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Females adjust maternal hormone concentration in eggs according to male condition in a burying beetle

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ABSTRACT

In birds and other vertebrates, there is good evidence that females adjust the allocation of hormones in their eggs in response to prenatal environmental conditions, such as food availability or male phenotype, with profound consequences for life history traits of offspring. In insects, there is also evidence that females deposit juvenile hormones (JH) and ecdysteroids (ESH) in their eggs, hormones that play a key role in regulating offspring growth and metamorphosis. However, it is unclear whether females adjust their hormonal deposition in eggs in response to prenatal environmental conditions. Here we address this gap by conducting an experiment on the burying beetle *Nicrophorus vespilloides*, in which we manipulated the presence of the male parent and the size of the carcass used for breeding at the time of laying. We also tested for effects of the condition (i.e., body mass) of the parents. We then recorded subsequent effects on JH and ESH concentrations in the eggs. We found no evidence for an effect of these prenatal environmental conditions (male presence and carcass size) on hormonal concentration in the eggs. However, we found that females reduced their deposition of JH when mated with heavier males. This finding is consistent with negative differential allocation of maternal hormones in response to variation in the body mass of the male parent. We encourage further work to investigate the role of maternally derived hormones in insect eggs.

1. Introduction

In many animals, including birds, fishes and insects, females deposit hormones, such as testosterone (T), corticosterone, thyroid hormones, juvenile hormones (JH), and ecdysteroids (ESH) into their eggs (De Loof et al., 2013; Gharib and de Reggi, 1983; Groothuis et al., 2005; Power et al., 2001; von Engelhardt and Groothuis, 2011). Maternal hormones play an important role in shaping the offspring's subsequent development, growth, survival and behaviour (Groothuis et al., 2005, 2019; Groothuis and Schwabl, 2007; Power et al., 2001; Schwander et al., 2008; von Engelhardt and Groothuis, 2011). Studies on several bird species and one fish species show that females adjust the deposition of such hormones in response to environmental cues available to females at the time of egg laying (Gasparini et al., 2007; Giesing et al., 2010; Gil et al., 1999). Studies on birds show that females adjust hormone deposition in response to cues that predict variation in the amount of food offspring are likely to receive after hatching, such as the quality of the male partner in species with biparental care (Gil et al., 1999) and the number of care-givers in cooperatively breeding species (Paquet et al., 2013). Such adjustments are often thought to be adaptive, providing females with a mechanism for altering the offspring's phenotype to match the environmental conditions offspring are likely to encounter after hatching (Groothuis et al., 2019; Meylan et al., 2012). In birds, maternal hormones affect the offspring's begging behaviour, which in turn influences offspring growth and development via the effect of offspring begging on the amount of food provisioned by male and female parents (Paquet and Smiseth, 2015; Smiseth et al., 2011). Thus, prior work on birds suggests that female adjustment of maternal hormone levels in eggs is associated with offspring begging and biparental food provisioning. These conditions are not unique to birds, as offspring begging and biparental provisioning of food for offspring also occurs in some insects, such as burying beetles of the genus *Nicrophorus* (Eggert and Müller, 1997; Scott, 1998). Thus, to determine whether female adjustment of maternal hormone levels in eggs is associated with biparental food provisioning and offspring begging, we need to extend the study of female adjustment of maternal hormones to relevant non-avian taxa, such as burying beetles.

