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Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps

Jennifer M. Jandt
University of Otago

Sainath Suryanarayanan
University of Wisconsin-Madison

John C. Hermanson
U.S. Department of Agriculture

Robert L. Jeanne
University of Wisconsin-Madison

Amy L. Toth
Iowa State University, amytoth@iastate.edu

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Abstract
The social and nutritional environments during early development have the potential to affect offspring traits, but the mechanisms and molecular underpinnings of these effects remain elusive. We used *Polistes fuscatus* paper wasps to dissect how maternally controlled factors (vibrational signals and nourishment) interact to induce different caste developmental trajectories in female offspring, leading to worker or reproductive (gyne) traits. We established a set of caste phenotype biomarkers in *P. fuscatus* females, finding that gyne-destined individuals had high expression of three caste-related genes hypothesized to have roles in diapause and mitochondrial metabolism. We then experimentally manipulated maternal vibrational signals (via artificial 'antennal drumming') and nourishment levels (via restricted foraging). We found that these caste-related biomarker genes were responsive to drumming, nourishment level or their interaction. Our results provide a striking example of the potent influence of maternal and nutritional effects in influencing transcriptional activity and developmental outcomes in offspring.

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Author for correspondence:
Jennifer M. Jandt
e-mail: jjandt2@gmail.com

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Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps

Jennifer M. Jandt[1,2], Sainath Suryanarayanan[4], John C. Hermanson[5,8], Robert L. Jeanne[6,7] and Amy L. Toth[2,3]

[1]Department of Zoology, University of Otago, Dunedin, New Zealand
[2]Department of Ecology, Evolution, and Organismal Biology, and [3]Department of Entomology, Iowa State University, Ames, IA, USA
[4]Population Health Institute, [5]Department of Civil and Environmental Engineering, [6]Department of Zoology, and [7]Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA
[8]USDA Forest Service, Madison, WI, USA

JMJ, 0000-0002-0991-8577; RLJ, 0000-0001-9908-7457; ALT, 0000-0003-0824-0933

The social and nutritional environments during early development have the potential to affect offspring traits, but the mechanisms and molecular underpinnings of these effects remain elusive. We used *Polistes fuscatus* paper wasps to dissect how maternally controlled factors (vibrational signals and nourishment) interact to induce different caste developmental trajectories in female offspring, leading to worker or reproductive (gyne) traits. We established a set of caste phenotype biomarkers in *P. fuscatus* females, finding that gyne-destined individuals had high expression of three caste-related genes hypothesized to have roles in diapause and mitochondrial metabolism. We then experimentally manipulated maternal vibrational signals (via artificial 'antennal drumming') and nourishment levels (via restricted foraging). We found that these caste-related biomarker genes were responsive to drumming, nourishment level or their interaction. Our results provide a striking example of the potent influence of maternal and nutritional effects in influencing transcriptional activity and developmental outcomes in offspring.

1. Introduction

An organism's social environment can have profound effects on its developmental fate. Compared with other forms of social interaction, maternal behaviour can exert an especially potent influence, as contact with offspring can be prolonged and occur throughout sensitive developmental stages [1]. There has been increasing interest in understanding how specific forms of mother–offspring interaction during critical periods in early development can affect the physiology, behaviour and health of offspring, and whether such effects are reversible [1–3]. To date, many studies on maternal effects have focused on mammals, especially humans and rodents [3], in which it can be difficult to experimentally manipulate maternal behaviour and to separate it from correlated factors such as nutritional deficiencies and other forms of stress. Thus, there is still much to be learned about how maternal and nutritional factors interact to influence offspring development and about the underlying physiological and molecular mechanisms mediating these effects.

Maternal care is also well developed in some insects, and such systems are highly amenable to experimental manipulation. Notably, in eusocial Hymenoptera and their closest solitary relatives, maternal care of brood in the nest has been identified as a critical pre-adaptation to the evolution of eusociality [4]. Brood care, including physical contact and level of brood provisioning, can influence endocrine pathways (such as juvenile hormone) during critical stages of development, resulting in physiologically and behaviourally distinct

Table 1. Worker and gyne caste traits.

phenotype	caste-related gene expression				physiology
	inositol oxygenase	*heat-shock protein 90a*	*rhodopsin*	*hexamerin 70b*	lipid
foundress-reared worker-like	low	low	low	low	low
worker-reared gyne-like	high	high	high	high	high

reproductive (queen) and non-reproductive (worker) castes [5–8]. Unlike highly eusocial species, where thousands of workers interact with developing larvae, queens of primitively eusocial species, such as *Polistes* wasps, have exclusive and close physical contact with their offspring for many weeks (see [9] for eusocial classifications). Despite great interest in maternal care in the context of eusocial evolution, little is known about the mechanisms by which primitively eusocial mothers influence caste development of their offspring. This topic is particularly interesting because in most social insect species, female offspring exhibit immense phenotypic plasticity (i.e. differential developmental paths towards queen or worker castes [10]). This sets up the possibility that maternal behaviour (via social interactions) or access to nutrition (via brood feeding) could strongly influence offspring caste fate, predisposing them either to remain at the nest, forgo reproduction and help rear siblings, or to become reproductive and found their own colonies.

