in regulating sterile female caste age polyethism (Robinson et al., 1991; Hartfelder, 2000). Such new functions of JH as a regulator of nonreproductive female worker behaviour have evolved in a wide range of social Hymenoptera (e.g. *Polybia occidentalis*, O'Donnell and Jeanne, 1993; *Polistes canadensis*, Giray et al., 2005; *Polistes dominula*, Shorter and Tibbetts, 2009). Given the functional diversity of JH in hymenopteran females, an outstanding question is how conserved JH is as regulator of reproduction and behaviour in their male counterparts.

JH has an important role in adult male sexual maturation and behaviour of non-social insects: for example, in accessory gland protein synthesis and courtship behaviour in *Drosophila* (Wilson et al., 2003; Wijesekera et al., 2016). The role of JH in regulating reproductive physiology and behaviour of hymenopteran males has received relatively little attention. What is mostly known comes from studies of Apidae bees, specifically the solitary carpenter bee *Xylocopa appendiculata* and the social *A. mellifera*. In these bees, JH appears to have retained a similar ancestral and conserved function found in some female Hymenoptera and other insects, regulating sexual maturation and subsequent dispersal behaviour (Giray and Robinson, 1996; de Oliveira Tozetto et al., 1997; Harano et al., 2008; Sasaki et al., 2012; Sasaki and Nagao, 2013). It is likely that JH is a key regulator of male dispersal across the social Hymenoptera. The dual role of JH in regulating sexual maturation and dispersal provides a robust mechanism that may maximise the fitness of both males and their female nestmates.

Here, we examine patterns of male natal dispersal in the Neotropical social paper wasp *Polistes lanio*, and test the hypothesis that JH co-regulates dispersal and sexual maturation. Males of *P. lanio* make an ideal model for determining dispersal processes because nesting cycles continue throughout the year, with males and females being produced concurrently across nests, such that dispersal by males is not seasonal or mate limited (Giannotti and Machado, 1994; Lucas and Field, 2013). Using observations of natural dispersal patterns of males in wild populations, examination of sexual maturity and experimental applications of the JH analogue methoprene, we determine the timing of adult male dispersal from natal nests and the potential co-regulatory role of JH. Specifically, we address three questions: (1) Is male dispersal age-dependent? (2) Is delayed dispersal associated with sexual maturation? (3) Does JH co-regulate dispersal and sexual maturation?

MATERIALS AND METHODS

Study sites

Post-emergence nests (established nests with emerged natal offspring) of *P. lanio* were studied during the tropical wet season in Trinidad, Trinidad and Tobago, in June to August 2014 and July to September 2015. Nests were among natural populations in semi-rural areas, with a total of 37 nests used across four sites (of approximately two hectares each): 22 nests at Verdant Vale (site VV) 2014–2015 (10°41'5.44"N, 61°17'24.95"W); nine nests at Eastern Main Rd (EM) 2014–2015 (10°39'1.21"N, 61°15'9.63"W); four nests at Cumuto Tamana Rd (CT) 2015 (10°34'48.01"N, 61°14'38.06"W); two nests at the University of the West Indies Field Station (UWI) 2015 (10°38'16.04"N, 61°25'37.94"W).

Age dependency of male dispersal

We recorded the age at which 154 natal males dispersed from 27 nests at sites VV, EM and CT. On each nest, all wasps were recorded via daily censuses; after each census, males were removed from the nest with forceps and given a four-coloured spot combination on the dorsal thorax using extra fine tip Uni POSCA markers. During marking, wing length was measured as a straight line between the intersection of a tegula and wing to the furthest apex tip in millimetres, to 2 decimal places (d.p.). A newly emerged adult male was identified by black glossy eyes, absence of wing wear (Garcia and Noll, 2013) and its appearance coinciding with a hatched pupal cell (i.e. a wasp had recently eclosed from the cell). After marking, individuals were immediately returned to the natal nest comb using forceps. The response of nestmates to males that had been marked and returned was observed for 5 min; if a recently marked male was attacked and ejected from the nest by nestmates or flew off the nest within 5 min, it was excluded from analysis as this would indicate that marking had likely interfered with natural dispersal ($n=9$ exclusions from 163 marked males). The age of dispersal was determined as the first day that an individual was observed absent from the nest during daily census. Between census days, no males returned to natal nests after putative dispersal. If a marked male was observed on another nest ($n=19$ males), and departed again from these new non-natal nests, the date from the natal nest was used as the true dispersal event.

A repeated-measures generalized linear mixed model (GZLMM1) (binomial distribution, logit model), with the binary response variable of a dispersal event occurring or not per census date, was used to test whether natal dispersal could be explained by age (fixed effect); natal nest of origin was included as a random effect. To visualise dispersal events in the population, a Kaplan–Meier estimation was used, reporting the dispersal probability with

age as a survival plot. A Cox model (Cox1, Efron method for ties, describing hazard ratio as dispersal rate) was used to test possible influences of body size and nest characteristics on age of male dispersal (Table S1). First, dimensional reduction was performed on nest characteristics using a principal component analysis (PCA). A correlation coefficient matrix of nest characteristic variables – number of eggs, larvae, pupae, empty cells, parasitised cells, mean females and mean males per nest – was scored. Numbers of empty and parasitized cells were removed from the PCA, as they had coefficients $r < 0.3$. Included PCA variables were centred and scaled, with Bartlett's sphericity test ($P < 0.001$) and Kaiser–Meyer–Olkin statistic ($KMO > 0.5$) being satisfied. The PCA generated a single component with an eigenvalue > 1 (explaining 77% of variance), in which a regression factor score was calculated – henceforth described as 'nest/group size' (see Table S2 for PCA results). The final Cox model (Cox1) tested whether age of dispersal differed by wing length (an indicator of body size, Fig. S1), nest/group size, number of empty and parasitised cells, clustered by nest. Proportional hazards were assumed, as dispersal was only recorded from the natal nest.

Effect of sexual maturation on delayed dispersal

To assess sexual maturation, we measured the sizes of multiple reproductive organs in males of different ages and states of dispersal. Typical indicators of sexual maturation in male social wasps include a reduction in the size of the testes area (via degeneration, with sperm production being non-continuous), and an increase in the size of seminal vesicles and accessory glands as they develop and fill (Moors et al., 2009).

A total of 166 males were collected for dissection at sites VV, EM and CT. We defined three dispersal states: 'natal-nesting' males on their natal nest; 'dispersed-nesting' males that had dispersed and were caught on another nest (i.e. not their natal nest); 'dispersed-flight' males caught around the site but not on a nest (although not mutually exclusive with dispersed nesting, as males may be in transit). Data were gathered for 141 natal-nesting males from 11 nests: newly emerged, $n=42$ (0-day-old males collected on day of emergence); 1 day old, $n=38$; 2 days old, $n=35$; 3 days old, $n=21$; 4 days old, $n=3$; 5 days old, $n=2$. We collected 13 dispersed-nesting males on six non-natal nests, and 12 dispersed-flight males found off nests. All males were preserved in 70% ethanol and stored at -20°C .