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Although there is evidence that female insects deposit hormones in their eggs (Schwander et al., 2008; De Loof et al., 2013; Gharib and de Reggi, 1983), it is currently unclear whether females *adjust* the deposition of maternal hormones in response to prenatal environmental cues (see below). Insect hormones are different from those in birds and other vertebrates, suggesting that female deposition of maternal hormones into eggs has independent evolutionary origins in these taxa. In insects, the main hormones deposited in eggs are JH and ESH, which are jointly involved in the regulation of numerous aspects of insect phenotype, such as metamorphosis and offspring growth and development (Nijhout, 1998). There are many functional similarities between JH and T, including evidence that female hormone levels vary in response to social environments (reviewed in Tibbetts et al., 2019; Tibbetts and Crocker, 2014). Furthermore, hormone levels in offspring affect their growth and begging behaviour in a species where both parents can provision their offspring with food after hatching (Crook et al., 2008). Prior work on insects provide evidence for an association between ESH levels in eggs and environmental conditions during development, such as population density in desert locusts (*Schistocerca gregaria*) (Hägele et al., 2004) and day length in migratory locusts (*Locusta migratoria*) (Tawfik et al., 2002). However, it is unclear whether these associations are caused by *adjustment* of female allocation of maternal hormones in response to environmental conditions as opposed to differential mortality of eggs with different ESH levels under different environmental conditions, or irreversible changes in ESH levels due to exposure of different environmental conditions during development. For example, Hägele et al. (2004) found marked differences in ESH levels of eggs produced by female migratory locusts that had been raised in a crowded or a solitary environment over several generations. However, there were no differences in ESH levels of eggs produced by solitary females and solitary females temporarily maintained in a crowded environment at the time of egg laying, suggesting that females did not adjust the allocation of ESH to the environmental conditions they were exposed to during egg laying. A recent study on house crickets (*Acheta domesticus*) found that the interaction between maternal and grand-maternal diets influenced the amount of ESH in eggs, suggesting that females adjust their deposition of ESH in eggs based on prenatal environmental cues (Crocker and Hunter, 2018). This study investigated effects on the content rather than concentrations of maternal hormones in eggs. Given that female insects often adjust the size of their eggs in response to prenatal conditions (Fox et al., 1997), it is therefore unclear whether the greater amount of ESH in eggs reflects that eggs had a higher concentration of ESH or whether larger eggs simply contain a greater amount of ESH. Finally, given that these insect species do not show parental food provisioning or offspring begging, it is unclear how these studies relate to our understanding based on prior work on maternal hormone deposition in birds. Thus, there is now a need for studies that investigate whether females adjust the deposition of maternal hormones in eggs in insects. Such studies should focus specifically on species with extensive post-hatching parental care involving food provisioning by both parents and offspring begging, and should control for potential confounding effects due to egg size.

Here we investigate whether females adjust hormone deposition in their eggs in response to prenatal environmental conditions in the burying beetle *Nicrophorus vespilloides*. This species is well suited to investigate this hypothesis as it exhibits offspring begging and biparental food provisioning after hatching (Eggert and Müller, 1997; Scott, 1998). Females only mature their oocytes once they encounter the carcass of a small vertebrate (Scott and Traniello, 1987), and females start laying eggs 3–28 h after encountering a carcass (Ford and Smiseth, 2017). Given that egg production starts after females encounter a carcass, females might adjust the deposition of hormones into their eggs based on various prenatal environmental cues that may predict the amount of food available to offspring after hatching. Firstly, the presence or absence of a male partner at the carcass at the start of egg laying provides females with a cue for the likelihood that the male will assist in food provisioning after hatching (Paquet and Smiseth, 2017). Females will store sperm from prior matings, allowing them to breed on their own if no male is present (Eggert, 1992). There is evidence that females adjust offspring mass at hatching (Paquet and Smiseth, 2017), but not egg size (Ford, 2019) or clutch size (Ford, 2019; Paquet and Smiseth, 2017), in response to the presence of the male during egg laying. Secondly, the size of the vertebrate carcass used for breeding determines the total amount of resources that will be available for the developing larvae. The size of the carcass used for breeding varies and there is evidence that females lay more but smaller eggs when breeding on large carcasses (Botterill-James et al., 2017). It is currently unknown whether females adjust the deposition of maternal hormones in response to the presence of the male and/or the size of the carcass. Here, we used a 2 × 2 factorial design where we manipulated the presence or absence of the male parent and the size of the carcass (small versus large) at the time of egg laying. We then measured subsequent effects on the concentration of JH and ESH in the eggs. We predicted that females would deposit more JH (and possibly more ESH) in their eggs when breeding on large carcasses and in the presence of the male. Prior work shows that JH stimulates larval begging in our study species (Crook et al., 2008), and that male parents respond to increased larval begging by provisioning more food (Smiseth and Moore, 2004). There is also evidence that both the presence of the male and access to a larger carcass have positive effects on larval growth (Paquet and Smiseth, 2017; Sieber et al., 2017). Prior work on birds suggest that females also may adjust the deposition of hormones in eggs depending on their condition (Pilz et al., 2003; Sandell et al., 2007) or the condition of their partner (Sheldon, 2000). Therefore, we tested for effects of the prenatal body mass of both parents on female deposition of maternal hormones in eggs, using body mass as a proxy of their condition.

2. Methods

2.1. Study population and animal husbandry

In these experiments, we used virgin beetles that had been reared in the laboratory. The beetles derived from lines originally collected in the wild in Edinburgh, UK. Non-breeding beetles were housed individually in transparent, plastic containers (124 × 82 mm and 20 mm high) containing moist soil and were maintained at 21 ± 2 °C under a 16:8 h light:dark cycle. We fed nonbreeding beetles small pieces of raw, organic beef twice a week.

