Juvenile hormone, but not nutrition or social cues, affects reproductive maturation in solitary alkali bees (*Nomia melanderi*)

Karen M. Kapheim* and Makenna M. Johnson

Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322, USA

*Correspondence: Karen M. Kapheim (karen.kapheim@usu.edu)

Key words: eusociality, alkali bees, Dufour's gland, nutrition, juvenile hormone, reproduction

Abstract

Eusocial insect colonies are defined by extreme variation in reproductive activity among castes, but the ancestral conditions from which this variation arose are unknown. Investigating the factors that contribute to variation in reproductive physiology among solitary insects that are closely related to social species can help to fill this gap. We experimentally tested the role of nutrition, juvenile hormone (JH), and social cues on reproductive maturation in solitary alkali bees (Halictidae: *Nomia melanderi*). We find that alkali bee females emerge from overwintering with undeveloped ovaries and Dufour's glands. Initial activation of these reproductive structures does not depend on pollen consumption, though dietary protein or lipids may be necessary for long-term reproductive activity. JH is likely to be a limiting factor in alkali bee reproductive activation, as only those females treated with JH developed mature oocytes and Dufour's glands. These females reached reproductive maturity without access to mates or nesting opportunities. Unlike for related social bees, the effects of JH were not suppressed by the presence of older, reproductive females. These results provide important insight into the factors that influence reproductive activity in an important native pollinator, and those that may have been particularly important in the evolution of reproductive castes.

Introduction

Complex social organization, such as that observed among honey bees (*Apis mellifera*), ants (Formicidae), and vespid wasps (Vespidae), is marked by a high degree of variance in reproductive activity among individuals within a colony. This variation is demarcated among reproductive castes, whereby workers do not reproduce, despite engaging in maternal behaviors (e.g., brood feeding or nest defense), and queens reproduce while largely refraining from brood care (Michener, 1974). Workers of many social insect species have similar reproductive anatomy to queens (e.g., ovaries, spermathecae, glands, ovipositor), yet remain functionally sterile. This suggests the factors that influence the function of these structures differ between queens and workers, and that understanding this variation may provide insights into the physiological basis for the origin of social insect castes.

The factors that differentially influence reproductive activation among social insect castes include nutritional, endocrine, and social cues (Kapheim, in review). For example, both queens and workers acting as nurses within a honey bee colony consume a protein-rich diet, but this protein contributes to egg-production only in the queens (Winston, 1987). Similarly, treatment with the juvenile hormone (JH) analog methoprene leads to accelerated ovarian development in queen paper wasps (*Polistes canadensis*), but instead increases foraging activity in workers (Giray et al., 2005). Finally, social cues, such as aggression from the queen, can repress endocrine pathways, and thus ovary development, in worker bumble bees (*Bombus impatiens*) and social halictid bees (*Megalopta genalis*), but aggression directed from workers toward queens does not have the same effect (Kapheim et al., 2016; Padilla et al., 2016; Smith et al., 2009). Understanding how these differences in sensitivity to physiological and environmental cues arises among females and contributes to variation in reproductive activity is thus key to understanding the origins of social insect castes.

One approach toward this goal is to investigate how these factors contribute to variation in reproductive development in solitary species representative of the ancestors that gave rise to social castes. We conducted two experiments to determine how variation in nutrition, JH, and the social environment influence reproductive development in a solitary bee that shares similarities with the ancestors of social bees. Alkali bees (*Nomia melanderi*) are semi-managed, native pollinators of alfalfa seed crops that range throughout the western U.S.A. (Cane, 2008). This species belongs to a basal subfamily of Halictidae (Nomiinae) in which eusociality has never evolved, but they are closely related to the Halictinae, in which eusociality evolved at least twice (Brady et al., 2006; Danforth et al., 2008; Gibbs et al., 2012). Importantly, these bees exhibit extended maternal behavior, characteristic of that which was a necessary pre-adaptation to sociality (Batra, 1970; Batra and Bohart, 1969; Schwarz et al., 2007).