We conducted dissections in phosphate-buffered saline using a Leica M165 C stereo microscope with a Leica IC80 HD digital camera attachment. Dissections were conducted by cutting a rectangular section into the ventral side of the abdomen and peeling back tergites, removing the testes and paired seminal vesicles with accessory glands. Removed organs were prepared on a slide. Using ImageJ 1.51j8, two-dimension measurements were taken of the area around the outer layer of the testes, seminal vesicles (SVs) and accessory glands (AGs) separately (mm^2 to 2 d.p.) (Fig. 1). Area of the seminal vesicles and accessory glands is reported as the mean area between paired organs for each structure, as the simultaneous function of ejaculation is unknown (SV and AG hereby referring to the mean size between respective organs). Measurements were taken blind to the age or dispersal state of the individual.

First, to determine whether body size was a potential confounding variable (i.e. if larger individuals had a head start over smaller conspecifics), we assessed if wing length was correlated with larger initial reproductive organ size (testes, SV and AG area) in 0-day-old males using Pearson's correlation (Pearson1–3). Second, to investigate if sexual maturation (i.e. smaller testes and larger SV and AG organs) was related to age in natal-nesting males, we ran three separate general linear mixed models (GLMM1/GLM1–2)

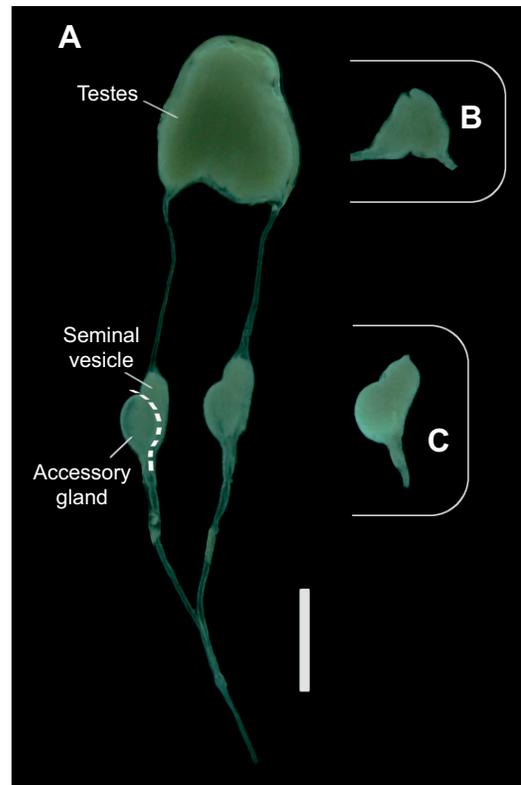


Fig. 1. Reproductive organs of newly emerged *Polistes lanio* male. (A) The testes connect to seminal vesicles, and the seminal vesicles and accessory glands (dashed line separates the two) connect into the ejaculatory duct. In older dispersed males, the testes size is reduced (B), whereas seminal vesicle and accessory gland sizes are increased (C). Scale bar: 1 mm.

with the response variables testes, and SV or AG size. Fixed effects for GLMMs consisted of wasp age, site location, wing length and year; natal nest origin was a random effect. Finally, to investigate differences in sexual maturation between natal-nesting, dispersed-nesting and dispersed-flight males, we ran three separate general linear models (GLM3–5) with the response variables testes, SV or AG size. The fixed effects for these models consisted of dispersal state, site location, wing length and year. We performed *post hoc* Tukey tests on the multilevel variable of dispersal state (single-step method for P -value adjustment).

Co-regulation effect of JH on dispersal and sexual maturation

Methoprene is a proven JH analogue in *Polistes* females (Giray et al., 2005; Shorter and Tibbetts, 2009). To find a suitable non-lethal dose in males, 72 natal males (~ 1 –7 days old) were collected from seven nests at sites VV, CT and UWI. Topical applications of $1 \mu\text{l}$ methoprene/acetone were made to the central dorsal side of the thorax using a micro-syringe. Treatments consisted of methoprene in acetone at 0, 250 and $500 \mu\text{g} \mu\text{l}^{-1}$ or a blank control ($n=18$ males per treatment, randomly assigned with respect to nest of origin). Dose range was based on a previous study in *A. mellifera* males (Sasaki et al., 2012) and its natural titre increase in males prior to flight behaviour was also confirmed in *A. mellifera* (Giray and Robinson, 1996). Males were marked as above. Males from the same nest were kept together in $25 \times 15 \times 15$ cm ventilated plastic enclosures, which were exposed to field-realistic conditions, but sheltered from direct sunlight, rain and ant predation. All males had *ad libitum* access to food, in the form of a cut piece of starch mango

Mangifera indica, and water. Mortality rates were recorded every 24 h for 10 days post-treatment. A Cox model (Cox2, Efron method for ties) was used to determine whether mortality rates between treatments, clustered by nest, significantly differed from that of the blank control group. Proportional hazards were assumed, as housing conditions did not change throughout the trial.

To observe the effect of methoprene on dispersal behaviour, 61 newly emerged males (0-day-old individuals) from seven nests at sites VV, EM and CT were selected. Each male was marked and treated as above during daylight hours, with a 1 μl topical application of either 0 $\mu\text{g } \mu\text{l}^{-1}$ ($n=30$ males) or 250 $\mu\text{g } \mu\text{l}^{-1}$ ($n=31$ males) methoprene in acetone. Treated males were returned to their natal nests and observed for 5 min (there were no instances of immediate departure). For each nest a census was conducted 24 and 48 h after treatment to determine timing of early dispersal. To test whether methoprene application induced departure, a Cox model (Cox3, Efron method for ties, describing hazard rate as dispersal rate) was used to determine whether dispersal rate of males treated with methoprene differed from that of the control treatment, clustered by nest. Proportional hazards were assumed, as dispersal was recorded from only the natal nest.

To ascertain whether methoprene accelerates sexual maturation, 19 males were collected from six nests at sites VV, CT and UWI. Each male was marked and treated as above with either 0 $\mu\text{g } \mu\text{l}^{-1}$ ($n=11$ males) or 250 $\mu\text{g } \mu\text{l}^{-1}$ ($n=8$ males) methoprene in acetone. Males were then housed in plastic enclosures as above. After 48 h, males were collected and their bodies stored in 70% ethanol at -20°C . Reproductive organ measurements were taken as described above. There was no male mortality observed in enclosures within the 48 h period. To test whether methoprene applications accelerated maturation, we used three separate GLMMs (GLMM2/GLM6–7) with response variables of testes, SV or AG size. As above, maturity is indicated by smaller testes, but larger SVs and AGs. The fixed effect for models was methoprene treatment, with natal nest of origin as a random effect.

Statistical analyses

Analyses were performed in R 3.3.3 (<https://www.r-project.org/>), using packages ‘car’ (Fox and Weisberg, 2011), ‘ggpubr’ (<https://CRAN.R-project.org/package=ggpubr>), ‘Hmisc’ (<https://CRAN.R-project.org/package=Hmisc>), ‘lme4’ (Bates et al., 2015), ‘lmerTest’ (<https://CRAN.R-project.org/package=lmerTest>), ‘multcomp’ (Hothorn et al., 2008), ‘RLRsim’ (Scheipl et al., 2008) and ‘survival’ (Therneau and Grambsch, 2000). Histograms and $Q-Q$ plots assessed deviations from normal distribution. When appropriate, model fit was assessed by checking the residual deviance against the degrees of freedom or residual versus fitted value plot. Variance of the random effect in models is reported with standard deviation (s.d.). If the random effect had a variance and s.d. of 0.00, the effect was dropped, and a subsequent non-mixed model used. All analyses were tested at $\alpha=0.05$, with averages reported as median with interquartile range (IQR), mean \pm s.d. or s.e.

