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Does your article include research that required ethical approval or permits?:
This article does not present research with ethical considerations

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It is a condition of publication that data, code and materials supporting your paper are made publicly available. Does your paper present new data?:
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H.T.P., K.B.M. & M.A.E. conceived and designed the study; H.T.P. conducted the experiment and collected the data; H.T.P. & K.B.M. analysed the data; H.T.P. wrote, and K.B.M. & M.A.E. revised the manuscript. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Socially cued anticipatory adjustment of female signalling effort in a moth

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Keywords: *Uraba lugens*, sex pheromone, mate search, life history, mating strategies

Abstract

Juvenile population density has profound effects on subsequent adult development, morphology, and reproductive investment. Yet, little is known about how the juvenile social environment affects adult investment into chemical sexual signalling. Male gumleaf skeletonizer moths, *Uraba lugens,* facultatively increase investment into antennae (pheromone receiving structures) when reared at low juvenile population densities, but whether there is comparable adjustment by females into pheromone investment is not known. We investigate how juvenile population density influences the 'calling' (pheromone-releasing) behaviour of females, and the attractiveness of their pheromones. Female *U. lugens* adjust their calling behaviour in response to socio-sexual cues: adult females reared in high juvenile population densities called earlier and for longer than those from low juvenile densities. Juvenile density also affected female pheromonal attractiveness: y-maze olfactometer assays revealed that males prefer pheromones produced by females reared at high juvenile densities. This strategic investment in calling behaviour by females, based on juvenile cues that anticipate the future socio-sexual environment, likely reflects a response to avoid mating failure through competition with neighbouring signallers.

1. Introduction

Female insects typically use pheromones to advertise their location to conspecific males, a mechanism that is widely thought to incur little physiological costs to the signaller [1-4]. Nevertheless, females adjust strategically their investment into pheromone production and release according to abiotic and biotic cues [5–9], suggesting there are costs of pheromone production. Indeed, females alter their signalling investment in the presence of competing signallers, a response referred to as pheromone autodetection [10] or chorusing [11], by increasing [7,11–13], or decreasing signalling behaviour [9,14], manifested by the duration [9] and timing of calling bouts [7,9,15,16] within the same scotophase. This evidence of density-dependent strategic investment into pheromone production is in response to adult conspecifics: whether females anticipate the risk of future signalling competition from their juvenile social environment is not known.

There is emerging interest in the facultative adjustment of mating effort that is anticipated by the juvenile social environment [17]. In populations where density fluctuates between generations, selection may favour individuals that can assess cues that provide information on their future reproductive environment and adjust their investment accordingly [17–19]. Studies of anticipatory investment have focussed exclusively on males. For example, male moths use larval population density as a cue of future sperm competition risk, increasing their investment in gametes when reared at high density [20–23], or in antennal length [24] and wing muscle [21] when reared at low density.

Do females similarly anticipate their future socio-sexual environment, using cues in the juvenile environment to adjust their reproductive investment accordingly? Like males, the resources required for female reproductive activities are typically acquired during the juvenile stage of development [25], and especially in capital-breeding species [where adults do not feed). For dioecious species, females must signal their location and receptivity for mating [26–29], and the rate of female mating failure is likely to be higher in those insect species where females are less mobile, and populations are sparse— the “mate-finding Allee effect” [30, 31]. Two density-dependent mechanisms, which may cause female mating failure, generate different predictions of female calling strategies. First, mate-finding Alee effects predict that females from low-density populations should increase their signalling effort to alert sparsely located mates. Second, females from high-density populations should increase their signalling effort to avoid mating failure through competition from neighbouring females. There is emerging evidence that females adjust their calling behaviour in response to the presence of adult conspecifics [7,12,14] and heterospecifics [13], but male responses to changes in female calling investment are not known.