The genus *Polistes* is an ideal system in which to dissect the mechanisms mediating maternal effects. Females (founding queens or 'foundresses') that initiate colonies have prolonged and easily observable mother–offspring interactions. Moreover, while queens and workers do not differ morphologically and caste fate is flexible into adulthood, the two castes are distinct behaviourally, physiologically and transcriptionally [11]. In temperate-zone *Polistes* species, larvae produced early in the colony cycle (foundress-reared, FR) tend to develop worker-caste traits (e.g. low fat stores; table 1). By contrast, later in the colony cycle, after workers emerge and take over foraging for the colony, worker-reared (WR) larvae tend to develop gyne- (pre-founding queen) caste traits (e.g. higher fat stores; table 1) [12–14]. Even though well-fed *Polistes* colonies produce more female offspring with gyne-like traits than do poorly fed colonies [15–17], recent work showed that nourishment differences alone do not fully explain differential expression of caste-related genes [18,19]. Jeanne and co-workers [19–21] hypothesized that vibrational signals produced by *Polistes* foundresses and directed at developing larvae are an additional source of environmental input affecting caste developmental trajectories.

In most species of *Polistes*, vibrational signalling takes the form of antennal drumming: the queen trills her antennae against the rims of the brood cells during larval feeding [22]. Jeanne's [19] suggestion that antennal drumming is a signal that biases larvae to develop into workers is supported by several observations. First, the foundress initiates vibrations when the oldest FR offspring reach the third (of five) larval instars [23], the stage at which caste developmental pathways probably begin to diverge [24]. Second, rates of vibrational signalling are high early in the colony cycle, when worker offspring are produced, and lower later in the season, when worker production ceases [23,25,26]. Third, experimental simulation of antennal drumming on *P. fuscatus* nests causes larvae to develop lower (more worker-like) fat stores [21]. The emerging picture of caste-biased development in *Polistes* is a dual-input model, in which both maternal factors—vibrational signals and nutrition—interact to affect caste-related traits of offspring [20].

Here, we test this model. We use an experimental approach with *Polistes fuscatus* to examine how maternal vibrational signals and nourishment interact to affect offspring gene expression and physiology. Our study involves a novel integration of the fields of behavioural ecology, electrical engineering (simulated maternal drumming using piezoelectric devices) and genomics. We first establish caste phenotype biomarkers related to gene expression and lipid stores. We examine the effects of simulated drumming and nutrition restriction on these caste-related biomarkers. We hypothesize that the combined effects of antennal drumming and low nourishment lead to worker-like phenotypes (table 1); that is, lower expression of gyne-biased biomarker genes and lower lipid stores. Moreover, we explore whether there is evidence that the two posited caste-influencing factors (drumming and nutrition) interact to influence caste-related gene expression.

2. Material and methods

For our basic experimental approach, we artificially exposed WR larvae to two conditions normally experienced only by FR larvae: antennal drumming and low nutrition. This results in a 2×2 matrix of treatments and controls. In our statistical models, we tested for effects of the two treatments and their interaction on colony growth, the expression of selected gyne-biased biomarker genes and lipid stores.

(a) Wasp colonies

We initiated the experiment by rearing 90 *P. fuscatus* colonies in the field at the Iowa State University Horticulture Farm, in Ames, Iowa (42 in 2013 and 48 in 2014; see the electronic supplementary material, S1, for set-up details). Of those, 43 colonies (all of which, except for two in 2013, were initiated by solitary foundresses) produced offspring that could be used in this study (15 from which we collected FR larvae only, 18 from which we collected WR larvae only and 10 from which we collected both FR and WR larvae; electronic supplementary material, S1).

Before experimental manipulations started, all wasps were allowed to forage freely in the surrounding fields. We monitored colonies for at least two weeks before beginning the drumming and/or nutrition treatments. All adult wasps were marked with a distinguishing paint mark to denote the nest of origin. Every 2–3 days, we recorded the developmental stage (egg, larval instar, pre-pupa (fifth-instar larva during cocoon construction) or pupa) of each immature individual in each nest. Detailed

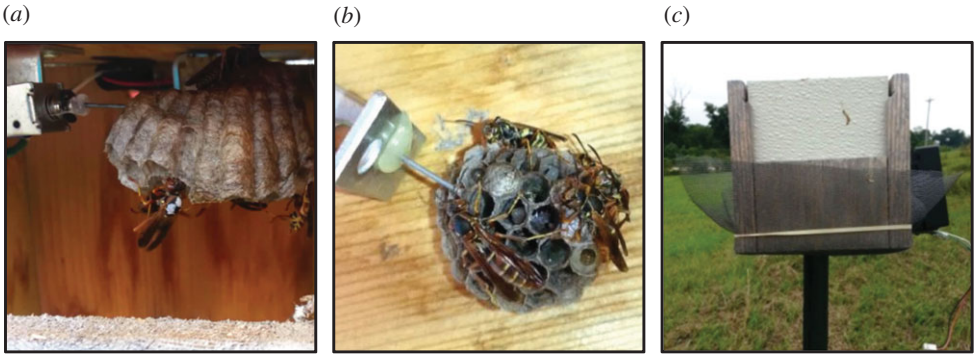

Figure 1. Nest treatment categories. (*a*) Drumming: antennal drumming was simulated through a battery-operated microcontroller attached to a solenoid device. The solenoid delivered 17 Hz pulse-width modulated square-wave vibration through a 1.5 cm, 16 or 18 G wire, glued between the push-pin of the solenoid and the edge of the nest. (*b*) No drumming: a 1.5 cm wire was glued between the edge of the nest and an L-bracket. (*c*) Restricted foraging: screens were attached to the base of the nest-box before sunrise and removed between 12.00 and 14.00. (Online version in colour.)

nest maps were used to track the stage of offspring in each cell throughout development, and to monitor nest growth and larval appearance and disappearance.