RESULTS

Dispersal is age dependent

All males eventually dispersed from natal nests. Age at dispersal ranged from 1 to 7 days old, with the median age of dispersal being 3 days (IQR=2.0). Male age had a significant positive effect on the likelihood of dispersal occurring (GZLMM1: $\chi^2=53.21$, $P<0.001$; random effect of natal nest origin variance \pm s.d.=0.38 \pm 0.62). Dispersal probability increased between each age category (Kaplan–Meier), ranging from 19% in 1 day old males to 100%

in 7 day old males (Fig. 2). Body size and recorded nest characteristics did not significantly alter male dispersal rates from the natal nest (Cox1: wing length, Wald $\chi^2=-0.013$, $P=0.990$, mean \pm s.e. wing length=18.24 \pm 0.09 mm; nest/group size, Wald $\chi^2=0.663$, $P=0.507$; number of empty cells, Wald $\chi^2=-0.764$, $P=0.445$; number of parasitised cells, Wald $\chi^2=0.471$, $P=0.638$).

In 12% of natal dispersal events ($n=19$), males appeared soon after on non-natal foundress and post-emergence nests. These non-natal nests ranged from 0.5 to 16.5 m away from natal origin nests (skewed towards lower dispersal distances). Males appeared either on the same day or up to 2 days after initial natal dispersal. Dispersed-nesting males had a median age of 2 days (IQR=1.5) and stayed on non-natal nests for 1–2 days. Males always eventually left non-natal nests, and in only two instances did a male appear on a second non-natal nest (both post-emergence nests, 5.0 and 10.0 m away from the first non-natal nest), where they remained for a day each before departure.

Delayed dispersal is associated with sexual maturity

On emergence, body size did not appear to influence initial reproductive organ size ($N=10$ nests, $n=41$ males). Wing length was not significantly correlated with testes (Pearson1: $r=0.09$, d.f.=39, $P=0.585$), SV (Pearson2: $r=0.12$, d.f.=39, $P=0.441$) or AG (Pearson3: $r=0.04$, d.f.=39, $P=0.814$) size in 0-day-old males (mean \pm s.e.: wing length, 18.05 \pm 0.13 mm; testes size, 1.84 \pm 0.07 mm 2 ; SV size, 0.06 \pm <0.01 mm 2 ; AG size, 0.09 \pm <0.01 mm 2).

Ageing in natal nests was associated with sexual maturation, with older males showing signs of being more sexually mature than younger males ($N=11$ nests, $n=141$ males), indicated by a decrease in testes and increase in SV and AG size (Fig. 3). There was a significant negative effect of age on testes size (GLMM1: $\chi^2=37.92$, $P<0.001$). No significant difference in testes size was detected between the three sites ($\chi^2=0.52$, $P=0.771$), with wing length ($\chi^2=1.41$, $P=0.234$, mean \pm s.e. wing length=17.98 \pm 0.07 mm) or between the two years ($\chi^2=0.10$, $P=0.754$); natal nest origin variance \pm s.d.=0.02 \pm 0.13. There was a significant positive effect of age on SV size (GLM1: $F_{1,133}=29.68$, $P<0.001$). No significant difference in SV size was detected between the three sites ($F_{2,133}=0.15$, $P=0.929$), with wing length ($F_{1,133}=2.21$, $P=0.137$) or between the two years ($F_{1,133}=0.33$, $P=0.569$); natal nest origin random effect was dropped as there was no detectable variance. There was a significant positive effect of age on AG size (GLM2: $F_{1,133}=33.83$, $P<0.001$). No significant

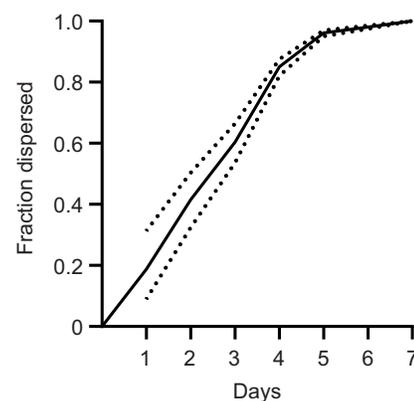


Fig. 2. Male *P. lanio* dispersal rates from natal nests in the population by age. Kaplan–Meier estimate with lower and upper 95% CIs (dotted lines) ($n=154$).

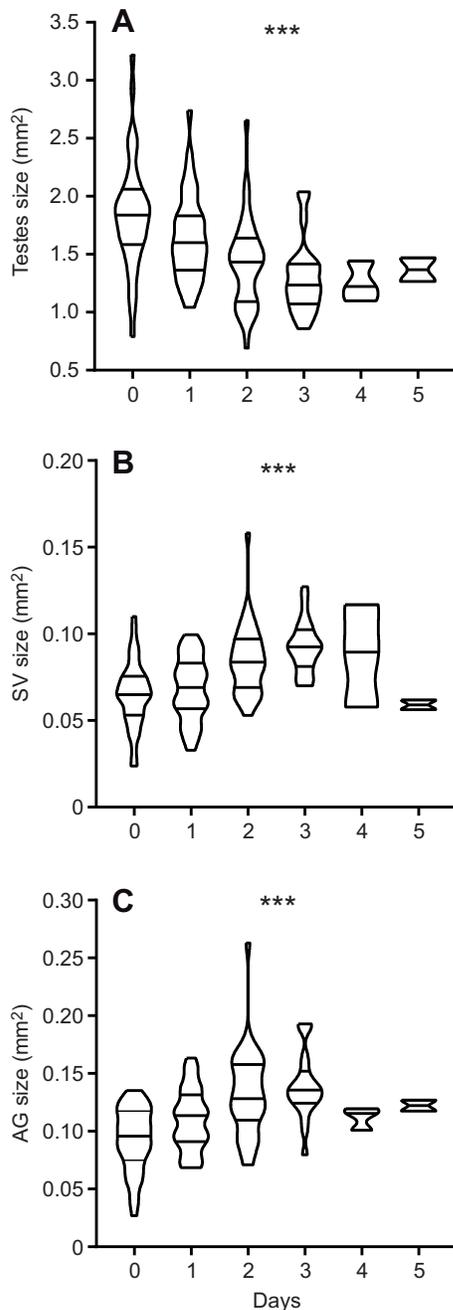


Fig. 3. Effect of age on size of testes, seminal vesicles (SVs) and accessory glands (AGs). Natal male age in days has a negative relationship with testes size (A; GLMM1, $***P < 0.001$), and a positive relationship with SV (B) and AG size (C; GLM1–2, $***P < 0.001$). Median centre line with IQR ($n=141$).

difference in AG size was detected between the three sites ($F_{2,133}=2.61$, $P=0.271$), with wing length ($F_{1,133}=2.00$, $P=0.157$) or between the two years ($F_{1,133}=1.03$, $P=0.310$); natal nest origin random effect was again dropped as there was no detectable variance.