We explore the impact of juvenile population density on female calling behaviour and sex pheromonal attractiveness in the capital breeding, gumleaf skeletonizer moth, *Uraba lugens,* (Lepidoptera: Nolidae). Early-instar caterpillars are highly gregarious, eating, moulting and moving as a group, and dispersing at later instars to live alone or in smaller groups [32]. Adult U. lugens eclose during late scotophase (dark period) or early in the morning [32], and female calling frequency peaks seven hours after the onset of scotophase [33]. Male U. lugens reared at low population densities have larger wings and antennae, the latter improving mate detection [24,34]. We ask: (1) is female investment in sexual signalling influenced by her juvenile social environment, and (2) do males distinguish between sex pheromones produced by females reared under different larval social environments?

2. Methods

Experimental animals were collected as eggs from eucalypt trees in Royal Park, Melbourne, Victoria, and maintained under standard conditions (25°C and 15 h light: 9 h dark light cycle; 70% humidity). First-instar offspring were haphazardly allocated to one of three experimental treatments that manipulated juvenile population density: 'Low' density (LD) (one larva/container); 'Medium' density (MD) (five larvae/container); or 'High' density (HD) (25 larvae/container). Containers were 1 L and filled *ad libitum* with *Eucalyptus* spp. leaves, which were replaced every two days. Pupae were transferred to individual vials (120 ml) and adult females were haphazardly allocated to one of two experiments (female calling behaviour or female pheromonal attractiveness). A single female only was selected from each container for each treatment, removing the need to account for container effects.

(a) Juvenile density-dependent calling strategies of virgin females

The effect of juvenile density on female investment in sex pheromone signalling was examined by monitoring female calling behaviour for four scotophases post eclosion. Newly eclosed females from each treatment (LD 24; MD = 21; HD = 28) were isolated in clear plastic containers (120 ml) and their calling recorded for the first 9 hours of the scotophase over four consecutive days under a red-filtered light. Females were spot-checked (over a 2–5 min window) on the hour, every hour for calling behaviour, which is unambiguous: the wings are expanded to reveal the raised tip of the abdomen and the everted gland. The number and duration of each calling bout were recorded. Not all females eclosed at the start of the first scotophase, so calling duration is expressed as a proportion of the time following eclosion, hereafter 'proportion of time calling'. Female wing vein length was measured as an index of body size, following [35] (see S2).

(b) The effect of female juvenile population density on pheromonal attractiveness

The effect of juvenile population density on female pheromone investment was explored by examining male preferences for pheromones produced by females from LD or HD populations using a glass Y-maze (specifications in S1). A standardized airflow was pushed over a single HD and LD female (each housed at the end of each arm of the Y-maze) toward a focal male located at the entry to the maze. Females were ≤ 48hrs post-eclosion and matched for body weight (mean percentage female weight difference = 3.34%; maximum = 9.97%). Once both females called (see above), a virgin MD male (≤ 36 hrs post-eclosion) was placed at the entry to the Y-maze: he was deemed to have made a choice when he flew at least 5 cm into one of the arms of the Y-maze, remaining there for > 1 min. If males made a choice before 10 s had elapsed or remained immobile for 30 mins, they were replaced with a novel male (*n* = 4). Males that did not complete the trial within 60 min were excluded. Males were used once only, and pairs of females were used for two trials. Trials were conducted mid-scotophase.

(c) Statistical Analysis

Analyses were conducted in R studio v3.5.2 [36] (details in S3). The effect of population density on latency until calling and the proportion of time spent calling were analysed with GLMMs with female identity incorporated as a random effect, and Poisson and normal distributions, respectively. All dependent variables were power transformed to maximize normality of model residuals. Female relative wing size ([individual wing length—mean treatment wing length]/standard deviation of treatment wing length) was used as a covariate, as female wing size was significantly affected by juvenile density (S2; Table I). Post-hoc differences in the interaction between juvenile density and female age were analysed with Tukey's HSD, using planned contrasts with significance levels adjusted using a sequential Bonferroni procedure to limit the Type 1 error rate.