Little is known about the factors that influence alkali bee reproductive development. However, a recent study demonstrated that JH accelerates reproductive maturation (Kapheim and Johnson, 2017). It was also recently documented that adult female alkali bees consume pollen on a daily basis (Cane et al., 2016). Pollen is the primary source of dietary protein and lipids for bees (Roulston and Cane, 2000), but whether pollen consumption is necessary for reproduction has not been experimentally tested. We investigated two aspects of reproductive physiology – oocyte growth, which requires proteins for egg-yolk (Badisco et al., 2013) and maturation of the Dufour's gland, which secretes lipids used for nest cell construction (Cane, 1981). Our results reveal that initiation of oogenesis and Dufour's gland maturation does not require dietary protein, but only females treated with JH reached reproductive maturity. This response to JH was not affected by variation in the social environment (i.e., co-housing with an older, reproductive female). This provides important insight into the physiological foundation from which social insect castes evolved, as well as the reproductive physiology of an important pollinator.

Methods

Collections

This study took place in Touchet, WA, U.S.A, where alkali bees nest in large soil beds near alfalfa seed fields (Cane, 2008). Alkali bees overwinter as prepupae in below-ground nests, and emerge as adults upon completion of development the following summer. We trapped newly eclosed adult females from 27 May-8 June 2016 by placing emergence traps on 3 bee beds prior to the start of emergence. Traps were checked at least 3 times a day, and new bees were transferred back to the laboratory in individually labeled 15 ml tubes placed inside a cooler with a single ice pack placed under a layer of cardboard.

Experiment 1 – nutrition effects on reproductive physiology

Upon arrival to the laboratory, bees were chilled at 4ºC for 5 min and randomly assigned to a treatment group: sugar water only (sterile 35% sucrose solution), sugar water with pollen (2.5 g sterile, finely-ground, honey-bee pollen in 30 ml of sterile 35% sucrose solution), sugar water with pollen plus 4 sprigs of fresh, untripped alfalfa flowers. (Bees were observed manipulating these flowers to release pollen on a regular basis throughout the experiment.) Bees were placed in perforated plastic deli containers (72 mm x 90 mm lower diameter x 113 mm upper diameter), and reared in the lab for 10 days (d). Sugar water or pollen mix and alfalfa flowers were changed daily. Pollen-sugar mixture was shaken vigorously before each feeding to achieve homogeneity, and then pipetted into feeding troughs made from 1.5 ml microcentrifuge tubes with the tapered tip removed. The cages were kept at 22-28ºC, 40-85% RH and full spectrum lighting 13 L: 11 D, as has been previously described (Kapheim and Johnson, 2017). At the end of the 10 d rearing period, bees were chilled for 3 min at 4ºC, placed in individually-labeled tubes, and flash-frozen in liquid nitrogen.

We also collected newly emerged females and reproductive females for comparison to lab-reared females. Newly emerged females were collected from emergence traps as described above, but were flash-frozen immediately upon return to the laboratory. Reproductive females were identified as those returning to a nest hole with pollen on their hind legs. They were captured by net, and flash-frozen immediately upon return to the laboratory.

Experiment 2 – social and endocrine effects on reproductive physiology

Newly emerged females were collected as in Experiment 1. Each bee was randomly assigned to a treatment group: sham control, solvent control, or JH. For JH treatments, JH-III (product E589400, Toronto Research Chemicals, Inc., Toronto, Ontario, Canada) was dissolved in dimethylformamide (DMF) at a concentration of 100 µg per µl. Bees in the solvent control group received 1 µl of DMF applied to the thorax with a pipette tip. Bees in the JH group received 100 µg JH in 1 µl of DMF applied to the thorax with a pipette tip. Bees in the sham group were touched lightly on the thorax with a clean pipette tip. Hormone treatments were repeated when bees were 5 d old. Bees in each treatment group were randomly assigned to be caged alone or with an older, reproductive female, defined as above. All bees were paint-marked on the dorsal abdomen with a uniquely colored Decocolor© paint pen (Uchida of America Co, Torrance, CA). All bees were reared in cages, and received 35% sugar water mixed with pollen and fresh alfalfa flowers for 10 d, as described for Experiment 1.