After workers began emerging, colonies were assigned to experimental treatments, as follows. Two treatments in 2013: drumming or no drumming (unmanipulated WR control). Four treatments in 2014: drumming/restricted foraging, drumming/unrestricted foraging, no drumming/restricted foraging or no drumming/unrestricted foraging (unmanipulated WR control). To control for variation in colony size, colonies were sorted from largest to smallest (ranked first by number of adults, then number of larvae) and put into groups of two (2013) or four (2014), such that the largest colonies were grouped together, the next largest colonies were grouped together, etc. Colonies within each size group were then randomly assigned to one of the two or four treatments. We collected WR samples from nine colonies in 2013 (four drumming, five no drumming) and 19 colonies in 2014 (four drumming/restricted, three drumming/unrestricted, five no drumming/unrestricted, seven no drumming/restricted; electronic supplementary material, S1).

(b) Artificial drumming

We modified hardware used to simulate antennal drumming in laboratory colonies [21] to operate on nests in the field. Each nest in the 'drumming' treatment had a battery-operated microcontroller (Sparkfun Logomatic v. 2 Serial SD Datalogger, Sparkfun Electronics) programmed to run a solenoid (Sparkfun 5v small) that drummed the nest at 17 Hz with a pulse-width-modulated square wave for 2 s, repeated every 10 s for the first 10 min of each hour during daylight hours (figure 1*a*). The width of the pulse was set to induce nest vibration equal in amplitude to that caused by natural antennal drumming [21]. The solenoid was attached to the outer edge of the paper nest by a 1.5 cm wire (16 or 18 G). Throughout the season, we used a USB Digital Multimeter (Sparkfun TOL-12967) to verify that the signal sent to the solenoid was the correct frequency and amplitude. Devices were activated on 19 July 2013 and on 18 July 2014. We verified solenoid function in 2013 on 13 August and in 2014 on 23 July and 24 August; in 2014, we excluded one colony with a non-functioning solenoid.

Only one control group—wasps subjected to no drumming—was used for this experiment. Although a second drumming control (with randomized timing or pulses) would have been desirable, samples sizes were limiting, and we decided not to include the second control because previous research had already established that individuals reared with 17 Hz drumming signal had significantly lower lipid stores (a worker-like phenotype; table 1) than those reared with random drumming [21]. These results suggested that the 17 Hz signal, and not the experience of an external vibrational stressor on the nest, affect caste development in *Polistes*. To control for the disturbance of having a device attached to the nest, nests assigned to the 'no drumming' treatment had a 1.5 cm wire (16 or 18 G) attached between the outer edge of the paper nest and an L-bracket, but no solenoid (figure 1*b*).

(c) Restricted foraging

Beginning on 12 August 2014, we wrapped the base of each nest-box assigned to the restricted foraging treatment with a fine-mesh screen (figure 1*c*) before wasp foraging began (before sunrise). The mesh screen was removed at approximately 12.00–14.00, and wasps were allowed to forage freely for the remainder of the day. This reduced the amount of time available for foraging by 5–7 h per day. Nests were restricted up to 5 days per week. Nests were not restricted on days we mapped nests or days that it rained, and restriction started later in the season than drumming treatments due to logistical constraints.

(d) Sample collection and preparation

Samples for gene expression and lipid analysis were collected at the pre-pupal stage of development (fifth instar larvae within the first 1–2 days of spinning the cocoon, before initiating metamorphosis). FR pre-pupae, defined as pre-pupae collected before workers were present on the nest, were collected from 21 June to 8 July 2013 and 28 June to 6 July 2014. WR pre-pupae were included in analyses only if individuals were in their third larval instar or earlier at the start of the artificial drumming or restricted foraging treatment [21]. WR pre-pupae were collected one to three times per week (7 August–12 September 2013 and 8 August–13 September 2014). At collection, half of the pre-pupae (2013) or a single pre-pupa (2014) were collected from the nest; the remaining pupae had their silken caps marked with a paint pen and were allowed to develop to adulthood. (Adult behaviour was subsequently observed and analysed, but sample sizes were too small to draw strong conclusions; see the electronic supplementary material, S2, for details on methods and results of adult behaviour.) Pre-pupal samples were kept on dry ice after collection and stored at −80°C. To preserve RNA, frozen pre-pupal heads were removed and kept on dry ice until processing for gene expression analyses. Frozen pre-pupal bodies were weighed, then sexed under a dissecting microscope to exclude males [27].

(e) Gene expression analyses

We quantified gene expression using real-time quantitative reverse transcriptase–polymerase chain reaction (qRT–PCR) with Sybr Green detection. Caste-related candidate genes were chosen based on previous gene expression data from several *Polistes* species [14,18,28–31]. Two genes, *heat-shock protein 90a* and *inositol oxygenase*, were of interest because they are hypothesized to have roles in diapause and mitochondrial energy production, respectively [29,31], and in previous studies [28,30] they both showed significant upregulation in WR fifth-instar larvae (i.e. gyne-biased gene expression). *Hexamerin 70b* codes for a storage protein, significantly upregulated in WR larvae and was chosen because it is hypothesized to be regulated via nutrition and be a biomarker of a diapause phenotype [14]. The fourth gene, *rhodopsin*, was of interest because it showed significant upregulation in WR larvae [28,30], but also in larvae that received lower nourishment [18]. For each sample, we also analysed the expression of two internal control genes, *elongation factor-1α* (*ef-1α*) and *ribosomal protein-49* (*rp-49*), and one external control gene, *mCherry*. We used both internal control genes, *ef-1α* and *rp-49*, to normalize expression levels of each candidate gene (see the electronic supplementary material, S3, for details on qRT–PCR methods, including replication, samples selected for assay, primer design and primer sequences, as well as raw data of internal control genes across treatments).