Male preferences were analysed using chi-square tests (conducted in Microsoft Excel). Four pairs of females were used once, and fourteen pairs of females were used twice, but it is not possible to incorporate female pair identity as a random effect in this experimental design. The qualitative pattern we report in the results remains unchanged if we exclude the second mating from these 14 twice-mating pairs, although it is not significant due to the drastically reduced sample size ($\chi^2 = 3.56$, $n = 18$, $p = 0.059$).

3. Results

(a) Juvenile density-dependent calling strategies

Female calling behaviour was significantly affected by an interaction between juvenile population density and female age (Table I; Fig. 1a, b): post-hoc tests revealed that on the first day only, HD females started calling earlier and called for a longer duration than LD females (Fig. 1a,b). The latency until calling was shorter, and the proportion of the scotophase spent calling was greater for relatively larger females (Table I).

(b) Males prefer the pheromones of females derived from high density juvenile populations

Thirty-two males (73%) completed the trials, with males showing a significant preference for the pheromones from HD females (HD females = 22, LD females = 10; $\chi^2 = 4.50$, $p = 0.03$).

4. Discussion

We provide novel evidence of female anticipatory investment in chemical signalling by altering both the nature and the timing of release of their sex pheromones, apparently in response to the risk of reproductive failure through competition for mates. Importantly, females were not exposed to conspecific calling during the experiments, and so this adjustment is in response to *perceived* future competition with other females, which is informed by juvenile population density. Adult female *U. lugens* that eclosed from high juvenile population densities commenced calling earlier and called for a longer time than females raised in low density populations, and males preferred the pheromones of females that had eclosed from high than low juvenile population densities. This preference, documented for young females, is likely to have significant fitness consequences because older females rarely attract males in field populations [34], and delays in the timing of mating have significant impact on fecundity in short-lived moths [38].

Competition between females may increase the risk of reproductive failure [37], especially for species such as *U. lugens*, where females have temporal constraints on mating [34]. Strategic adjustment of calling behaviour during the scotophase may reduce this risk [39] by extending the “mating window” in which females encounter males [4]. Female *U. lugens* anticipating high population densities called for a greater proportion of the scotophase by commencing their calling several hours earlier in the evening. More males are likely to be searching early in the scotophase because copulation can take up to four hours and males can mate once only per scotophase. Interestingly, this pattern changed as females aged, with the calling duration of older females being shorter, irrespective of their larval social environment; a pattern consistent with a fitness cost of releasing pheromone [26,40].

Other lines of evidence indicate that female moths adjust their signalling behaviour in response to the potential for competition [7,15,26,39]. This is manifested by an increase in time spent calling in the presence of adult conspecifics [7] or conspecific sex pheromones [39]. These data are consistent with the view that increased investment in calling at higher densities of conspecific signallers is advantageous [11,12,39,41–43], although this is mostly likely because less intensive signalling guarantees the female will not attract a male.

It is unsurprising that the signalling behaviour of female *U. lugens* was not consistent with mate-finding Allee effects. Although the mating success of female moths is typically lower at lower population densities [42-44], the evidence for a greater investment in signalling with decreasing population density is equivocal. While pickleworms *Diaphania nitidalis* emit more concentrated pheromone components at lower adult female densities [8], other moths spend less time calling in the presence of conspecific pheromones [16], and the likelihood of calling in other species does not change in the presence of conspecific competitors [15,45]. Similarly, the calling behaviour of *Plodia interpunctella* did not respond to experimental selection through juvenile population density [46].

The preferences of male *U. lugens* for pheromones produced by females eclosed from high population densities presumably arises from quantitative and/or qualitative [component ratio) differences in the pheromone. Chemical analysis of the pheromone output of females reared under different juvenile population density treatments was not possible, but field experiments indicate that the concentration of pheromones may be important. Males arriving at traps containing a solitary female *U. lugens* bait had longer antennae than those arriving at traps containing two females [34], suggesting that males can more readily detect higher concentrations of pheromone. Theory predicts that under conditions of male competition, females benefit from releasing small quantities of pheromone as this may attract higher quality males [18], but it may pay females to release a greater quantity of pheromone when there is competition for males.