Upon collection, all bees from both experiments were stored in liquid nitrogen until return to Utah State University, where they were transferred to a -80 ºC freezer.

Dissections

Dissections followed previously reported methods (Kapheim and Johnson, 2017). Briefly, bees were

129 dissected under a Leica M80 stereomicroscope fitted with an IC80HD camera (Leica Microsystems,
130 Buffalo Grove, IL, USA). We measured Dufour's gland and terminal oocyte lengths from images, using
131 software in the Leica Application Suite (v. 4.5). The observer was blind to the treatment group of each
132 bee during dissections, and both authors concurred on measurements. Mating status was determined
133 by examination of the spermatheca under a compound microscope. We excluded newly emerged
134 females with sperm and reproductive females without sperm from further analyses. The hindgut was
135 removed during dissection and stored at -80 ºC until further analysis.
136
137 *Pollen quantification*
138 To determine whether our diet treatments were effective, we quantified the amount of pollen
139 consumed by our lab-reared females receiving pollen, relative to reproductive females, by estimating
140 the number of pollen grains in the hindgut. We followed previously described methods (Cane et al.,
141 2016) to estimate pollen grains in 6 hindguts from each group. Individual hindguts were placed in 0.5 ml
142 microcentrifuge tubes with 50 µl of 70% ethanol and torn apart with forceps. After guts were shredded,
143 the mix was vortexed for 5 sec, using Vortex Genie 2 on highest setting of 10. The shredded gut was
144 then removed using forceps, dabbing tissue on sides of the tube to remove excess ethanol and pollen.
145 Each sample was vortexed on the highest setting for 10 seconds immediately prior to loading 10 µl of
146 the solution into one chamber of a hemocytometer for pollen counting. Pollen grains were counted
147 across the entire chamber under a compound microscope at 20X magnification. Three different 10 µl
148 aliquots were counted for each sample, using the entire chamber each time. For each sample, the
149 average of these three counts was divided by 0.0009 ml, the volume of each hemocytometer chamber,
150 and then multiplied by the volume of ethanol used per sample (0.05 ml).
151
152 *Statistical analyses*

All statistical analyses were performed in R version 3.2.5 (Team, 2016). Visual inspection of a qq-plot (R package "car", (Fox and Weisberg, 2011)) and an Anderson-Darling normality test (R package "nortest", (Gross and Ligges, 2015)) revealed significant departures from normality in the distribution of terminal oocyte and Dufour's gland lengths for Experiment 1, but not Experiment 2. We therefore applied a Box-Cox transformation to the data for Experiment 1 before running the final model (Venables and Ripley, 2002). For both Experiment 1 and 2, we modeled maximum terminal oocyte and Dufour's gland lengths with separate linear mixed effects regressions that initially included intertegular width and treatment (coded as factors: diet for Experiment 1, JH*social for Experiment 2) as fixed effects, with bee bed of origin as a random effect (Bates et al., 2015). In each case, the variance and standard deviation for the intercept of bee bed was zero, so a linear model without random effects was used for subsequent analyses. Intertegular width was removed from the final models in the cases where it was non-significant ($p > 0.05$) – all except Dufour's gland length in Experiment 2. We used post-hoc tests to investigate significant differences between treatment groups (Hothorn et al., 2008).

For Experiment 2, we repeated the analyses after removing cases where the older, reproductive partner had a smaller intertegular width, terminal oocyte, or Dufour's gland than the newly emerged cage-mate to determine whether relative size or reproductive development influenced the outcome of the social treatment.

Final estimates of pollen counts in the hindgut were compared between groups (nesting females, sugar + pollen mix, sugar + pollen + alfalfa flowers) with a linear model function (lm), after applying a Box-Cox transformation of the data (Venables and Ripley, 2002).