(f) Lipid quantification

We quantified lipids of individual pre-pupae by a colorimetric assay with a sulphophosphovanillin reagent, a method that has previously provided consistent and sensitive results for *Polistes* [11,30] (see the electronic supplementary material, S4, for additional details of this method). Absolute quantities of lipid content were divided by body weight to achieve the proportion of body weight made up of lipids. All proportion data were arcsine square root transformed ($y = \arcsin\sqrt{x}$ [32]) before they were included in the analysis, but figures depict non-transformed data.

(g) Data analysis

(i) Colony growth

To determine whether artificial drumming, restricted foraging or the combination caused unintended (in the case of drumming) or intended (in the case of restricted foraging) stress on colonies, we used the appearance of new larvae or disappearance of existing larvae as a metric of colony growth. We calculated colony growth as change in larval number on the nest between one observation period and the next. To do this, we summed the larvae in the nest each week, and subtracted the previous week from the current week (beginning the week before artificial drumming began), while accounting for larvae that had undergone pupation or experimental removal. A positive change in larval number means new larvae were produced; a negative change represents larval removal (due to natural enemies, ejection from the nest or cannibalism). Drumming and restricted foraging were both manipulated in 2014; therefore, we focus colony growth analyses on 2014 data only. However, as we began foraging restriction after drumming started, early weeks for all colonies were technically 'unrestricted', so we could not include all data into a single analysis to test for additive treatment effects. Instead, we analysed data across weeks for each treatment group separately to determine whether changes occurred after drumming or restricted foraging began. We used mixed-model ANOVAs with time (week during the season, between 15 July and 2 September 2014) as a fixed factor and colony as a random factor. We treated each week as a discrete variable, and used Tukey's HSD post hoc tests to examine pairwise comparisons when 'time' was a significant factor ($p < 0.05$). All focal colonies were used to examine colony growth, except for one for which we had incomplete data.

(ii) Gene expression and lipid stores

To avoid pseudo-replication, data from pre-pupal samples were averaged for each colony, and colony was used as the 'sample'. Data were analysed using generalized linear models. Gene expression data were from the lognormal distribution (Kolmogorov's D: $p > 0.10$), so we fitted those models using the log-link function for μ. Weight, total lipid and proportion of lipid (arcsine transformed) were normally distributed (Shapiro–Wilk goodness-of-fit test: $p > 0.05$), so we fit those models using the Identity link function for μ. To verify our caste-biased metrics, we compared FR with 'unrestricted/no drumming' (unmanipulated WR control) groups. We did not include colony as a random factor in these analyses as only three of the 10 colonies from which we collected both FR and WR larvae were sorted into the unmanipulated control group (electronic supplementary material, S1). To test for main effects and interactions of our artificial drumming and restricted foraging manipulations, we used full factorial models. In all models, year was included as a factor.

All analyses were run with JMP v. 12. All descriptive statistics from this study are available in the electronic supplementary material, S5, and raw data are available from Dryad Digital Repository (doi:10.5061/dryad.m82qm).

3. Results

(a) Caste phenotype biomarkers

Three of four caste-related candidate genes exhibited the expected (table 1) lower expression in *P. fuscatus* FR pre-pupae compared with unmanipulated WR pre-pupae (*heat-shock protein 90a*: FR versus WR: $\chi^2_1 = 4.63$, $p = 0.031$, year: $\chi^2_1 = 0.60$, $p = 0.44$; *hexamerin 70b*: FR versus WR: $\chi^2_1 = 5.43$, $p = 0.02$, year: $\chi^2_1 = 2.52$, $p = 0.11$; *inositol oxygenase*: FR versus WR: $\chi^2_1 = 4.16$, $p = 0.04$, year: $\chi^2_1 = 0.01$, $p = 0.91$; figure 2*a–c*). Expression of *rhodopsin* was not significantly different (FR versus WR: $\chi^2_1 = 0.34$, $p = 0.56$, year: $\chi^2_1 = 1.34$, $p = 0.25$; figure 2*d*).

Also as predicted (table 1), FR pre-pupae had significantly lower lipid levels, both in terms of total lipid and proportion of lipid per body size, than unmanipulated WR pre-pupae (total lipid: $\chi^2_1 = 5.06$, $p = 0.025$, year: $\chi^2_1 = 0.24$, $p = 0.62$; proportion of lipid: $\chi^2_1 = 3.79$, $p = 0.05$, year: $\chi^2_1 = 0.29$, $p = 0.59$; figure 2*e,f*). FR pre-pupae did not differ from WR pre-pupae in terms of weight ($\chi^2_1 = 0.94$, $p = 0.33$, year: $\chi^2_1 = 0.41$, $p = 0.52$).

(b) Treatment effects on colony growth

As expected, all colonies regardless of treatment showed trends towards peak colony growth in the mid-season, and growth slowed and/or larvae began disappearing at the end of the season (figure 3), known as the 'decline phase' [33,34]. The weekly trends indicate the foraging restriction treatment (black bar) coincided with a significant decrease in colony growth, to levels at or below zero growth (restricted foraging/no drumming treatment: $F_{7,41.1} = 5.92$, $p < 0.0001$; restricted foraging/drumming treatment: $F_{7,21} = 5.64$, $p < 0.001$; figure 3). Colony decline was not significant for the unrestricted