Ethics. The experiments were conducted in accordance with the University of Melbourne procedures for ethical animal experimentation. All moths died naturally.

Data accessibility. Data are located in Dryad (https://datadryad.org/stash/share/_mP1F5BZ4eyiWcrgRyUelQt8ul5J112Dw3wJWLFLJU0) [47]. R code are provided in Supplementary Materials.

Authors' contribution. H.T.P., K.B.M. & M.A.E. conceived and designed the study; H.T.P. conducted the experiment and collected the data; H.T.P. & K.B.M. analysed the data; H.T.P. wrote, and K.B.M. & M.A.E. revised the manuscript. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. We declare we have no competing interests.

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Table I. Summary of models examining the impact of juvenile rearing density and age on female calling behaviour

	Model					
Model parameters	Latency until calling [a]			Proportion of time calling [b]		
	χ^2	df	P	χ^2	df	P
Density	$\chi^2 = 4.05$	2	0.13	$\chi^2 = 2.10$	2	0.35
Female age	$\chi^2 = 86.90$	3	<0.001	$\chi^2 = 63.48$	3	<0.001
Female size	$\chi^2 = 7.35$	1	0.007	$\chi^2 = 4.92$	1	0.03
Density x female age	$\chi^2 = 12.59$	6	0.049	$\chi^2 = 18.83$	6	0.004

[a] raised to the exponent 0.09

[b] raised to the exponent 1.72

Figure legend

Figure 1. The effect of the interaction between population density (Low, Medium and High) and female age (one to four-days old) on (a) the latency to female calling, and (b) the proportion of the scotophase spent calling. Values are means ± SE.

Figure 1a.

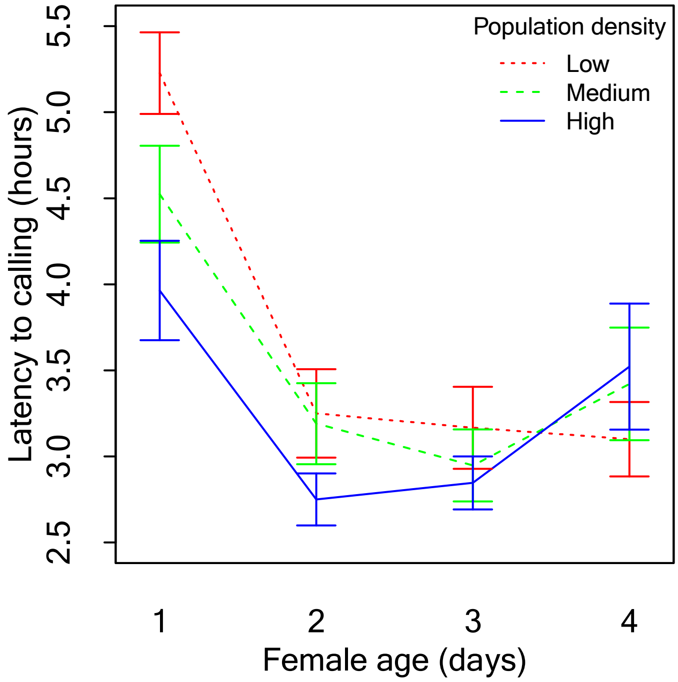
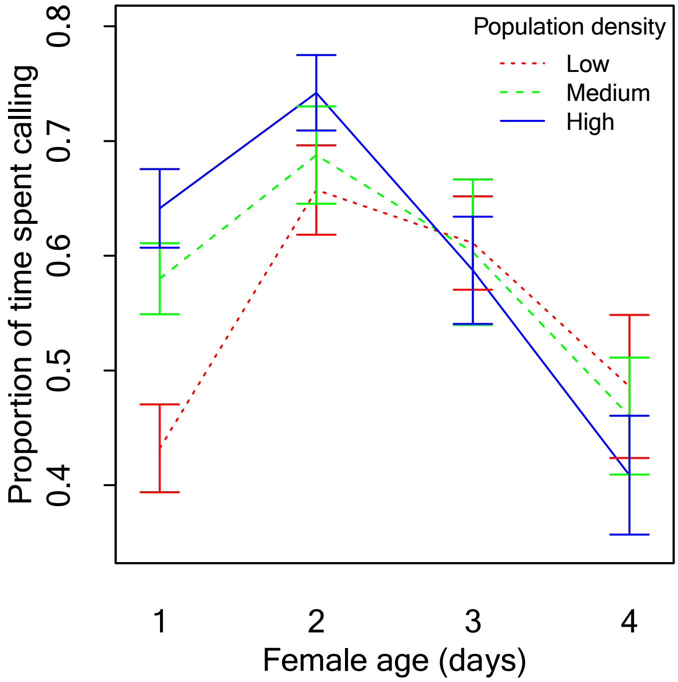