Signs of greater sexual maturity were detected in dispersed males found in flight, compared with males found on nests (both natal nesting and dispersed nesting) (Fig. 4). Dispersal state had a significant effect on testes size (GLM3: $F_{2,157}=13.49$, $P < 0.001$). Dispersed-flight males had significantly smaller testes than natal-nesting (Tukey: $P < 0.001$) and dispersed-nesting ($P=0.015$) males;

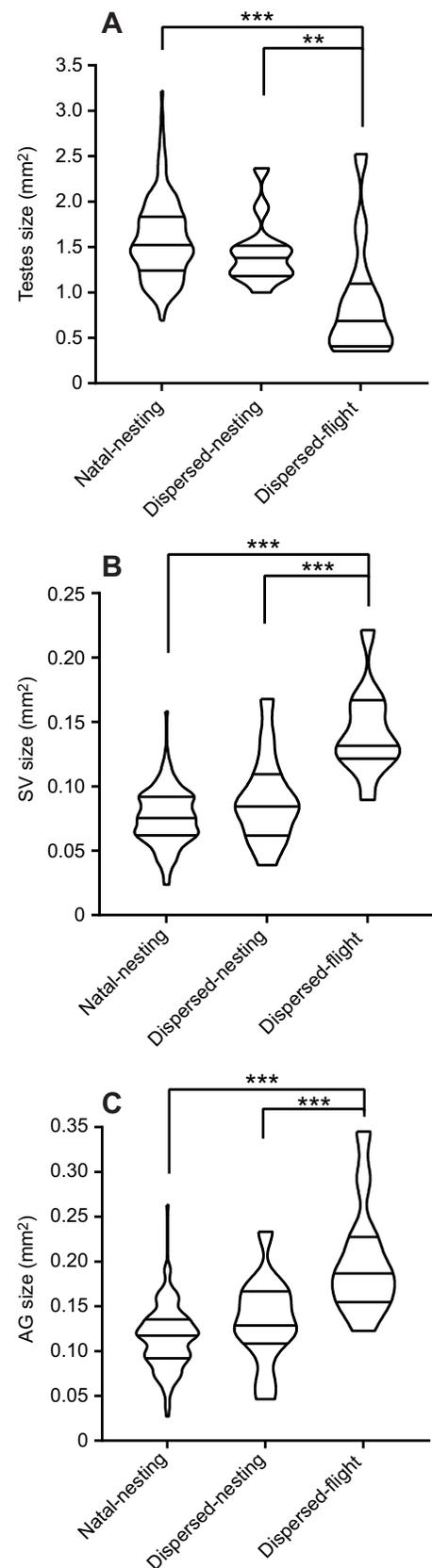


Fig. 4. Size of testes, SVs and AGs in dispersed, natal-nesting and dispersed-nesting males. Dispersed males caught in flight have significantly smaller testes (A) and larger SVs (B) and AGs (C) than both natal-nesting and dispersed-nesting males (GLM3–5, Tukey: $**P < 0.05$; $***P < 0.001$). Median centre line with IQR ($n=166$).

testes size was not significantly different between natal-nesting and dispersed-nesting males ($P=0.290$). No significant difference in testes size was detected between the three sites ($F_{2,157}=0.21$, $P=0.811$), with wing length ($F_{1,157}=0.92$, $P=0.338$, mean±s.e. wing length=18.06±0.07 mm) or between the two years ($F_{1,157}=0.12$, $P=0.728$). Dispersal state had a significant effect on SV size (GLM4: $F_{2,156}=37.25$, $P<0.001$). Dispersed-flight males had significantly larger SV sizes than natal-nesting (Tukey: $P<0.001$) and dispersed-nesting ($P<0.001$) males; SV size was not significantly different between natal-nesting and dispersed-nesting males ($P=0.201$). No significant difference in SV size was detected between the three sites ($F_{2,156}\leq 0.01$, $P=0.995$), with wing length ($F_{1,156}=1.97$, $P=0.163$) or between the two years ($F_{1,156}=0.19$, $P=0.661$). Dispersal state had a significant effect on AG size (GLM5: $F_{2,157}=24.69$, $P<0.001$). Dispersed-flight males had significantly larger AG sizes than natal-nesting (Tukey: $P<0.001$) and dispersed-nesting ($P<0.001$) males; AG size was not significantly different between natal-nesting and dispersed-nesting males ($P=0.347$). No significant difference in AG size was detected between the three sites ($F_{2,157}=0.57$, $P=0.565$), with wing length ($F_{1,157}=1.20$, $P=0.276$) or between the two years ($F_{1,157}=0.71$, $P=0.401$).

JH co-regulates dispersal and sexual maturation

In determining appropriate levels of methoprene ($n=72$ males), males treated with 500 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene had significantly higher mortality rates compared with blank controls (Cox2: Wald $\chi^2=3.54$, HR=6.91, lower 95% CI=2.37, upper 95% CI=20.14, $P<0.001$), confirming that the treatment had been successfully absorbed. Males treated with 500 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene were seven times more at risk of mortality than blank control treated males. Survival of males treated with 0 $\mu\text{g}\ \mu\text{l}^{-1}$ (Wald $\chi^2=1.51$, HR=1.87, lower 95% CI=0.83, upper 95% CI=4.31, $P=0.132$) and 250 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene (Wald $\chi^2=-0.60$, HR=0.66, lower 95% CI=0.17, upper 95% CI=2.61, $P=0.552$) was not significantly different from blank control treated males. Treatment with 250 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene was considered appropriate for use in further methoprene experiments (Fig. S2).

Treatment of newly emerged 0-day-old males with methoprene on natal nests ($N=7$ nests, $n=61$ males) resulted in faster rates of dispersal (Fig. 5). Males treated with 250 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene were

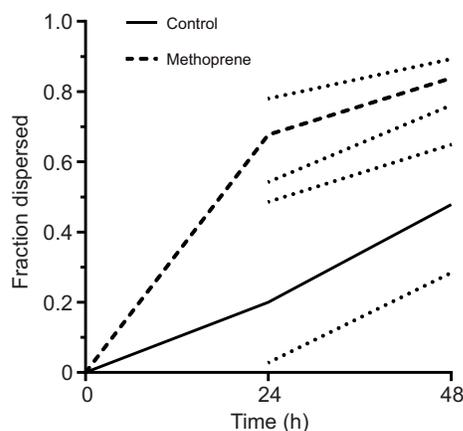


Fig. 5. Effect of methoprene treatment on dispersal of male natal wasps. Earlier dispersal rates are observed in natal 0-day-old males treated with 250 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene (dashed line) after 48 h compared with 0 $\mu\text{g}\ \mu\text{l}^{-1}$ treated control males (solid line). Cox3 plot with lower and upper 95% CIs (dotted lines), $P<0.001$ ($n=61$).

three times more likely to disperse earlier than 0 $\mu\text{g}\ \mu\text{l}^{-1}$ control males (Cox3: Wald $\chi^2=3.82$, HR=3.16, lower 95% CI=1.76, upper 95% CI=5.80, $P<0.001$).