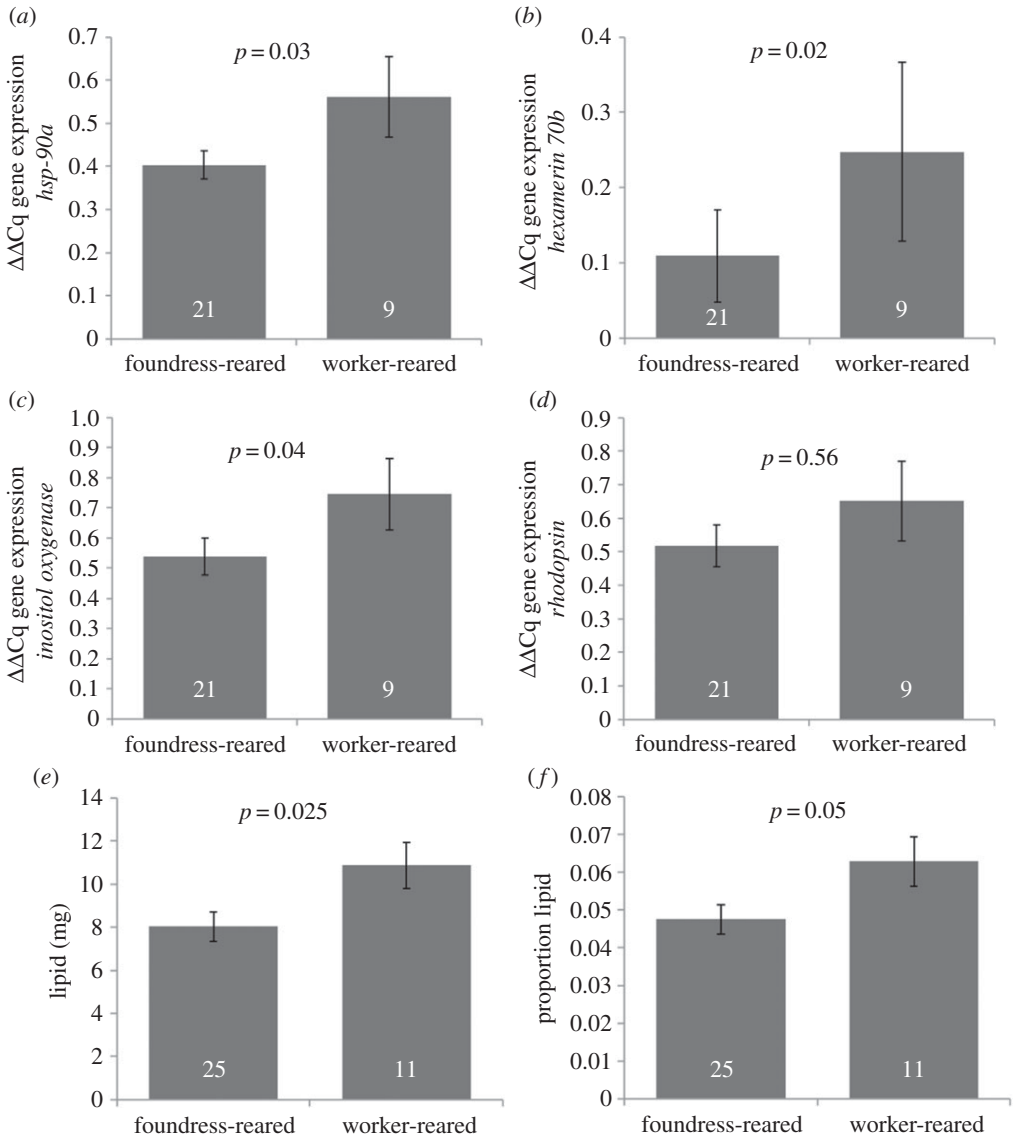

Figure 2. Normalized gene expression and lipid content among unmanipulated (no drumming/unrestricted foraging) FR and WR pre-pupae. Expression levels of (*a*) *heat-shock protein 90a*, (*b*) *hexamerin 70b* and (*c*) *inositol oxygenase* were significantly higher among WR compared with FR individuals. (*d*) Expression of *rhodopsin* was not significantly different between groups. (*e*) Total lipid and (*f*) proportion lipid (total lipid divided by body mass) were significantly higher among WR compared with FR individuals. Results from generalized linear models, where 'year' was included as a factor. Numbers inside bars represent sample sizes in number of colonies.

treatment group ($F_{7,28}$= 1.85, $p = 0.12$) nor in response to drumming alone (unrestricted foraging/drumming treatment: $F_{7,14}$= 1.86, $p = 0.15$).

(c) Treatment effects on gene expression

Artificial drumming was associated with significantly lower expression of *heat-shock protein 90a* (drumming: $\chi^2_1 = 3.88$, $p = 0.049$, restricted foraging: $\chi^2_1 = 0.26$, $p = 0.62$, drumming × restricted foraging: $\chi^2_1 = 0.13$, $p = 0.71$, year: $\chi^2_1 = 0.87$, $p = 0.35$; figure 4*a*) and *rhodopsin* (drumming: $\chi^2_1 = 5.16$, $p = 0.023$, restricted foraging: $\chi^2_1 = 0.55$, $p = 0.46$, drumming × restricted foraging: $\chi^2_1 = 1.62$, $p = 0.20$, year: $\chi^2_1 = 1.19$, $p = 0.27$). Restricted foraging was associated with lower expression of *hexamerin 70b* (drumming: $\chi^2_1 = 0.71$, $p = 0.40$, restricted foraging: $\chi^2_1 = 3.94$, $p = 0.047$, drumming × restricted foraging: $\chi^2_1 = 0.02$, $p = 0.88$, year: $\chi^2_1 = 4.35$, $p = 0.037$; figure 4*b*). Finally, the interaction of artificial drumming × restricted foraging was associated with significantly reduced expression of *inositol oxygenase* (drumming: $\chi^2_1 = 3.08$, $p = 0.08$, restricted foraging: $\chi^2_1 = 6.49$, $p = 0.001$, drumming × restricted foraging: $\chi^2_1 = 7.56$, $p = 0.006$, year: $\chi^2_1 = 2.89$, $p = 0.09$; figure 4*c*).