Figure 1b.



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Abstract

Juvenile population density has profound effects on subsequent adult development, morphology, and reproductive investment. Yet, little is known about how the juvenile social environment affects adult investment into chemical sexual signalling. Male gumleaf skeletonizer moths, *Uraba lugens,* facultatively increase investment into antennae (pheromone receiving structures) when reared at low juvenile population densities, but whether there is comparable adjustment by females into pheromone investment is not known. We investigate how juvenile population density influences the 'calling' (pheromone-releasing) behaviour of females, and the attractiveness of their pheromones. Female *U. lugens* adjust their calling behaviour in response to socio-sexual cues: adult females reared in high juvenile population densities called earlier and for longer than those from low juvenile densities. Juvenile density also affected female pheromonal attractiveness: y-maze olfactometer assays revealed that males prefer pheromones produced by females reared at high juvenile densities. This strategic investment in calling behaviour by females, based on juvenile cues that anticipate the future socio-sexual environment, likely reflects a response to avoid mating failure through competition with neighbouring signallers.

1. Introduction

Female insects typically use pheromones to advertise their location to conspecific males, a mechanism that is widely thought to incur little physiological costs to the signaller [1-4]. Nevertheless, females adjust strategically their investment into pheromone production and release according to abiotic and biotic cues [5–9], suggesting there are costs of pheromone production. Indeed, females alter their signalling investment in the presence of competing signallers, a response referred to as pheromone autodetection [10] or chorusing [11], by increasing [7,11–13], or decreasing signalling behaviour [9,14], manifested by the duration [9] and timing of calling bouts [7,9,15,16] within the same scotophase. This evidence of density-dependent strategic investment into pheromone production is in response to adult conspecifics: whether females anticipate the risk of future signalling competition from their juvenile social environment is not known.

There is emerging interest in the facultative adjustment of mating effort that is anticipated by the juvenile social environment [17]. In populations where density fluctuates between generations, selection may favour individuals that can assess cues that provide information on their future reproductive environment and adjust their investment accordingly [17–19]. Studies of anticipatory investment have focussed exclusively on males. For example, male moths use larval population density as a cue of future sperm competition risk, increasing their investment in gametes when reared at high density [20–23], or in antennal length [24] and wing muscle [21] when reared at low density.

Do females similarly anticipate their future socio-sexual environment, using cues in the juvenile environment to adjust their reproductive investment accordingly? Like males, the resources required for female reproductive activities are typically acquired during the juvenile stage of development [25], and especially in capital-breeding species [where adults do not feed). For dioecious species, females must signal their location and receptivity for mating [26–29], and the rate of female mating failure is likely to be higher in those insect species where females are less mobile, and populations are sparse— the "mate-finding Allee effect" [30, 31]. Two density-dependent mechanisms, which may cause female mating failure, generate different predictions of female calling strategies. First, mate-finding Alee effects predict that females from low-density populations should increase their signalling effort to alert sparsely located mates. Second, females from high-density populations should increase their signalling effort to avoid mating failure through competition from neighbouring females. There is emerging evidence that females adjust their calling behaviour in response to the presence of adult conspecifics [7,12,14] and heterospecifics [13], but male responses to changes in female calling investment are not known.