Males treated with 250 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene at 0 days of age showed some signs of accelerated sexual maturity relative to 0 $\mu\text{g}\ \mu\text{l}^{-1}$ control males of the same age 48 h after application from emergence ($n=19$ males) (Fig. 6). AG size in males treated with 250 $\mu\text{g}\ \mu\text{l}^{-1}$ methoprene was significantly larger than 0 $\mu\text{g}\ \mu\text{l}^{-1}$ control males (GLM6: $F_{1,17}=4.60$, $P=0.047$; random effect dropped). However, there was no significant difference in SV size (GLM7: $F_{1,17}=0.47$, $P=0.502$; random effect dropped)

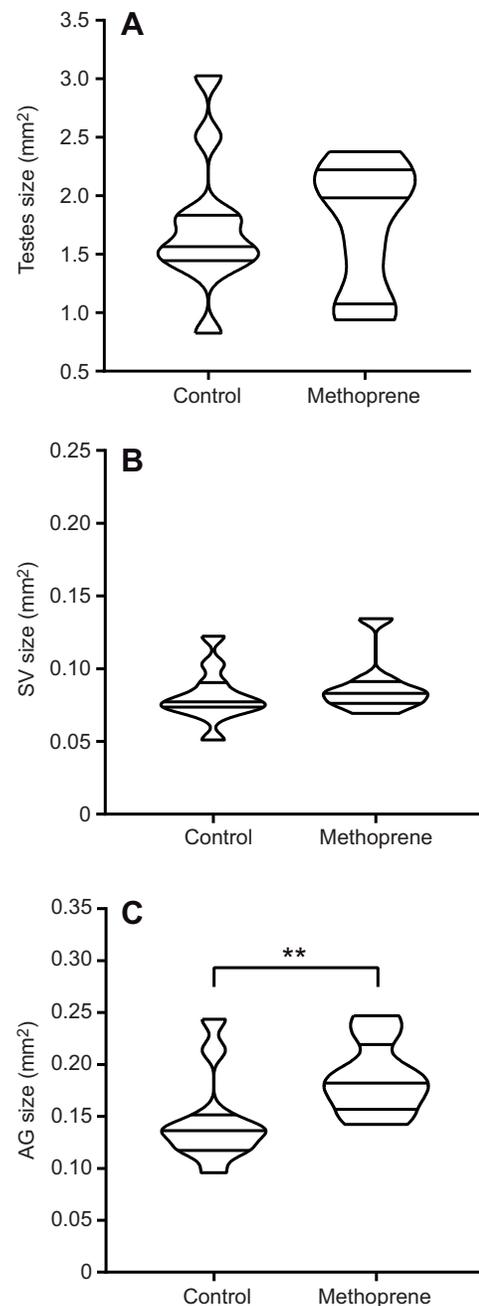


Fig. 6. Effect of methoprene treatment on size of testes, AGs and SVs in 0-day-old male wasps. AG size increased (GLM6, $**P<0.05$) in 0-day-old males treated with methoprene after 48 h (C), but no effect was seen on size of testes (A) or SV (B; GLMM2 and GLM7, n.s., $P>0.05$). Median centre line with IQR ($n=19$).

or testes size (GLMM2: $\chi^2_1=0.04$, $P=0.851$; natal nest origin variance \pm s.d.=0.01 \pm 0.08).

DISCUSSION

In the social Hymenoptera, delayed male dispersal from the natal nest is likely to be correlated with a period of sexual maturation post-pupation. Here, we provide evidence that males of the social paper wasp *P. lanio* delay dispersal from the natal nest, and that dispersed males found in flight tend to be more mature compared with males that are still on the nest. We show through experimental manipulation that the JH analogue methoprene induces precocious dispersal in males and can accelerate parts of the sexual maturation process within 48 h of application. These results suggest that JH plays a key mechanistic role in regulating dispersal and sexual maturation in males of this species, co-regulating physiology and behaviour, and potentially helping to maximise fitness. These findings support the theory that the JH family is crucial in regulating insect behaviour and life histories.

Male dispersal was predicted by age. A pattern of age-determined dispersal of males indicates that dispersal is strongly regulated internally by chronology in *P. lanio*. Animals typically disperse from natal groups within a fixed age range, correlating with sexual maturation or first breeding attempts (Martín and Bucher, 1993; González et al., 2006; Fernandez-Duque, 2009). Males of *P. lanio* dispersed from the natal nest at 1–7 days of age, which is similar to that for other closely related wasps in both temperate and tropical climates (*Polistes ferreri*, 0–5 days; *Polistes jokahamae*, 0–6 days; *Polistes major*, 0–13 days; *Ropalidia marginata*, 0–8 days; *Ropalidia cyathiformis*, mean dispersal 19 days; Gadagkar and Joshi, 1984; Cameron, 1986; Makino, 1993; Sinzato et al., 2003; Sen and Gadagkar, 2006, 2011). Males that leave the nest early may be individuals that later appear on non-natal nests, but early dispersal is likely to be sub-optimal as males will not be fully mature. Of the dispersed males caught in flight around the field sites, a single male was known to be particularly young at 1 day old; that male showed evidence of sexual immaturity with larger testes along with smaller seminal vesicles and accessory glands than other likely older dispersed males caught in flight. Overall, delayed dispersal appears to allow *P. lanio* males the opportunity to mature further on the nest (O'Donnell, 1999). It is likely that delayed dispersal for sexual maturation is a general strategy in social wasp species.

Dispersed males caught in flight showed significant signs of sexual maturation in comparison to natal-nesting males. With ageing, testes size decreased whilst seminal vesicle and accessory gland size increased, which matches findings of male post-pupation sexual maturation in other social wasps such as *Vespa vulgaris* and *Vespa velutina* (Moors et al., 2009; Poidatz et al., 2018). Completion of male sexual maturation varies across hymenopteran species but is usually >7 days of age in social bees and wasps (see Table S3), whilst *P. lanio* males disperse on average before they reach 7 days. Male *Polistes* have a range of mating strategies both within and between species (Beani and Turillazzi, 1988, 1990; Lee and Starr, 2007; Molina and O'Donnell, 2009), and sexual maturation or fully mature reproductive organ size may vary subtly among dispersed males. Although no correlation has been found so far to traits such as body size and initial reproductive organ size (*P. lanio* in this study) or sperm length (*Polistes simillimus* in De Souza et al., 2018). There may be selection for males to disperse just before completing maturation in order to reduce chances of inbreeding (Pusey and Wolf, 1996). Genetic studies have detected no significant levels of inbreeding in *P. lanio* or the sister species *P. canadensis* (Lengronne et al., 2012; Southon et al., 2019, 2020).

JH appeared to co-regulate dispersal and sexual maturation, with methoprene treatments causing earlier departure and increased accessory gland sizes. JH is likely to play a similar role in *P. lanio* males as it does in other male insects, including previously studied bee species (Giray and Robinson, 1996; de Oliveira Tozetto et al., 1997; Harano et al., 2008; Sasaki et al., 2012; Sasaki and Nagao, 2013), where JH is thought to regulate not only dispersal (e.g. milkweed bugs *Oncopeltus fasciatus*; Caldwell and Rankin, 1972) and courtship behaviour (e.g. *Drosophila melanogaster*; Wijesekera et al., 2016) but also sexual maturation such as in accessory gland activity (e.g. red flour beetles *Tribolium castaneum* and *D. melanogaster*; Wilson et al., 2003; Parthasarathy et al., 2009). Accessory gland fluids facilitate sperm transfer, but in many species also contain a concoction of biochemicals (e.g. sex peptides) that increase mating success and influence post-copulation female behaviour (Gillott, 2003). There are also likely to be a number of other hormones at play; for example, ecdysteroids and dopamine, the latter of which is expected to be regulated by JH and linked to the behavioural and physiological changes observed in this study (Harano et al., 2008). Because of the apparent coupled effect of JH on dispersal and sexual maturation in our study, we were not able to determine cause and effect; i.e. if JH independently regulates both these processes or if one (e.g. dispersal) is a downstream effect of the sexual maturation process. In *P. canadensis*, JH titres are higher in reproductives than guards, which in turn have higher levels than nonreproductive foragers (Giray et al., 2005). Although the effects of JH in regulating behaviour in *P. lanio* are unknown, it is likely that the same dual action will function in this sister species. Whilst JH appears to have a critical influence on male dispersal, there are likely to be several other physiological and environmental factors that could also explain delayed dispersal. Physiologically, flight for newly emerged adult holometabolous insects is severely limited until cuticle sclerotization and waterproofing is complete (Vincent and Wegst, 2004). Environmentally, nests undergoing social turmoil or in the declining phase may provide reduced nutritional resource opportunities; equally, hazardous weather conditions such as prolonged periods of heavy rainfall will stop flight activity. Additionally, dispersal decisions may involve resident females, who could force newly mature males to depart the nest to avoid inbreeding with sisters or to avoid depleting nest resources (Hakala et al., 2019). Future work arising from this study involves quantifying the natural JH titre changes of ageing males, and investigating whether the functionality of JH is specific to reproductive life history or if it also regulates other male behaviours observed during their brief tenure on the nest – for example, newly emerged male interactions with brood and female nestmates (Cameron, 1986; Beani et al., 2014; Southon, 2018).