(d) Treatment effects on lipid stores

WR pre-pupae in the drumming treatment had lower mean total lipid, proportion lipid and weight than those reared without drumming (see the electronic supplementary material, S5, for descriptive statistics), but these differences were not statistically significant (total lipid: drumming: $\chi^2_1 = 1.57$, $p = 0.21$, restricted foraging: $\chi^2_1 = 0.86$, $p = 0.35$, drumming × restricted foraging: $\chi^2_1 = 0.15$, $p = 0.70$, year: $\chi^2_1 = 9.04$, $p = 0.003$; proportion lipid: drumming: $\chi^2_1 = 0.65$, $p = 0.42$, restricted foraging: $\chi^2_1 = 0.25$, $p = 0.61$, drumming × restricted foraging: $\chi^2_1 = 0.10$, $p = 0.75$, year: $\chi^2_1 = 26.99$, $p < 0.001$; weight: drumming: $\chi^2_1 = 2.06$, $p = 0.15$, restricted foraging: $\chi^2_1 = 0.31$, $p = 0.58$, drumming × restricted foraging: $\chi^2_1 = 0.50$, $p = 0.48$; year: $\chi^2_1 = 1.38$, $p = 0.24$).

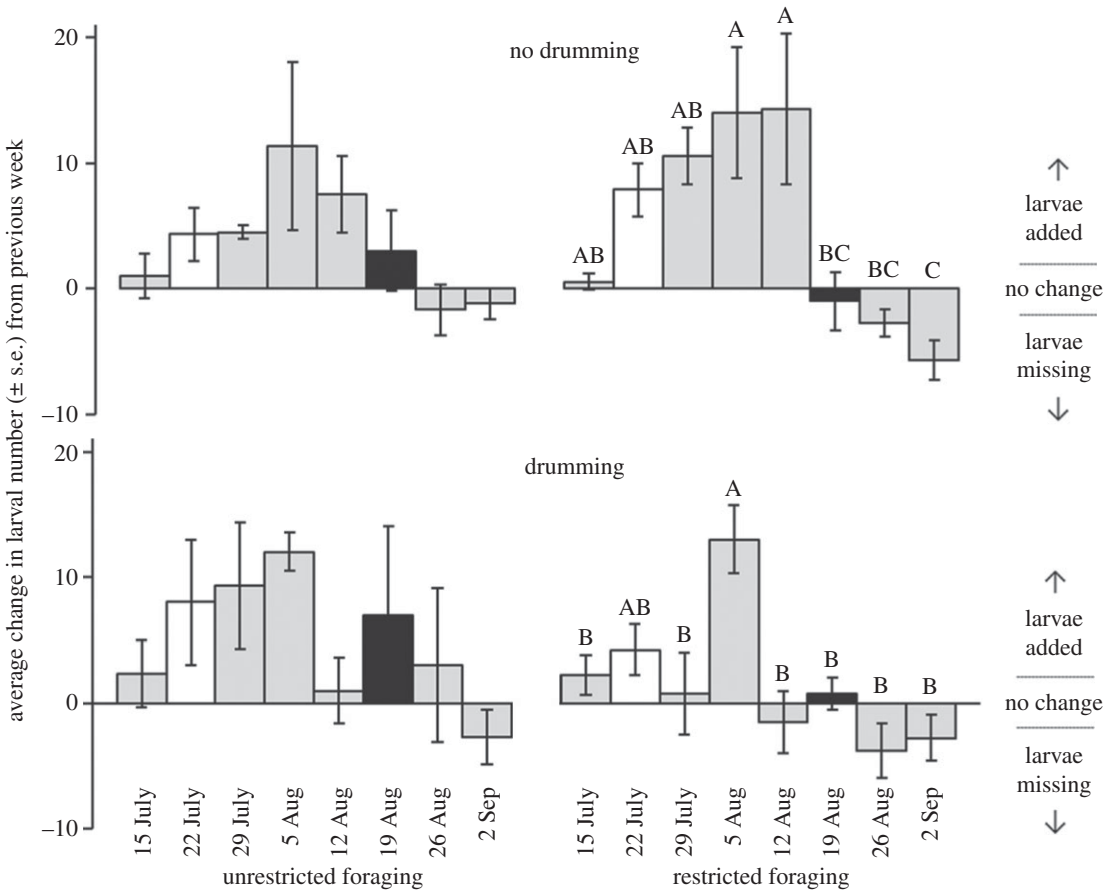

Figure 3. Colony development (change in larvae on the nest over time) in 2014. Treatments were analysed separately. Bars represent average change in the number of larvae (±s.e.) each week. White bars represent first set of data collected after artificial drumming began; black bars denote first set of data collected after foraging was restricted. Artificial drumming and/or foraging restriction treatments continued until all larvae had pupated or disappeared. Values above zero represent an increase in larvae added to the nest; values below zero represent larval disappearance. When time was a significant predictor of colony growth, we include capital letters above the bars to denote pairwise differences (Tukey's HSD post hoc analysis, $p < 0.05$).