2.2. Experimental design and procedures

We used a 2 × 2 factorial design to investigate whether females adjust deposition of maternal hormones in their eggs in response to whether the male partner was present or absent at the time of egg laying and whether females were provided with a large or a small mouse carcass for breeding. We randomly selected pairs of non-sibling males and females for use in the experiments. We paired beetles at random to exclude any potential effect of assortative mating between males and females (Smiseth and Moore, 2004). At the beginning of the experiment, we weighed all males and females to record their pre-breeding body mass, using this as a proxy of their body condition. To ensure that females were able to lay fertilized eggs regardless of whether a male was present or absent at the time of egg laying, we placed all pairs in plastic containers (110 × 110 mm and 30 mm high) with approximately 10 mm deep moist soil for at least 24 h (range: 25.16–28.40 h) before moving females to a larger plastic container (170 × 120 and 60 mm high) filled with a 10–20 mm layer of soil and provided with a previously frozen mouse carcass (supplied by Livefoods Direct Ltd., Sheffield, UK) to initiate breeding (Paquet and Smiseth, 2017; Steiger, 2013). We assigned all females at random to the different treatment groups. We moved both parents to the new container for those females that were assigned to the treatments where the male was

present, while we moved the female only for those females that were assigned to the treatments where the male was absent. Females assigned to the treatments involving a small carcass were provided with a mouse carcass with a mean mass of 6.57 g (range 4.54–9.23 g), and females assigned to the treatments involving a large carcass were provided with a mouse carcass with a mean mass of 23.24 g (range 19.00–27.34 g).

To record the time of the initiation of egg laying, we placed the boxes on flat-bed scanners (Canon Canoscan 9000F Mark II, Canon Inc., Tokyo, Japan) (Ford and Smiseth, 2016). We scanned the breeding boxes every hour using Vuescan professional edition software (Hamrick Software, Sunny Isles Beach, FL) and recorded the time of appearance of the first laid eggs in the bottom of the box. We set up 134 experimental females across the experiment. We excluded 12 experimental females because they laid fewer than 5 eggs (7 from the treatment where the female only bred on a small carcass, 3 for the treatment where the female only bred alone on a large carcass, and 2 for the treatment where both parents bred on a large carcass). Thus, the final sample sizes for each treatment were as follows: both parents breeding on a small carcass (n = 30 clutches), both parents breeding on a large carcass (n = 28 clutches), female only breeding on a small carcass (n = 32), and female only breeding on a large carcass (n = 32). When possible, we collected 10 eggs within a day from initiation of laying to limit potential effects due to egg development (mean: 11.35 h since start of laying, range: 5.50–25.25 h). We collected 2 × 5 eggs (5 for each hormone analysis) that were gently collected with forceps, weighed in groups of five in an Eppendorf tube (in order to later calculate hormonal concentrations per gram of eggs) and kept frozen until further analyses. When there were fewer than 10 eggs for a given female (N = 16 clutches), we collected 5 eggs that were randomly assigned to the analysis for one of the two hormones.

2.3. Hormones assay

2.3.1. Juvenile hormone radio-immunoassay

We assigned five eggs from each clutch at random for the analyses of JH. The eggs were crushed in glass tubes with 500 μL of distilled water. We extracted JH by adding 3 mL of diethyl-ether to the tubes and by vortexing the mixture. The solvent and the aqueous phases were separated by centrifuging the tubes for 5 min at 2000 rpm (4 °C). The aqueous phase contained water, eggshells and proteins, while JH, which is a lipidic hormone, remains in the solvent. We then placed the tubes in a cold bath to freeze the water. The diethyl-ether phase containing the hormone was decanted and poured off in new glass tubes. This step was performed twice for each sample and the resultant was then evaporated at 37 °C. We dissolved the dried extracts in 400 μL of phosphate buffer and JH concentrations were assayed in duplicates. Specifically, 100 μL of extract or JHIII standard (Sigma Aldrich, US) were incubated overnight with 4000 cpm of the $_3$H-juvenile hormone III (Perkin Elmer, US) and polyclonal antiserum (provided by Prof. Walter Goodman, Wisconsin-Madison University). The bound fraction was then separated from the free fraction by addition of dextran-coated charcoal and activity was counted on a tri-carb 2810 TR scintillation counter (Perkin Elmer, US). Inter- and intra-assay variation in JH concentrations were 19.47% and 15.86%, respectively. Intra-assay measurements were highly repeatable (Pearson correlation coefficient = 0.82, 95%CI = 0.75–0.87). The JH lowest detectable concentration was 57.84 pg/100 μL of extract. Sample dilution displacement curves were parallel to the standard curve showing that the sample hormone is recognized in the same way as the JHIII standard.