Results

177 *Experiment 1 – nutrition effects on reproductive physiology*

178 Mortality was not significantly different among lab-reared females on different diet treatments
179 (mortality: sugar – 40%, sugar + pollen – 32%, sugar + pollen + flowers – 46%; $\chi^2 = 1.29$, $p = 0.52$, $n = 92$).
180 There were significant differences in both maximum terminal oocyte and Dufour's gland length among
181 treatment groups (oocytes: $F_{4,101} = 82.42$, $r^2 = 0.77$, $p < 2.2x10^{-16}$, Table S1; Dufour's: $F_{4,101} = 45.55$, $r^2 =$
182 0.64, $p < 2.2x10^{-16}$, Table S2). Among these groups, lab-reared, 10 d old females had significantly longer
183 terminal oocytes and Dufour's glands than newly emerged females (Table 1, Fig. 1). However, actively
184 nesting reproductive females had significantly more developed reproductive anatomy than either newly
185 emerged or lab-reared females (Table 1, Fig. 1). We did not observe significant differences in oocyte or
186 Dufour's gland length among females reared in the lab for 10 d on different diets (Table 1, Fig. 1).

187

188 The estimated number of pollen grains detected in the hindguts was not significantly different among
189 reproductive females and the two groups of lab-reared females that received pollen in their diet ($F_{2,15} =$
190 3.32, $r^2 = 0.31$, $p = 0.06$, Fig. S1).

191

192 **Table 1** Summary of results from Experiment 1. Values are mean ± 1 standard deviation (s.d.).

Group	n	Maximum terminal oocyte length (mm)	Dufour's gland length (mm)
Newly emerged	36	0.77 ± 0.54	3.80 ± 0.46
Sugar water only	14	1.81 ± 0.42	4.47 ± 0.52
Sugar water + pollen	22	1.98 ± 0.64	4.66 ± 0.47
Sugar water + pollen + flowers	14	2.01 ± 0.47	4.50 ± 0.60
Nesting, reproductive	20	3.35 ± 0.30	5.55 ± 0.30