Males occasionally dispersed from their natal nest to nearby non-natal nests. It is possible that non-natal males are related to the nests they visit, as neighbouring nests in aggregations of *P. canadensis* form viscous genetic population structures (Lengronne et al., 2012; Southon et al., 2019). Males may be accepted onto neighbouring nests because of recognition errors (by nestmate females) or they may provide some non-genetic benefits to females, through defence or low levels of cooperation (Cameron, 1986; Southon, 2018). Alternatively, males could be seeking opportunistic mating (although mating attempts are rarely observed on the nest; R.J.S. and S.S. personal observations) or may be cheating nestmates to obtain forage and protection. Nest drifting is a common behaviour in females of tropical *Polistes* spp., whereby workers split time between natal and non-natal nests (Sumner et al., 2007). However, non-natal nest visitation by males differs from female drifting in

that males were never observed to return to nests (at least between days). It is likely that the mechanisms permitting females to join new nests predisposes males to be able to do the same.

Our study provides insights into the mechanisms regulating male dispersal in tropical *P. lanio*. Why delayed male dispersal is tolerated by females, and the role of males (if any) during their brief period on natal and non-natal nests, are outstanding questions for *P. lanio* and for males in social hymenopteran societies in general. Future research should address the potential adaptive (or maladaptive) significance of delayed male dispersal in social Hymenoptera societies.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.J.S., A.N.R., S.S.; Methodology: R.J.S., A.N.R., S.S.; Validation: R.J.S.; Formal analysis: R.J.S.; Investigation: R.J.S.; Resources: R.J.S.; Data curation: R.J.S.; Writing - original draft: R.J.S., A.N.R., S.S.; Writing - review & editing: R.J.S., A.N.R., S.S.; Visualization: R.J.S., A.N.R., S.S.; Supervision: A.N.R., S.S.; Project administration: R.J.S., A.N.R., S.S.; Funding acquisition: R.J.S., A.N.R., S.S.

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Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.226472.supplemental>

References

- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. doi:10.18637/jss.v067.i01
- Beani, L. and Turillazzi, S. (1988). Alternative mating tactics in males of *Polistes dominulus* (Hymenoptera: Vespidae). *Behav. Ecol. Sociobiol.* **22**, 257–264. doi:10.1007/BF00209840
- Beani, L. and Turillazzi, S. (1990). Male swarms at landmarks and scramble competition polygyny in *Polistes gallicus* (Hymenoptera: Vespidae). *J. Insect Behav.* **3**, 545–556. doi:10.1007/BF01052017
- Beani, L., Dessi-Fulgheri, F., Cappa, F. and Toth, A. (2014). The trap of sex in social insects: from the female to the male perspective. *Neurosci. Biobehav. Rev.* **46**, 519–533. doi:10.1016/j.neubiorev.2014.09.014
- Bloch, G., Borst, D. W., Huang, Z.-Y., Robinson, G. E., Cnaani, J. and Hefetz, A. (2000). Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. *J. Insect Physiol.* **46**, 47–57. doi:10.1016/S0022-1910(99)00101-8
- Bowler, D. E. and Benton, T. G. (2005). Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biol. Rev.* **80**, 205–225. doi:10.1017/S1464793104006645
- Caldwell, R. L. and Rankin, M. A. (1972). Effects of a juvenile hormone mimic on flight in the milkweed bug, *Oncopeltus fasciatus*. *Gen. Comp. Endocrinol.* **19**, 601–605. doi:10.1016/0016-6480(72)90264-X
- Cameron, S. A. (1986). Brood care by males of *Polistes major* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* **59**, 183–185.
- Clobert, J., Le Galliard, J.-F., Cote, J., Meylan, S. and Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecol. Lett.* **12**, 197–209. doi:10.1111/j.1461-0248.2008.01267.x
- Costamagna, A. C. and Landis, D. A. (2004). Effect of food resources on adult *Glyptapanteles militaris* and *Meteorus communis* (Hymenoptera: Braconidae), parasitoids of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). *Environ. Entomol.* **33**, 128–137. doi:10.1603/0046-225X-33.2.128
- de Oliveira Tozetto, S., Rachinsky, A. and Engels, W. (1997). Juvenile hormone promotes flight activity in drones (*Apis mellifera carnica*). *Apidologie* **28**, 77–84. doi:10.1051/apido:19970204
- De Souza, A. R., Folly, C., Dias, G., dos Santos, E. F. and Lino-Neto, J. (2018). Sperm morphometric in males of the paper wasp *Polistes simillimus*. *Bull. Insectol.* **71**, 51–56.
- Dingle, H. and Winchell, R. (1997). Juvenile hormone as a mediator of plasticity in insect life histories. *Arch. Insect Biochem. Physiol.* **35**, 359–373. doi:10.1002/(SICI)1520-6327(1997)35:4<359::AID-ARCH2>3.0.CO;2-N
- Fernandez-Duque, E. (2009). Natal dispersal in monogamous owl monkeys (*Aotus azarai*) of the Argentinean Chaco. *Behaviour* **146**, 583–606. doi:10.1163/156853908X397925
- Fox, J. and Weisberg, S. (2011). *An {R} Companion to Applied Regression*, 2nd edn. Thousand Oaks, CA: Sage.
- Gadagkar, R. and Joshi, N. V. (1984). Social organisation in the Indian wasp *Ropalidia cyathiformis* (Fab.) (Hymenoptera: Vespidae). *Ethology* **64**, 15–32. doi:10.1111/j.1439-0310.1984.tb00350.x
- Garcia, Z. J. and Noll, F. B. (2013). Age and morphological changes in the epiponini wasp *Polybia paulista* Von Ihering (Hymenoptera: Vespidae). *Neotrop. Entomol.* **42**, 293–299. doi:10.1007/s13744-013-0124-6
- Giannotti, E. and Machado, V. L. L. (1994). Colonial phenology of *Polistes lanio lanio* (Fabricius, 1775) (Hymenoptera, Vespidae). *Rev. Brasil. Entomol.* **38**, 639–643.
- Gillott, C. (2003). Male accessory gland secretions: modulators of female reproductive physiology and behavior. *Annu. Rev. Entomol.* **48**, 163–184. doi:10.1146/annurev.ento.48.091801.112657
- Giray, T. and Robinson, G. E. (1996). Common endocrine and genetic mechanisms of behavioral development in male and worker honey bees and the evolution of division of labor. *Proc. Natl Acad. Sci. USA* **93**, 11718–11722. doi:10.1073/pnas.93.21.11718
- Giray, T., Giovanetti, M. and West-Eberhard, M. J. (2005). Juvenile hormone, reproduction, and worker behavior in the neotropical social wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci. USA*, **102**, 3330–3335. doi:10.1073/pnas.0409560102
- González, L. M., Oria, J., Margalida, A., Sánchez, R., Prada, L., Caldera, J., Aranda, A. and Molina, J. I. (2006). Effective natal dispersal and age of maturity in the threatened Spanish Imperial Eagle *Aquila adalberti*: conservation implications. *Bird Study* **53**, 285–293. doi:10.1080/00063650609461444
- Goodman, W. G. and Cusson, M. (2012). The juvenile hormones. In *Insect Endocrinology* (ed. L. I. Gilbert), pp. 310–365. London: Academic Press.
- Gros, A., Hovestadt, T. and Poethke, H. J. (2008). Evolution of sex-biased dispersal: the role of sex-specific dispersal costs, demographic stochasticity, and inbreeding. *Ecol. Model.* **219**, 226–233. doi:10.1016/j.ecolmodel.2008.08.014
- Hakala, S. M., Seppä, P. and Helanterä, H. (2019). Evolution of dispersal in ants (Hymenoptera: Formicidae): a review on the dispersal strategies of sessile superorganisms. *Myrmecol. News* **29**, 35–55.
- Hamilton, W. D. (1964). The genetical evolution of social behaviour. I & II. *J. Theor. Biol.* **7**, 1–52. doi:10.1016/0022-5193(64)90038-4
- Hamilton, W. D. (1972). Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* **3**, 193–232. doi:10.1146/annurev.es.03.110172.001205
- Hansson, L. A. and Åkesson, S. (2014). *Animal Movement Across Scales*. New York, NY: Oxford University Press.
- Harano, K.-I., Sasaki, K., Nagao, T. and Sasaki, M. (2008). Influence of age and juvenile hormone on brain dopamine level in male honeybee (*Apis mellifera*): association with reproductive maturation. *J. Insect Physiol.* **54**, 848–853. doi:10.1016/j.jinsphys.2008.03.003
- Hartfelder, K. (2000). Insect juvenile hormone: from “status quo” to high society. *Braz. J. Med. Biol. Res.* **33**, 157–177. doi:10.1590/S0100-879X2000000200003
- Heinze, J. (2016). The male has done his work — the male may go. *Curr. Opin. Insect Sci.* **16**, 22–27. doi:10.1016/j.cois.2016.05.005
- Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* **50**, 346–363. doi:10.1002/bimj.200810425
- Hughes, W. O. H., Oldroyd, B. P., Beekman, M. and Ratnieks, F. L. (2008). Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* **320**, 1213–1216. doi:10.1126/science.1156108
- Hunt, J. H. (1999). Trait mapping and salience in the evolution of eusocial vespid wasps. *Evolution* **53**, 225–237. doi:10.1111/j.1558-5646.1999.tb05348.x
- Hunt, J. H. (2007). *The Evolution of Social Wasps*. New York, NY: Oxford University Press.
- Hunt, J. H., Baker, I. and Baker, H. G. (1982). Similarity of amino acids in nectar and larval saliva: the nutritional basis for trophallaxis in social wasps. *Evolution* **36**, 1318–1322. doi:10.1111/j.1558-5646.1982.tb05501.x
- Johnstone, R. A., Cant, M. A. and Field, J. (2012). Sex-biased dispersal, haplodiploidy and the evolution of helping in social insects. *Proc. R. Soc. B* **279**, 787–793. doi:10.1098/rspb.2011.1257
- Keller, L. F. and Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241. doi:10.1016/S0169-5347(02)02489-8