2.3.2. ESH immuno-assay

We assigned the remaining five eggs from each clutch for the analyses of ESH. Given the lipidic nature of this hormone, specific solvents were used to extract it from the eggs. First, we crushed the eggs in glass tubes with 5 mL of methanol. The mixture was then sonicated for 30 min and incubated overnight at 42 °C. After agitation and centrifugation (10 min, 4000 rpm, RT), we filtered the methanol containing the hormone with a specific syringe-filter (membrane PTFE, 0.45 μm) in new glass tubes. This step was then done twice with 2 mL of methanol. The methanol was then evaporated at 50 °C under nitrogen. The dried extracts were dissolved in 250 μL of assay buffer (1 M phosphate with BSA, NaCl, EDTA). We then assayed the ESH in duplicates with a commercial Enzyme Immunoassay (SpiBio, Bertin Pharma, France) and a microplate reader (Berthold, France). This assay is more specific to 20-hydroxy-ecdysone and ecdysone, but the antibody cross-reacts with other ecdysteroids: 20-hydroxy-ecdysone 100%, ecdysone 100%, 2-deoxy-20-hydroxy-ecdysone 88%, polypodine B 70%, 2-deoxy-ecdysone 63%, ponasterone A 43%, Cyasterone 5%, podecdysone C 4.5%, makisterone A 4%, 26-hydroxy-ecdysone 1.4%, muristerone A 1.2%, kaladasterone 1%, 22-epi-ecdysone < 0.1%, posterone < 0.1%. Inter- and intra-assay variation in ESH concentrations were 16.16% and 12.70%, respectively. Intra-assay measurements were highly repeatable (Pearson correlation coefficient = 0.97, 95%CI = 0.96–0.98). ESH lowest detectable concentration was 31 pg/100 μL of extract. Samples dilution displacement curves were parallel to the standard curve showing that the sample hormone is recognized in the same way as the standard.

2.4. Statistical analyses

We conducted all statistical analyses in a Bayesian framework using JAGS, version 4.2.0, via the 'rjags' package (Plummer, 2013) in R version 3.3 (R Core Team, 2013). To investigate whether females adjust the deposition of JH and ESH in response to the presence or absence of the male and carcass size (large or small), we built linear mixed models with treatment as a four-level fixed effect. We did this to test for the main effects of carcass size and male presence, as well as for effects of the interaction between them. We also added the female's own weight, as well as the weight of the male partner as fixed effects (scaled) in all models. In addition, we included time from laying until egg collection as a fixed effect (scaled), hereafter termed 'time since the onset of laying'. This variable reflects the age of the first-laid eggs in a given clutch and we included this to control for potential confounding effects due to the age of the eggs caused by differences in times of egg laying between females (Ford, 2019). There was no significant correlation between male weight and the age of the first-laid egg in the clutch (Pearson product moment correlation: −0.02 [−0.20,0.15], p-value = .79) and between male weight and the time interval between mating and egg laying (Pearson product moment correlation: −0.06 [−0.24,0.12], p-value = .53). We included clutch ID as a random effect given that we obtained 2 measures per clutch per hormone (except for 4 clutches where only one measure of ESH could be taken). These two measures acted as two observations of the underlying hormonal concentration of the sample and the fixed effects were applied on these estimated concentrations of the samples. As male weight may be an indicator of his parental quality, we also initially investigated whether female adjustment of maternal hormone deposition in response to male weight is conditional upon his presence at egg laying. We did this by including an interaction between male weight and male presence or absence. Given that we found no evidence for such interaction effects (12.60 ng/g [−22.82–48.31], P(> 0) = 0.76 and −0.42 ng/g [−4.10–3.23], P(> 0) = 0.41 for JH and ESH respectively), we removed this interaction from the final models. There was no indication that egg mass varied in response to male presence, carcass size or their interaction (all credible intervals largely overlapped zero), and we therefore excluded information on egg mass from the final models. Additionally, we found no evidence for an effect of the interaction between male and female body mass on concentrations of JH and ESH (−0.44 ng/g [−21.99–20.98], P(> 0) = 0.47and −0.12 ng/g [−2.12–1.88], P(> 0) = 0.46 for JH and ESH, respectively).