193

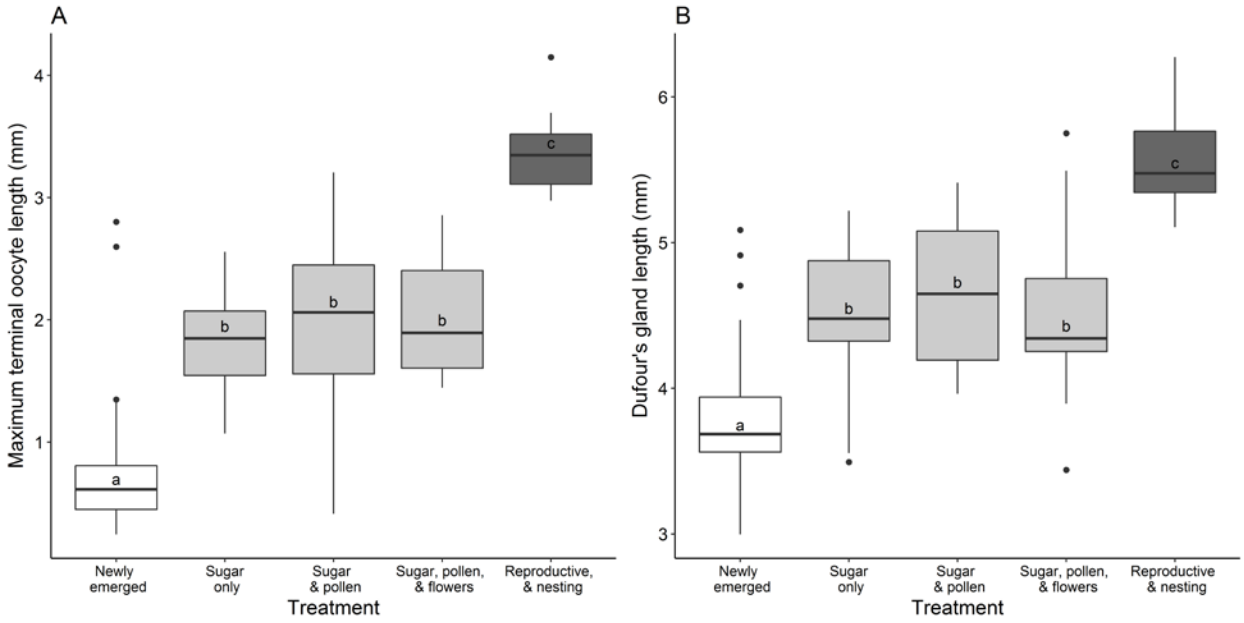


Figure 1 Effects of diet on reproductive development in alkali bees. (A) Maximum terminal oocyte length and (B) Dufour's gland length were significantly different between newly emerged, lab-reared, and nesting females. Diet did not have a significant effect on reproductive development when newly emerged females were reared in the lab for 10 d. Boxes represent the interquartile range, with the line as the median. Whiskers extend to 1.5 times the interquartile range. Circles are outliers. Letters indicate significant differences ($p < 0.05$). White boxes = newly emerged females, Grey boxes = lab-reared 10 d old females; Dark grey boxes = Reproductive, nesting females; Full model results are available in the supplementary materials.

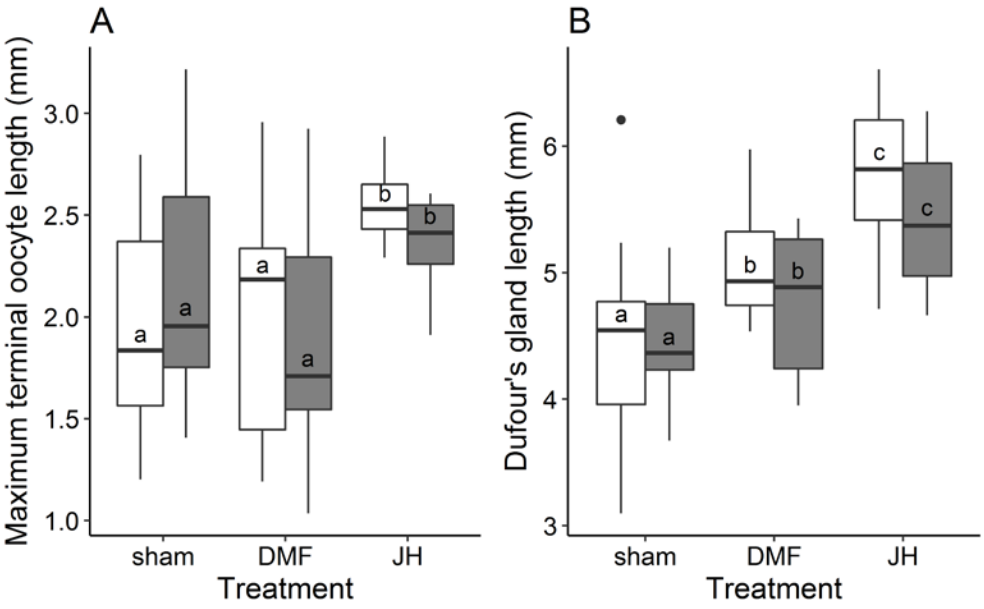
Experiment 2 – social and endocrine effects on reproductive physiology

Mortality was not significantly different among treatment groups in Experiment 2 (sham/solitary – 19%, sham/social – 19%, DMF/solitary – 20%, DMF/social – 47%, JH/solitary – 35%, JH/social – 44%; $\chi^2 = 7.04$, $p = 0.22$, $n = 101$). There were significant differences in maximum terminal oocyte and Dufour's gland length among treatment groups (oocytes: $F_{5,58} = 2.81$, $r^2 = 0.19$, $p = 0.02$, Table S3; Dufour's: $F_{6,57} = 8.77$, $r^2 = 0.19$, $p = 8.97 \times 10^{-7}$, Table S4). Females treated with JH had significantly longer terminal oocytes and Dufour's glands than females in control groups, but variation in the social environment did not have a significant effect on these measures of reproductive physiology (Table 2, Fig. 2).

214 **Table 2** Summary of results from experiment 2. Values are mean ± 1 standard deviation (s.d.).