We explore the impact of juvenile population density on female calling behaviour and sex pheromonal attractiveness in the capital breeding, gumleaf skeletonizer moth, *Uraba lugens,* (Lepidoptera: Nolidae). Early-instar caterpillars are highly gregarious, eating, moulting and moving as a group, and dispersing at later instars to live alone or in smaller groups [32]. Adult U. lugens eclose during late scotophase (dark period) or early in the morning [32], and female calling frequency peaks seven hours after the onset of scotophase [33]. Male U. lugens reared at low population densities have larger wings and antennae, the latter improving mate detection [24,34]. We ask: (1) is female investment in sexual signalling influenced by her juvenile social environment, and (2) do males distinguish between sex pheromones produced by females reared under different larval social environments?

2. Methods

Experimental animals were collected as eggs from eucalypt trees in Royal Park, Melbourne, Victoria, and maintained under standard conditions (25°C and 15 h light: 9 h dark light cycle; 70% humidity). First-instar offspring were haphazardly allocated to one of three experimental treatments that manipulated juvenile population density: 'Low' density (LD) (one larva/container); 'Medium' density (MD) (five larvae/container); or 'High' density (HD) (25 larvae/container). Containers were 1 L and filled *ad libitum* with *Eucalyptus* spp. leaves, which were replaced every two days. Pupae were transferred to individual vials (120 ml) and adult females were haphazardly allocated to one of two experiments (female calling behaviour or female pheromonal attractiveness). A single female only was selected from each container for each treatment, removing the need to account for container effects.

(a) Juvenile density-dependent calling strategies of virgin females

The effect of juvenile density on female investment in sex pheromone signalling was examined by monitoring female calling behaviour for four scotophases post eclosion. Newly eclosed females from each treatment (LD 24; MD = 21; HD = 28) were isolated in clear plastic containers (120 ml) and their calling recorded for the first 9 hours of the scotophase over four consecutive days under a red-filtered light. Females were spot-checked (over a 2–5 min window) on the hour, every hour for calling behaviour, which is unambiguous: the wings are expanded to reveal the raised tip of the abdomen and the everted gland. The number and duration of each calling bout were recorded. Not all females eclosed at the start of the first scotophase, so calling duration is expressed as a proportion of the time following eclosion, hereafter ‘proportion of time calling’. Female wing vein length was measured as an index of body size, following [35] (see S2).

(b) The effect of female juvenile population density on pheromonal attractiveness

The effect of juvenile population density on female pheromone investment was explored by examining male preferences for pheromones produced by females from LD or HD populations using a glass Y-maze (specifications in S1). A standardized airflow was pushed over a single HD and LD female (each housed at the end of each arm of the Y-maze) toward a focal male located at the entry to the maze. Females were ≤ 48hrs post-eclosion and matched for body weight (mean percentage female weight difference = 3.34%; maximum = 9.97%). Once both females called (see above), a virgin MD male (≤ 36 hrs post-eclosion) was placed at the entry to the Y-maze: he was deemed to have made a choice when he flew at least 5 cm into one of the arms of the Y-maze, remaining there for > 1 min. If males made a choice before 10 s had elapsed or remained immobile for 30 mins, they were replaced with a novel male (*n* = 4). Males that did not complete the trial within 60 min were excluded. Males were used once only, and pairs of females were used for two trials. Trials were conducted mid-scotophase.