We estimated parameters using vague priors (that is, prior

distributions allowing for a wide range of values, see https://doi.org/10.1016/j.yhbeh.2020.104708 script in supplementary material for more details). Posterior samples from three Markov Chain Monte Carlo (MCMC) chains were based on 3000 iterations after an adaptation period of 5000, burn-in of 5000 and thinning interval of 3 for each model. Model convergence was confirmed both visually and by using the 'R hat' Gelman–Rubin statistic (Gelman and Rubin, 1992). To assess the goodness of fit of our models, we performed post predictive checks using the χ^2 discrepancy metric (Gelman et al., 1996). We found no evidence for lack of fit (Bayesian *p* values: 0.492 and 0.498, values close to 0 or 1 would indicate lack of fit). We present the means [and 95% Credibility Intervals] from the posterior distributions of interest, as well as P(> 0) the proportion of the posterior distribution that was higher than zero (all posterior distributions are symmetrical). We interpret effects as 'statistically clear when 95% CI did not overlap zero and we report estimates for all parameters of interest regardless of their statistical clarity (Dushoff et al., 2019). We estimated effect sizes of continuous fixed effect variables by dividing their effect (for each posterior sample) by the standard deviation of the estimated true underlying hormone concentrations (mean and 95% Credibility Intervals of the estimated standard deviations were 89.68 ng/g [83.09–96.84] for JH and 9.05 ng/g [8.63–9.65] for ESH). To estimate the proportion of variation in the concentration of JH and ESH explained by our models, we computed R^2 following Gelman and Pardoe (2006). We note that negative values of R^2 are possible when the model has a poor ability to predict the response variable (Gelman and Pardoe, 2006).

3. Results

There was no evidence that females adjusted the concentrations of either JH or ESH in their eggs in response to the presence or absence of a male partner, the size of the carcass (small or large), or the interaction between them (Table 1, Fig. 1). However, females deposited less JH in eggs when they were mated with heavier males (effect size: −0.21 [−0.40 to −0.02], Table 1; Fig. 2). There were also some indication that heavier females deposited less JH in eggs, although this evidence was inconclusive as the 95% credibility intervals overlapped zero (effect size: −0.16 [−0.35 to 0.04], Table 1; Fig. 2). There was no evidence that females adjusted the concentration of ESH in the eggs in response to either their body mass or the body mass of their male partner (effect sizes: 0.01 [−0.19–0.21] and −0.03 [−0.24–0.16], respectively Table 1; Fig. 3). There were some indications that concentration of JH in eggs increased with time since the onset of laying, although this evidence was inconclusive as the 95% credibility intervals overlapped zero (effect size: 0.16 [−0.06–0.37], Table 1). There was no evidence that the concentration of ESH increased or decreased with time since the onset of laying (effect size: −0.11 [−0.32–0.11], Table 1). Our estimated R^2 values suggested that the fixed effects included in our models explained 6.2% of the variation in JH concentration in the eggs (R^2 = 0.062), while the fixed effects failed to explain any variation in ESH concentration (R^2 = −0.02).

4. Discussion

Here we found no evidence that females adjusted the concentration of maternal hormones in response to either the presence or absence of the male partner at the time of egg laying or the size of the carcass used for breeding in *N. vespilloides*. However, we found that females deposited less JH when they were mated with heavier males. We also found some weak indication that heavier females laid eggs with lower JH concentrations. Our study provides evidence for female adjustment of maternal hormone concentrations in eggs in an insect. Our results suggest that female adjustment of maternal hormones in response to environmental cues is not unique to birds but may be more generally associated with offspring begging and biparental provisioning of food for offspring after hatching. Below, we provide a more detailed discussion of the wider implications of our results for our understanding of female adjustment of maternal hormones in eggs.

We found that females deposited *more* JH when they were mated to lighter males. Given that lighter males are likely to be in poorer condition than heavier males, our results suggest that females compensate for the potential detrimental effects of poor male condition by depositing more JH in eggs. Thus, our study provides evidence of reproductive compensation or negative differential allocation in *N. vespilloides*; that is, a reduction in female allocation to reproduction in response to their male partner being in better condition (Groothuis et al., 2005; Haaland et al., 2017). We note that our results derive from an experimental design where we paired males and females at random. This aspect of our design is important because it allowed us to exclude any potential effects due to assortative mating, such as females depositing more hormones mating assortatively with heavier males. Thus, our results provide evidence that females facultatively adjust hormone levels in their eggs in response to prenatal cues about the condition of their male partner. We note that our study provides no information on the potential adaptive value of female adjustment of maternal hormones in eggs given that we collected the eggs for use in the hormone assays. Thus, there is now a need for studies that investigate potential fitness consequences of maternal hormone levels for parents and offspring.