Endocrine treatment	Social environment	n	Maximum terminal oocyte length (mm)	Dufour's gland length (mm)
Sham control	Solitary	12	1.91 ± 0.51	4.46 ± 0.83
	Social	12	2.15 ± 0.62	4.47 ± 0.41
Solvent control	Solitary	12	2.01 ± 0.58	5.05 ± 0.42
	Social	10	1.87 ± 0.64	4.48 ± 0.55
100 µg JH	Solitary	10	2.55 ± 0.20	5.78 ± 0.58
	Social	8	2.37 ± 0.24	5.43 ± 0.59

215



216

217 **Figure 2** Effects of endocrine and social treatments on reproductive development in alkali
218 bees. (A) Maximum terminal oocyte length and (B) Dufour's gland length were
219 significantly different between lab-reared females treated with JH versus controls, but
220 variation in the social environment did not significantly affect reproductive development.
221 Boxes represent the interquartile range, with the line as the median. Whiskers extend to
222 1.5 times the interquartile range. Circles are outliers. Letters indicate significant
223 differences ($p < 0.05$). Grey bars = social treatment, white bars = solitary treatment; Full
224 model results are available in the supplementary materials.

225

226 Reproductive females and newly emerged females paired in the social treatments were similar in size to
227 one another (mean ratio of intertegular width = 1.03 ± 0.11 s.d.). On average, the reproductive females
228 had larger oocytes and Dufour's glands than their newly emerged cage-mates (mean ratio of oocyte
229 length = 1.36 ± 0.42, mean ratio of Dufour's glands = 1.13 ± 0.17). However, there were 13 cases for

230 which the reproductive female was smaller and/or had smaller ovaries or Dufour's glands than their
231 newly emerged female cage-mates. Elimination of these 13 cases from the dataset did not change the
232 results (Table S5-S6).

233

234 **Discussion**

235 Variation in reproductive physiology is a hallmark of the social insect societies, in which just one or a few
236 individuals out of thousands within a colony are reproductively active, despite shared genetic influences.
237 Investigating the factors that contribute to reproductive variation in solitary relatives of social insects
238 can provide clues as to how reproductive castes evolve (Kapheim, in review). Our results demonstrate
239 that solitary alkali bees do not require dietary protein during the initial stages of reproductive
240 development, but that JH may be a limiting factor in completion of reproductive maturation. We also
241 find that, unlike for social bees, interactions between conspecifics do not influence reproductive
242 physiology. This provides important information about the physiological foundation from which
243 reproductive castes emerged.

244

245 Access to dietary protein did not limit reproductive activation among newly emerged alkali bees, but it
246 also was not sufficient for reproductive maturation. Most of the lab-reared females in our study did not
247 develop mature oocytes or Dufour's glands during the 10 d study period, despite having similar amounts
248 of pollen in their hindguts as actively nesting, reproductive females, indicating they had consumed
249 ecologically relevant amounts of pollen during the experiment. Alkali bees commonly begin laying eggs
250 within a few days of eclosion, thus our study period provided ample time for oocyte maturation (Bohart
251 and Cross, 1955). It is possible that the completion of reproductive maturation requires ecological cues,
252 such as nesting substrate, or access to mates. Seminal fluid is known to trigger oogenesis in several
253 insect species (Avila et al., 2011), and mating limitation is known to influence reproductive activity in

254 other halictid bees (Yanega, 1989; Yanega, 1992). However, lab-reared females of another halictid bee,
255 *Megalopta genalis*, reached reproductive maturity when reared, unmated, in the lab for 10 days
256 (Kapheim et al., 2012). If mating is a reproductive limitation in alkali bees, it can apparently be
257 overridden by JH treatments, as some JH-treated females in our study reached reproductive maturity,
258 even in the absence of mating or ecological cues. Regardless of the role of dietary protein in the
259 initiation of reproductive activation, the fact that alkali bees and other closely related halictid bees
260 consume pollen on a daily basis suggests that protein is likely necessary for sustained reproductive
261 activity throughout the breeding season (Cane et al., 2016; Wuellner, 1999).
262
263 Our results are in contrast to results of similar studies of solitary bees in the family Megachilidae, *Osmia*
264 *californica* and *Megachile rotundata*, which showed access to dietary protein is essential for
265 reproductive maturation among newly emerged females (Cane, 2016; Richards, 1994). Unlike alkali
266 bees, *Osmia* overwinter as adults, begin oogenesis prior to eclosion, and thus eclose with depleted
267 protein stores (Wasielewski et al., 2011). Protein stores have not been measured in newly emerged
268 alkali bees, but remaining reproductively quiescent until eclosion is likely to be less energetically
269 expensive and may be associated with increased availability of nutrient stores for post-eclosion
270 maturation (Hahn and Denlinger, 2007). Alkali bees may therefore be better poised to initiate oogenesis
271 without a dietary protein source. Conversely, pollen is necessary to stimulate vitellogenesis in *M.*
272 *rotundata*, which also remains reproductively quiescent until ecolosion. The apparent difference in
273 nutritional requirements for oogenesis among megachilid and halictid bees indicates that the
274 physiological basis for reproductive activity is highly variable among solitary bees. This suggests that
275 assumptions about reproductive physiology among the ancestors of social bees should be made with
276 caution. Additional research on solitary bees from additional families in which eusociality has evolved
277 (e.g., Apidae) are necessary to fully understand variation in nutritional requirements for reproduction.