(c) Statistical Analysis

Analyses were conducted in R studio v3.5.2 [36] (details in S3). The effect of population density on latency until calling and the proportion of time spent calling were analysed with GLMMs with female identity incorporated as a random effect, and Poisson and normal distributions, respectively. All dependent variables were power transformed to maximize normality of model residuals. Female relative wing size ([individual wing length—mean treatment wing length]/standard deviation of treatment wing length) was used as a covariate, as female wing size was significantly affected by juvenile density (S2; Table I). Post-hoc differences in the interaction between juvenile density and female age were analysed with Tukey's HSD, using planned contrasts with significance levels adjusted using a sequential Bonferroni procedure to limit the Type 1 error rate.

Male preferences were analysed using chi-square tests (conducted in Microsoft Excel). Four pairs of females were used once, and fourteen pairs of females were used twice, but it is not possible to incorporate female pair identity as a random effect in this experimental design. The qualitative pattern we report in the results remains unchanged if we exclude the second mating from these 14 twice-mating pairs, although it is not significant due to the drastically reduced sample size ($\chi^2 = 3.56$, $n = 18$, $p = 0.059$).

3. Results

(a) Juvenile density-dependent calling strategies

Female calling behaviour was significantly affected by an interaction between juvenile population density and female age (Table I; Fig. 1a, b): post-hoc tests revealed that on the first day only, HD females started calling earlier and called for a longer duration than LD females (Fig. 1a,b). The latency until calling was shorter, and the proportion of the scotophase spent calling was greater for relatively larger females (Table I).

(b) Males prefer the pheromones of females derived from high density juvenile populations

Thirty-two males (73%) completed the trials, with males showing a significant preference for the pheromones from HD females (HD females = 22, LD females = 10; $\chi^2 = 4.50$, $p = 0.03$).

4. Discussion

We provide novel evidence of female anticipatory investment in chemical signalling by altering both the nature and the timing of release of their sex pheromones, apparently in response to the risk of reproductive failure through competition for mates. Importantly, females were not exposed to conspecific calling during the experiments, and so this adjustment is in response to *perceived* future competition with other females, which is informed by juvenile population density. Adult female *U. lugens* that eclosed from high juvenile population densities commenced calling earlier and called for a longer time than females raised in low density populations, and males preferred the pheromones of females that had eclosed from high than low juvenile population densities. This preference, documented for young females, is likely to have significant fitness consequences because older females rarely attract males in field populations [34], and delays in the timing of mating have significant impact on fecundity in short-lived moths [38].

Competition between females may increase the risk of reproductive failure [37], especially for species such as *U. lugens*, where females have temporal constraints on mating [34]. Strategic adjustment of calling behaviour during the scotophase may reduce this risk [39] by extending the “mating window” in which females encounter males [4]. Female *U. lugens* anticipating high population densities called for a greater proportion of the scotophase by commencing their calling several hours earlier in the evening. More males are likely to be searching early in the scotophase because copulation can take up to four hours and males can mate once only per scotophase. Interestingly, this pattern changed as females aged, with the calling duration of older females being shorter, irrespective of their larval social environment; a pattern consistent with a fitness cost of releasing pheromone [26,40].

Other lines of evidence indicate that female moths adjust their signalling behaviour in response to the potential for competition [7,15,26,39]. This is manifested by an increase in time spent calling in the presence of adult conspecifics [7] or conspecific sex pheromones [39]. These data are consistent with the view that increased investment in calling at higher densities of conspecific signallers is advantageous [11,12,39,41–43], although this is mostly likely because less intensive signalling guarantees the female will not attract a male.

It is unsurprising that the signalling behaviour of female *U. lugens* was not consistent with mate-finding Allee effects. Although the mating success of female moths is typically lower at lower population densities [42-44], the evidence for a greater investment in signalling with decreasing population density is equivocal. While pickleworms *Diaphania nitidalis* emit more concentrated pheromone components at lower adult female densities [8], other moths spend less time calling in the presence of conspecific pheromones [16], and the likelihood of calling in other species does not change in the presence of conspecific competitors [15,45]. Similarly, the calling behaviour of *Plodia interpunctella* did not respond to experimental selection through juvenile population density [46].