There are several potential explanations for why females deposited *more* JH when mated to lighter males in *N. vespilloides*. First, females may do so to speed up larval development, thereby compensating for the detrimental effects of poor male condition. For example, there is evidence that larger males are better at protecting the brood against conspecific intruders that would kill the brood if they succeed in taking over the carcass (Otronen, 1988). However, this explanation seems unlikely given that females were mated before they were given a carcass for breeding and that there was no evidence that the effect of male

Table 1
Estimated effects of male presence, carcass size and parents' weight on hormonal concentrations in the eggs. B represents treatments where both parents were present at egg laying, F when only females where present, L represent treatments provided with Large carcass and S with small carcasses.

Response variable	Explanatory variable	Mean estimate [95%CRI]	P(> 0)
JH concentration	Male presence	B-F = 21.74 [−33.42–77.56]	0.78
	Carcass size	L-S = −16.58 [−68.54–36.83]	0.27
	Interaction	(B−F)-(L-S) = 37.46 [−12.49–90.34]	0.93
	Male weight (scaled)	−18.95 [−37.08 to −1.95]	0.013
	Female weight (scaled)	−13.95 [−32.14–3.94]	0.064
	Time since onset of laying (scaled)	14.36 [−4.66–32.72]	0.93
ESH concentration	Male presence	B-F = 1.19 [−4.10–6.60]	0.67
	Carcass size	L-S = −0.13 [−5.29–5.08]	0.48
	Interaction	(B−F)-(L-S) = 1.32 [−3.95–6.54]	0.69
	Male weight (scaled)	−0.29 [−2.17–1.56]	0.38
	Female weight (scaled)	0.10 [−1.72–1.98]	0.54
	Time since onset of laying (scaled)	−0.98 [−2.95–1.06]	0.17

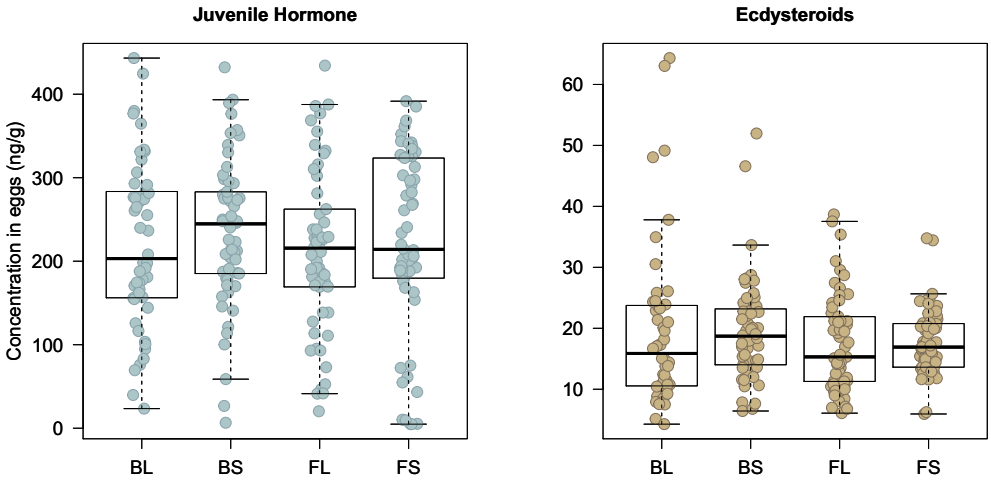

Fig. 1. No clear evidence for effects of the experimental treatments on hormonal concentrations in the eggs. B represents treatments where both parents were present at egg laying, F when only females where present, L represent treatments provided with Large carcass and S with small carcasses.

prenatal mass was conditional on whether the male was present or absent when females were provided with a carcass. Second, females may deposit more JH in their eggs to compensate for the effects of poor male condition if male condition serves as an indicator of the offspring's subsequent growth and development. Differential allocation of JH could be mediated by different sperm quality or quantity from males of different sizes. For example, there is growing evidence that males can affect offspring phenotype via sperm or seminal fluids (see e.g. Simmons and Lovegrove, 2019). Finally, males could alter female condition and hormonal levels through their behaviour during mating if for example heavier males have higher copulation rates (Pitnick and García-González, 2002). Future work is needed to understand the underlying mechanism of the effect of male weight on JH levels in eggs (e.g. whether due to genetic differences between males or due to paternal effects resulting from the male's phenotype), as well as its adaptive value for parents and offspring. Such studies could manipulate the body mass of parents (Steiger, 2013) and measure subsequent consequences on maternal hormone levels in eggs and the fitness consequences for parents and offspring.