Our results suggest that JH is a limiting factor in reproductive maturation among alkali bees. Females treated with JH were the only lab-reared bees in our study that developed fully mature oocytes and Dufour's glands. This confirms earlier results (Kapheim and Johnson, 2017), and provides evidence of a conserved gonadotropic role for JH in alkali bees. In most insects, including bumble bees (*B. terrestris*), JH stimulates the synthesis of vitellogenin, an egg-yolk precursor protein necessary for oocyte maturation (Amsalem et al., 2015; Badisco et al., 2013). This suggests the gonadotropic response to JH may depend on a dietary source of protein. All of the females receiving JH treatments in our study also received dietary protein from pollen, and thus had the nutritional resources necessary to complete vitellogenesis. Future studies are needed to determine how nutrition and JH pathways interact in alkali bee oogenesis.

The path by which JH stimulates Dufour's gland maturation is less clear, as Dufour's gland evolved in the ancestor of Hymenoptera, and secretes chemicals with a wide range of functions within this group (Mitra, 2013). Dufour's gland is likely derived from the colleterial accessory gland in other insects (Mitra, 2013), and reproductive maturity of this gland is induced by JH in cockroaches (*Byrsotria fumigate, Periplaneta americana*) (Bell and Barth, 1970; Willis and Brunet, 1966). Moreover, JH influences the chemical composition of Dufour's gland secretions in bumble bee (*B. terrestris*) workers (Shpigler et al., 2014). This, along with our results, suggests that endocrine regulation of Dufour's gland is deeply conserved among insects. Additional research is needed to determine the molecular mechanisms by which JH affects Dufour's gland function.

Unlike for social bees, variation in the social environment does not influence reproductive physiology among solitary alkali bees. Research with a facultatively eusocial halictid bee, *M. genalis*, suggests that

aggression from older, reproductive females can limit reproductive development via JH-suppression in newly emerged females (Kapheim et al., 2016; Smith et al., 2009; Smith et al., 2013). Although we did not directly measure behavioral interactions among pairs as part of our study, we routinely observed aggressive exchanges among pairs of females in cages. Our results thus suggest that sensitivity to cues from the social environment observed in social halictid bees are not conserved in their solitary relatives. Alkali bees nest in extremely dense aggregations, with up to 713 nests per square meter in study area (Cane, 2008). At high density, these ground-nesting females are likely to encounter each other regularly as they dig tunnels and build cells, and there is thus likely to be strong selection against physiological sensitivity to social interactions in these populations. Similar studies with additional solitary bees are necessary to identify the circumstances under which sensitivity to the social environment influences reproductive physiology.