4. Discussion

In this study, we provide novel information on the mechanisms by which maternal behaviour and nourishment influence offspring developmental fate. In the context of caste phenotypic plasticity in the social wasp *P. fuscatus*, in which prolonged maternal care is provided to developing larvae, we demonstrate that both maternal vibrational cues and nutritional cues can influence offspring gene expression, setting up the potential for differential caste phenotype as adults. In addition, our data suggest that different inputs (vibrational and nutritional) influence development in *Polistes* via different molecular pathways, but these factors also show significant interactions. These results provide evidence that maternal behaviour has the potential to interact synergistically with nourishment factors, leading to novel transcriptional responses in offspring that are not the result of maternal factors or nourishment alone.

We also identified consistent transcriptional biomarkers of caste-biased development in *Polistes* wasps, a model genus for social evolution. We confirmed that three of four candidate genes, *heat-shock protein 90a* (*hsp90a*), *hexamerin 70b* and *inositol oxygenase*, were more highly expressed in WR (gyne-destined) *P. fuscatus* pre-pupae than in FR (worker-destined) pre-pupae. We also verified that FR *P. fuscatus* females have lower lipid stores than WR individuals. Our data agree with previously published transcriptomic and physiological data on four species of *Polistes*, suggesting mechanisms associated with caste development are conserved to some degree across *Polistes* species [11,14,28–31,35]. Specifically, high lipid stores and high expression of some genes related to metabolism and diapause appear to be conserved gyne-related traits across *Polistes*, and thus robust caste-bias phenotype biomarkers for this study and future studies of caste development in paper wasps.

The main finding of this study is that the dual inputs of vibrational signals and food provisions affect caste-related traits in *Polistes*. Using an experimental paradigm in which we simulated the maternal influences of antennal drumming and reduced nutrition by restricting foraging, we were able to examine each factor alone, and their interaction, on caste-related traits. Exposure to artificial drumming significantly decreased gyne-biased gene expression of *hsp90a* in WR individuals to levels similar to those of FR individuals. In *Nasonia* wasps, an increase in expression of *hsp90a* corresponds to early stages of diapause [36]. If we consider expression of *hsp90a* as an indicator of a diapause phenotype [29], our results suggest antennal drumming could inhibit diapause-associated gene expression. Antennal drumming was also previously observed to be associated with reduced offspring lipid stores [21], and lipid stores are another key diapause-related trait [14]. Inhibition of diapause has been proposed to be an evolutionary mechanism for the origin of the worker caste in wasps [14], thus our results suggest that maternal vibrational signals, diapause and caste evolution could be evolutionarily linked.

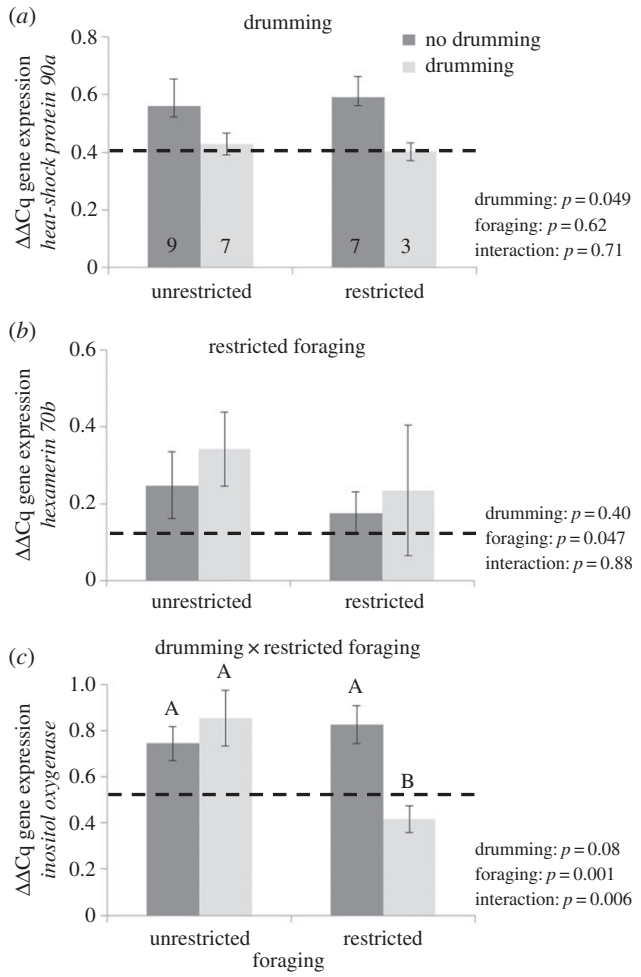

Figure 4. Differences in normalized expression levels of gyne-biased genes among WR pre-pupae that developed with or without artificial drumming and under unrestricted or restricted colony foraging. (*a*) Expression of *heat-shock protein 90a* was significantly reduced when individuals experienced artificial drumming. (*b*) Expression of *hexamerin 70b* was significantly reduced when individuals were reared in an environment with restricted colony foraging. (*c*) Expression of *inositol oxygenase* was significantly reduced when individuals experienced both artificial drumming and restricted colony foraging. Capital letters above bars in (*c*) denote pairwise differences (Tukey's HSD post hoc analysis, $p < 0.05$). Dotted lines represent average values calculated for FR pre-pupae (figure 2). Numbers inside the bars in (*a*) represent sample size (number of colonies) for all analyses.