Contrary to what we predicted, we found no evidence that females adjusted the deposition of maternal hormones in response to the presence or absence of the male at the time of egg laying or the size of the carcass used for breeding. This is surprising given that these two factors are major determinants of food availability for offspring after hatching in this species (Paquet and Smiseth, 2017; Sieber et al., 2017). Previous work shows that larvae were smaller at hatching but nevertheless compensated for their initial lower mass during growth (at the expense of male weight gain) when females laid the eggs in the presence rather than the absence of a male parent (Paquet and Smiseth, 2017). Our results show that differential allocation of JH or ESH is unlikely to be the mechanism responsible for this maternal effect. Future studies could assess whether females insects alter their allocation of other egg compounds such as proteins (vitellin) and lipids, in response to male presence and carcass size.

An alternative explanation for why we found no evidence for differential hormonal deposition in eggs in response to male presence and carcass size is that females may adjust their allocation in response to other key factors indicating the conditions experienced by offspring after hatching, such as temperature (Grew et al., 2019) or carcass decomposition (Ford and Smiseth, 2017). This suggestion is supported by

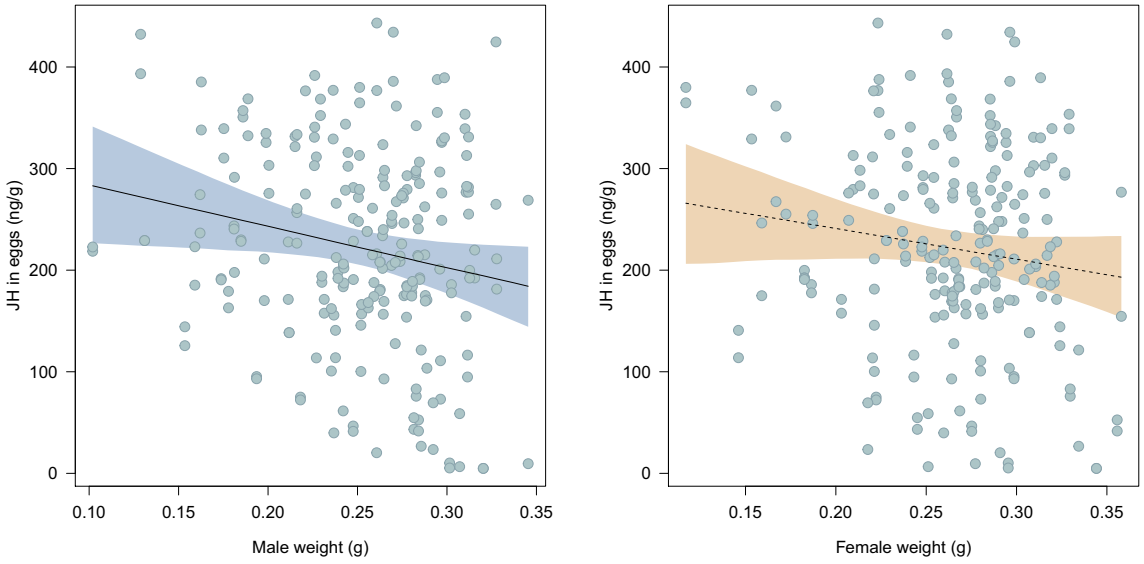

Fig. 2. Relationship between male weight (left panel) and female weight (right panel) and JH concentrations in the eggs. Lines show predicted means and shaded areas the 95% Credibility Intervals. The solid line represents effects for which the 95% C.I. of the slope did not span zero whereas the dashed line represents effects for which the 95% C.I. of the slope included zero.