- Lawson Handley, L. J. and Perrin, N.** (2007). Advances in our understanding of mammalian sex-biased dispersal. *Mol. Ecol.*, **16**, 1559-1578. doi:10.1111/j.1365-294X.2006.03152.x
- Leatemia, J. A., Laing, J. E. and Corrigan, J. E.** (1995). Effects of adult nutrition on longevity, fecundity, and offspring sex ratio of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae). *The Canadian Entomologist* **127**, 245-254. doi:10.4039/Ent127245-2
- Lee, J. X. and Starr, C. K.** (2007). Violent combat among *Polistes gigas* males (Hymenoptera: Vespidae). *Sociobiology* **50**, 337-342.
- Lehmann, J. and Boesch, C.** (2009). Sociality of the dispersing sex: the nature of social bonds in West African female chimpanzees, Pan troglodytes. *Anim. Behav.* **77**, 377-387. doi:10.1016/j.anbehav.2008.09.038
- Lengronne, T., Leadbeater, E., Patalano, S., Dreier, S., Field, J., Sumner, S. and Keller, L.** (2012). Little effect of seasonal constraints on population genetic structure in eusocial paper wasps. *Ecol. Evol.* **2**, 2615-2624. doi:10.1002/ece3.366
- Litte, M.** (1977). Aspects of the social biology of the bee *Halictus ligatus* in New York State (Hymenoptera, Halictidae). *Insectes Soc.* **24**, 9-36. doi:10.1007/BF0223277
- Lucas, E. R. and Field, J.** (2013). Caste determination through mating in primitively eusocial societies. *J. Theor. Biol.* **335**, 31-39. doi:10.1016/j.jtbi.2013.05.032
- Makino, S. I.** (1993). Sexual differences in larval feeding behavior in a paper wasp, *Polistes jadwigae* (Hymenoptera, Vespidae). *J. Ethol.* **11**, 73-75. doi:10.1007/BF02350009
- Martín, L. F. and Bucher, E. H.** (1993). Natal dispersal and first breeding age in monk parakeets. *The Auk* **110**, 930-933. doi:10.2307/4088651
- Matthysen, E.** (2012). Multicausality of dispersal: a review. In *Dispersal Ecology and Evolution* (ed. J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock), pp. 3-18. Oxford: Oxford University Press.
- Molina, Y. and O'Donnell, S.** (2009). Males exhibit novel relationships of dominance with nest departure in the social paper wasp *Mischocyttarus mastigophorus* (Hymenoptera: Vespidae). *Ethology* **115**, 738-746. doi:10.1111/j.1439-0310.2009.01659.x
- Moors, L., Schoeters, E., Coudron, K. and Billen, J.** (2009). Morphological changes in the male accessory glands and testes in *Vespula vulgaris* (Hymenoptera, Vespidae) during sexual maturation. *Invertebr. Biol.* **128**, 364-371. doi:10.1111/j.1744-7410.2009.00178.x
- Nagy, M. and Knörnschild, M.** (2016). Sex-biased dispersal and social systems of neotropical emballonurids. In *Sociality in Bats* (ed. J. Ortega), pp. 47-63. Switzerland: Springer International Publishing.
- O'Donnell, S.** (1999). The function of male dominance in the eusocial wasp, *Mischocyttarus mastigophorus* (Hymenoptera: Vespidae). *Ethology* **105**, 273-282. doi:10.1046/j.1439-0310.1999.00382.x
- O'Donnell, S. and Jeanne, R. L.** (1992). The effects of colony characteristics on life span and foraging behavior of individual wasps (*Polybia occidentalis*, Hymenoptera: Vespidae). *Insectes Soc.* **39**, 73-80. doi:10.1007/BF01240532
- O'Donnell, S. and Jeanne, R. L.** (1993). Methoprene accelerates age polyethism in workers of a social wasp (*Polybia occidentalis*). *Physiol. Entomol.* **18**, 189-194. doi:10.1111/j.1365-3032.1993.tb00467.x
- Parthasarathy, R., Tan, A., Sun, Z., Chen, Z., Rankin, M. and Palli, S. R.** (2009). Juvenile hormone regulation of male accessory gland activity in the red flour beetle, *Tribolium castaneum*. *Mech. Dev.* **126**, 563-579. doi:10.1016/j.mod.2009.03.005
- Poidatz, J., Bressac, C., Bonnard, O. and Thiéry, D.** (2018). Delayed sexual maturity in males of *Vespa velutina*. *Insect Sci.* **25**, 679-689. doi:10.1111/1744-7917.12452
- Pusey, A. E.** (1987). Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends Ecol. Evol.* **2**, 295-299. doi:10.1016/0169-5347(87)90081-4
- Pusey, A. and Wolf, M.** (1996). Inbreeding avoidance in animals. *Trends Ecol. Evol.* **11**, 201-206. doi:10.1016/0169-5347(96)10028-8
- Queller, D. C. and Strassmann, J. E.** (1998). Kin selection and social insects. *Bioscience* **48**, 165-175. doi:10.2307/1313262
- R Core Team.** (2017). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. URL: <https://www.R-project.org/>.
- Robinson, G. E., Strambi, C., Strambi, A. and Feldlaufer, M. F.** (1991). Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (*Apis mellifera*). *J. Insect Physiol.* **37**, 929-935. doi:10.1016/0022-1910(91)90008-N
- Ronce, O.** (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annu. Rev. Ecol. Syst.* **38**, 231-253. doi:10.1146/annurev.ecolsys.38.091206.095611