Hexameric storage proteins have also been associated with insect diapause [14,36,37], and are related to caste membership in the eusocial wasp genera *Polistes* and *Vespula* [29,31,38], and female size in the solitary wasp *Monobia quadridens* [39]. Restricted colony foraging led to a significant decrease in the expression of this gyne-biased gene. Because larvae cannot forage and feed themselves, the accumulation of macronutrients such as lipids or storage proteins is a direct result of feeding by adults. Although the foraging restriction treatment did not affect lipid stores, there was evidence that restriction treatment did impose a resource limitation. Colonies with restricted foraging exhibited decreased colony growth resulting from larval disappearance, whereas the drumming treatment did not cause observable stress to colony growth (figure 3). As colonies decline late in the season, fewer larvae are reared [34], and larval disappearance is a symptom of the documented *Polistes* starvation-response mechanism of larval cannibalism [40]. Reduced expression of *hexamerin 70b* in individuals from restricted colonies may thus also reflect nutritional stress during development. Oxidoreductase genes, which are involved in mitochondrial energy production, tend to be upregulated among *Polistes* WR larvae [29] and reproductive adults [31], suggesting that they may be important pathways for caste-biased development. We show that the expression of *inositol oxygenase* (an oxidoreductase) is responsive to the interaction of both maternal (artificial drumming) and nutritional (restricted foraging) factors.

Our data support a dual-input model of caste development [20], showing that vibrational signals and nourishment level experienced by developing larvae affect molecular pathways related to diapause and metabolism, processes that have been implicated in the evolution from solitary to eusocial behaviour [14,28]. We further suggest antennal drumming could have evolved as a mechanism for mothers to 'create workers' by inhibiting the first brood from entering a state of diapause during adulthood. In the 'diapause ground plan' scenario envisaged by Hunt *et al*. [14], poorly nourished, non-diapausing adults may be more likely to remain at the nest. There, they may stay to feed on nutritious, amino-acid-rich saliva produced by larvae [41], but may also engage in 'worker-like' allo-maternal care of brood. Later in the season, an increase in nourishment associated with being fed by a team of 'workers' (and absence of drumming) would lead to the upregulation of metabolic and diapause-related pathways. These social and nutritional inputs could produce 'gyne-like' individuals that were physiologically prepared to diapause and subsequently reproduce. More data are needed to test this hypothetical scenario of maternally driven eusocial evolution in wasps, including studies in solitary and highly eusocial wasps.

Substrate-borne vibration and tactile communication is common in insects [42], and may be an underappreciated mechanism regulating social organization in insect colonies [43,44]. For example, in queenright *Harpegnathos saltator* ant colonies, workers repeatedly bite larvae developing into queens, which results in those larvae changing their developmental trajectories and emerging as workers [45]. Termite soldiers use substrate-borne vibrations to communicate alarm or threat to conspecifics [46], and *Myrmica* ant pupae stridulate to communicate to workers in the nest their late developmental stage and social status in the colony [47]. Rapid antennation is also observed among adults engaged in non-lethal intra- or interspecific aggressive contests (e.g. *Polistes* co-foundresses [48–50], workers in queenless ant colonies [51,52], *Odontomachus* guards [53]). Our data provide a new dimension to the role of vibrational communication in the regulation of social phenotypes in insect societies, which now extends to influencing developmental transcriptional responses associated with adult caste fate.

Although we observed transcriptional responses of wasp offspring to our maternal and nourishment restriction treatments, we did not detect significant effects on lipid stores. Suryanarayanan *et al*. [21] showed that simulated drumming on gyne-stage laboratory nests produced worker-like adult wasps with significantly lower fat stores. Although drumming did not lead to significantly lower lipids in this study, it was associated with a trend towards lower lipid stores compared with those reared without drumming. The lack of significance in this study may be attributable to an observed large variation in baseline lipid stores in WR

individuals across years. The potential sources of this variation may be due to inconsistent environmental factors from year to year (e.g. weather, vegetation, pesticide usage) that could have affected prey availability for developing larvae. Although such environmental factors are a major challenge of working in the field, another recent study demonstrated perturbed caste-related gene expression when rearing *Polistes* in the laboratory [30]. Thus, while our field-based approach may have contributed to high lipid-level variation, it nonetheless allowed for the collection of highly informative data on caste-related gene expression patterns.

In sum, the data from our three caste-related genes provide evidence that genes can be: (i) socially responsive, changing only in response to substrate-borne vibrational maternal interactions, (ii) responsive to nourishment factors, or (iii) unique, with a transcriptional response that is the result of both maternal and nourishment factors combined. To our knowledge, this is the first demonstration of an offspring transcriptional response that is the result of interactions between maternal behaviour and nourishment during development. Our work provides support for the idea that direct physical interaction with offspring during early development, including mechanical or vibrational signals, can influence offspring developmental fate and adult phenotype. The importance of physical mother–offspring interactions may be a common theme in maternal effects in species with prolonged maternal care [3]. In addition, we suggest that mother–offspring physical interaction is an underappreciated mechanism affecting caste development and its evolution in social insects. This study underlines the importance of maternal interactions and feeding during larval development in caste development in a eusocial insect, and highlights the potent role of maternal influences during development in shaping the developmental fate of offspring through changes in gene expression during development.

Ethics. Wasps in this study were collected and euthanized humanely, according to standard collection protocols.

Data accessibility. Information on wasps collected is given in the electronic supplementary material. Raw data files related to colony size, lipid stores and gene expression are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.m82qm [54].

Authors' contributions. J.M.J. performed study conception, field and laboratory work, data analysis and writing; S.S. performed experimental design, advice and input on writing; R.L.J. performed study conception and experimental design, advice and input on writing; J.C.H. performed design and building of piezo devices, assistance with fieldwork and data analysis, and writing; A.L.T. performed study conception and experimental design, assistance with fieldwork and writing.

Competing interests. We declare we have no competing interests.

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