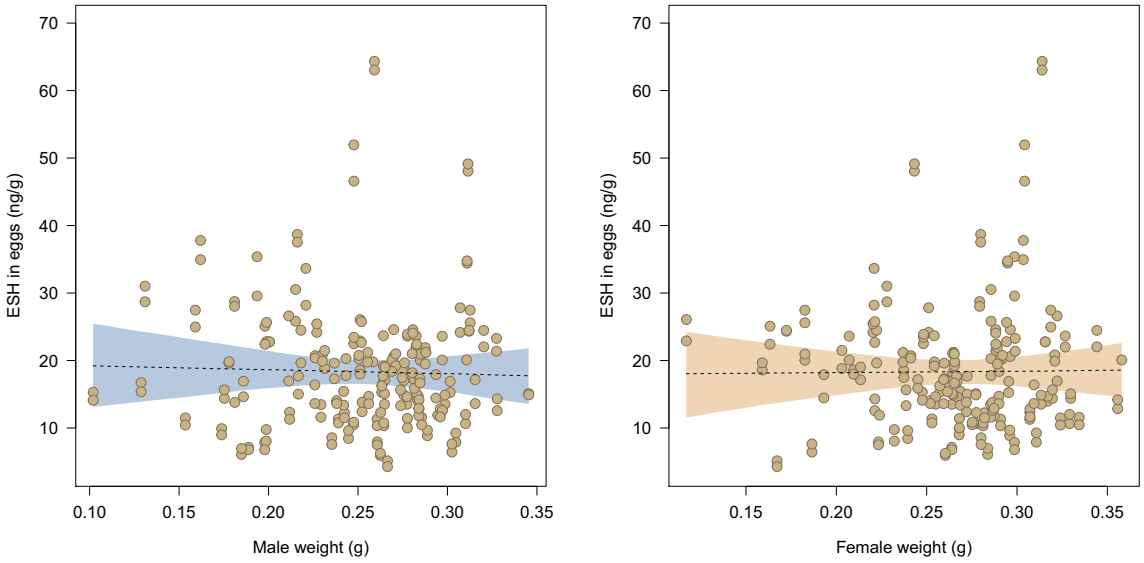

Fig. 3. Relationship between male weight (left panel) and female weight (right panel) and ESH concentrations in the eggs. Lines show predicted means and shaded areas the 95% Credibility Intervals. The dashed lines represents effect for which the 95% C.I. of the slope included zero.

the observation that most of the estimated variation in JH and ESH concentrations remains unexplained in our study. Finally, we cannot exclude the possibility that females may adjust the allocation of maternal hormones for later-laid eggs given that we only collected eggs laid within 26 h after the onset of egg laying to limit potential effect of embryo development. Such within-clutch variation may arise as a consequence of physiological constraints or they may represent an adaptive strategy as suggested in prior studies on birds (Groothuis and Schwabl, 2002; Love et al., 2008). In our study species, females lay their eggs asynchronously for a period of up to 60 h (Ford and Smiseth, 2017). Currently, there is little (if any) evidence from any taxa that females differentially adjust hormone deposition in early and late eggs in response to environmental cues (Schmaltz et al., 2008; van Dijk et al., 2013; Verboven et al., 2003, 2005). We encourage future work to investigate the presence and fitness consequences of such patterns in invertebrates.

Our study was motivated by prior work on birds, suggesting that female adjustment of maternal hormone levels evolved in the context of biparental food provisioning and offspring begging (Groothuis et al., 2019). We found evidence for female adjustment of maternal hormone levels in *N. vespilloides*; an insect with biparental food provisioning and offspring begging. However, we urge caution in interpreting our results as evidence that female adjustment of maternal hormone levels is causally associated with biparental food provisioning and offspring begging. The main reason for this is that there are alternative adaptive and non-adaptive explanations for why females appear to adjust maternal hormone levels in response to environmental conditions. For example female hormonal deposition may influence how dispersing offspring respond to the prenatal environment as reported for common lizards (*Zootoca vivipara*), where experimentally manipulated maternal corticosterone levels increased offspring philopatry (De Fraipont et al., 2000). Furthermore, maternal hormones may be passively transferred to the eggs with deleterious consequences for offspring. For example, a study on the tropical damselfish *Pomacentrus amboinensis* shows that maternal cortisol reduces the body size of fry at hatching (McCormick, 1998). Concurring with this possibility, prior work on our study species shows that an experimental increase in larval levels of methoprene (a JH analogue) induced reduced larval growth (Crook et al., 2008). Thus, there is now a need for more work to determine whether female adjustment of maternal hormones is a general phenomenon across insect species either with or without parental care.

To conclude, we provide the first clear evidence for female adjustment of maternal hormone levels in eggs in an insect species. Given the independent evolutionary origins of both biparental care and hormones in insects and birds, our results suggest that this is a case of convergence based on similarities in ecology and/or life histories. More work is clearly needed to understand the generality of such patterns across different insect species with and without parental care, as well as its underlying mechanisms and fitness consequences. Insects represent formidable systems to experimentally investigate the causes and consequences of hormonal allocation in eggs under diverse ecological conditions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2020.104708.

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