The preferences of male *U. lugens* for pheromones produced by females eclosed from high population densities presumably arises from quantitative and/or qualitative [component ratio) differences in the pheromone. Chemical analysis of the pheromone output of females reared under different juvenile population density treatments was not possible, but field experiments indicate that the concentration of pheromones may be important. Males arriving at traps containing a solitary female *U. lugens* bait had longer antennae than those arriving at traps containing two females [34], suggesting that males can more readily detect higher concentrations of pheromone. Theory predicts that under conditions of male competition, females benefit from releasing small quantities of pheromone as this may attract higher quality males [18], but it may pay females to release a greater quantity of pheromone when there is competition for males.

Ethics. The experiments were conducted in accordance with the University of Melbourne procedures for ethical animal experimentation. All moths died naturally.

Data accessibility. Data are located in Dryad (https://datadryad.org/stash/share/_mP1F5BZ4eyiWcrgRyUelQt8ul5J112Dw3wJWLFLJU0 [47]. R code are provided in Supplementary Materials.

Authors' contribution. H.T.P., K.B.M. & M.A.E. conceived and designed the study; H.T.P. conducted the experiment and collected the data; H.T.P. & K.B.M. analysed the data; H.T.P. wrote, and K.B.M. & M.A.E. revised the manuscript. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. We declare we have no competing interests.

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Table I. Summary of models examining the impact of juvenile rearing density and age on female calling behaviour

	Model					
Model parameters	Latency until calling [a]			Proportion of time calling [b]		
	χ^2	df	P	χ^2	df	P
Density	$\chi^2 = 4.05$	2	0.13	$\chi^2 = 2.10$	2	0.35
Female age	$\chi^2 = 86.90$	3	<0.001	$\chi^2 = 63.48$	3	<0.001
Female size	$\chi^2 = 7.35$	1	0.007	$\chi^2 = 4.92$	1	0.03
Density x female age	$\chi^2 = 12.59$	6	0.049	$\chi^2 = 18.83$	6	0.004

[a] raised to the exponent 0.09

[b] raised to the exponent 1.72

Figure legend

Figure 1. The effect of the interaction between population density (Low, Medium and High) and female age (one to four-days old) on (a) the latency to female calling, and (b) the proportion of the scotophase spent calling. Values are means ± SE.

Figure 1a.

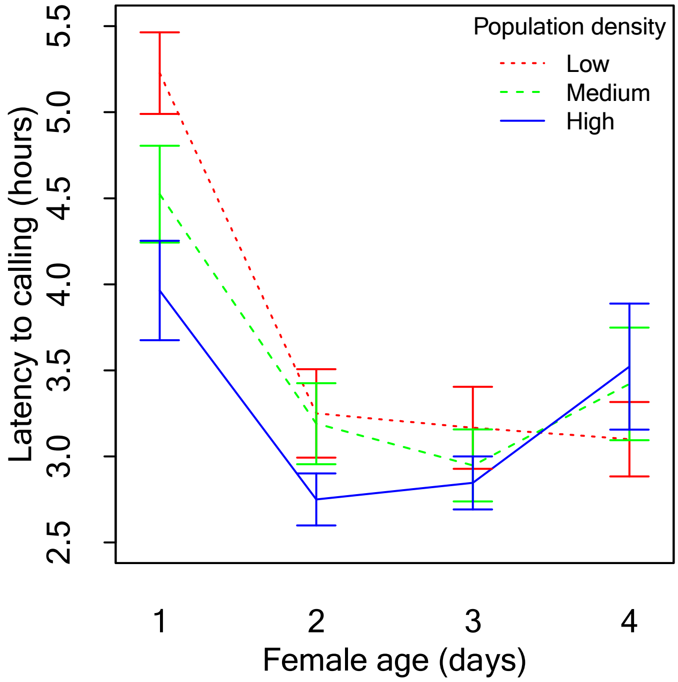

Figure 1b.