- Sasaki, K. and Nagao, T.** (2013). Juvenile hormone–dopamine systems for the promotion of flight activity in males of the large carpenter bee *Xylocopa appendiculata*. *Naturwissenschaften* **100**, 1183-1186. doi:10.1007/s00114-013-1116-4
- Sasaki, K., Akasaka, S., Mezawa, R., Shimada, K. and Maekawa, K.** (2012). Regulation of the brain dopaminergic system by juvenile hormone in honey bee males (*Apis mellifera* L.). *Insect Mol. Biol.* **21**, 502-509. doi:10.1111/j.1365-2583.2012.01153.x
- Sen, R. and Gadagkar, R.** (2006). Males of the social wasp *Ropalidia marginata* can feed larvae, given an opportunity. *Anim. Behav.* **71**, 345-350. doi:10.1016/j.anbehav.2005.04.022
- Sen, R. and Gadagkar, R.** (2011). Behavioural and morphological dimorphism of the sexes: an account of two primitively eusocial wasps. *J. Nat. Hist.* **45**, 1295-1309. doi:10.1080/00222933.2011.552808
- Scheipl, F., Greven, S. and Küchenhoff, H.** (2008). Size and power of tests for a zero random effect variance or polynomial regression in additive and linear mixed models. *Comput. Stat. Data Anal.* **52**, 3283-3299. doi:10.1016/j.csda.2007.10.022
- Shorter, J. R. and Tibbetts, E. A.** (2009). The effect of juvenile hormone on temporal polyethism in the paper wasp *Polistes dominulus*. *Insectes Soc.* **56**, 7-13. doi:10.1007/s00040-008-1026-1
- Sinzato, D. M., Prezoto, F. and Del-Claro, K.** (2003). The role of males in a neotropical paper wasp, *Polistes ferreri* Saussure, 1853 (Hymenoptera, Vespidae, Polistinae). *Rev. Brasil. Zoolociências* **5**, 89-100.
- Smith, A. R., Kapheim, K. M., Pérez-Ortega, B., Brent, C. S. and Wcislo, W. T.** (2013). Juvenile hormone levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Horm. Behav.* **63**, 1-4. doi:10.1016/j.yhbeh.2012.08.012
- Southon, R. J.** (2018). The adaptive value of males in simple eusocial insect societies. *PhD thesis*, University of Bristol, Bristol, UK.
- Southon, R. J., Bell, E. F., Graystock, P., Wyatt, C. D. R., Radford, A. N. and Sumner, S.** (2019). High indirect fitness benefits for helpers across the nesting cycle in the tropical paper wasp *Polistes canadensis*. *Mol. Ecol.* **28**, 3271-3284. doi:10.1111/mec.15137
- Southon, R. J., Radford, A. N. and Sumner, S.** (2020). High reproductive skew in the Neotropical paper wasp *Polistes lanio*. *Insectes Soc.* **67**, 451-456. doi:10.1007/s00040-020-00780-7
- Sumner, S., Lucas, E., Barker, J. and Isaac, N.** (2007). Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. *Curr. Biol.* **17**, 140-145. doi:10.1016/j.cub.2006.11.064
- Therneau, T. M. and Grambsch, P. M.** (2000). *Modelling Survival Data: Extending the Cox Model*. New York: Springer. ISBN 0-387-98784-3.
- Tibbetts, E. A.** (2007). Dispersal decisions and predispersal behavior in *Polistes* paper wasp 'workers'. *Behav. Ecol. Sociobiol.* **61**, 1877-1883. doi:10.1007/s00265-007-0427-x
- Tibbetts, E. A. and Izzo, A. S.** (2009). Endocrine mediated phenotypic plasticity: condition-dependent effects of juvenile hormone on dominance and fertility of wasp queens. *Horm. Behav.* **56**, 527-531. doi:10.1016/j.yhbeh.2009.09.003
- Vincent, J. F. V. and Wegst, U. G. K.** (2004). Design and mechanical properties of insect cuticle. *Arthropod. Struct. Dev.* **33**, 187-199. doi:10.1016/j.asd.2004.05.006
- Walton, A., Tumulty, J. P., Toth, A. L. and Sheehan, M. J.** (2020). Hormonal modulation of reproduction in *Polistes fuscatus* social wasps: duel functions in both ovary development and sexual receptivity. *J. Insect Physiol.* **120**, 103972. doi:10.1016/j.jinsphys.2019.103972
- Wijesekera, T. P., Saurabh, S. and Dauwalder, B.** (2016). Juvenile hormone is required in adult males for *Drosophila* courtship. *PLoS ONE* **11**, e0151912. doi:10.1371/journal.pone.0151912
- Wilson, E. O.** (1971). *The Insect Societies*. Cambridge, MA: Harvard University Press.
- Wilson, T. G., DeMoor, S. and Lei, J.** (2003). Juvenile hormone involvement in *Drosophila melanogaster* male reproduction as suggested by the Methoprene-tolerant²⁷ mutant phenotype. *Insect Biochem. Mol. Biol.* **33**, 1167-1175. doi:10.1016/j.ibmb.2003.06.007
- Wyatt, G. R. and Davey, K. G.** (1996). Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. In *Advances in Insect Physiology* (ed. P. D. Evans), pp. 213-274. San Diego, CA: Academic Press, Inc.
- Yuval, B., Kaspi, R., Field, S. A., Blay, S. and Taylor, P.** (2002). Effects of post-tenner nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Fla. Entomol.* **85**, 165-170. doi:10.1653/0015-4040(2002)085[0165:EOPTNO]2.0.CO;2
- Zera, A. J. and Cisper, G.** (2001). Genetic and diurnal variation in the juvenile hormone titer in a wing-polymorphic cricket: implications for the evolution of life histories and dispersal. *Physiol. Biochem. Zool.* **74**, 293-306. doi:10.1086/319664