Conclusions

This study is the first experimental investigation of dietary, endocrine, and social effects on reproductive maturation in a solitary bee closely related to lineages in which sociality evolved. Our results reveal that the factors contributing to the initiation of reproductive activation and completion of reproductive maturity may be different. Specifically, dietary protein was not essential for the initiation of reproductive activation, but also was not sufficient for reproductive maturation. JH, however, may be a limiting factor in maturation of both oocytes and Dufour's gland. This provides important insight into how sensitivity to these cues evolved with the origin of reproductive castes in social insects. For example, the effects of JH on Dufour's gland are apparently conserved between solitary alkali bees and bumble bee workers, but this is not the case for the effects of JH on oocyte maturation (Amsalem et al., 2013b; Kapheim and Johnson, 2017; Shpigler et al., 2014). This suggests that endocrine pathways influencing different aspects of reproductive physiology were independently modified during social

326 evolution. Also, nutrition and cues from the social environment are some of the most important factors
327 in reproductive suppression of workers among social bees (Amsalem et al., 2013a; Kapheim et al., 2016;
328 Lawson et al., 2016; Padilla et al., 2016), but these factors did not have a significant influence on
329 variation in reproductive activation in solitary alkali bees. This suggests that changes in how nutrient-
330 sensing and environment-sensing pathways regulate reproductive physiology were especially important
331 in the evolutionary origins of reproductive castes. Further comparisons of the molecular networks
332 underlying the physiological response to nutritional, endocrine, and social cues across species are likely
333 to provide key insight into how reproductive division of labor evolves.
334
335 **Acknowledgements**
336 This research was supported by the Utah Agricultural Experiment Station, Utah State University, and
337 approved as journal paper number 8999. This work was also supported by the USDA-ARS Alfalfa
338 Pollinator Research Initiative. Thanks to A. Tripodi for help with imaging spermathecae, E. Klinger for
339 help with pollen counting, and J. Cane for helpful discussion. Thanks to A. Tripodi and D. Cox-Foster for
340 helpful comments on a previous version of this manuscript. We are grateful to John Dodd and Forage
341 Genetics International for providing lab space and logistical support in Touchet, WA. We thank Mike
342 Ingham, Mark Wagoner, and Mike Buckley for access to their bee beds and bees. M. Jolley and F.
343 Dowsett provided valuable assistance in the field.
344
345 **References**
346 Amsalem, E., Grozinger, C.M., Padilla, M., Hefetz, A., 2015. Chapter Two - The physiological and genomic
347 bases of bumble bee social behaviour, in: Amro, Z., Clement, F.K. (Eds.), Advances in Insect
348 Physiology. Academic Press, pp. 37-93.
349 Amsalem, E., Shamia, D., Hefetz, A., 2013a. Aggression or ovarian development as determinants of

reproductive dominance in *Bombus terrestris*: interpretation using a simulation model. Insect Soc 60, 213-222.

Amsalem, E., Shpigler, H., Bloch, G., Hefetz, A., 2013b. Dufour's gland secretion, sterility and foraging behavior: correlated behavior traits in bumblebee workers. J Insect Physiol 59, 1250-1255.

Avila, F.W., Sirot, L.K., LaFlamme, B.A., Rubinstein, C.D., Wolfner, M.F., 2011. Insect seminal fluid proteins: identification and function. Annu Rev Entomol 56, 21-40.

Badisco, L., Van Wielendaele, P., Vanden Broeck, J., 2013. Eat to reproduce: a key role for the insulin signaling pathway in adult insects. Front Physiol 4, 202.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1-48.

Batra, S.W.T., 1970. Behavior of alkali bee, *Nomia-melanderi*, within nest (Hymenoptera-Halictidae). Ann Entomol Soc Am 63, 400-406.

Batra, S.W.T., Bohart, G.E., 1969. Alkali bees: response of adults to pathogenic fungi in brood cells. Science 165, 607.

Bell, W.J., Barth, R.H., 1970. Quantitative effects of juvenile hormone on reproduction in the cockroach *Byrsotria fumigata*. J Insect Physiol 16, 2303-2313.

Bohart, G.E., Cross, E.A., 1955. Time relationships in the nest construction and life cycle of the alkali bee. Ann. Ent. Soc. Amer. 48, 403-406.

Brady, S.G., Sipes, S., Pearson, A., Danforth, B.N., 2006. Recent and simultaneous origins of eusociality in halictid bees. Proc R Soc Lond B Biol Sci 273, 1643-1649.

Cane, J.H., 1981. Dufour's gland secretion in the cell linings of bees (Hymenoptera: Apoidea). J Chem Ecol 7, 403-410.

Cane, J.H., 2008. A native ground-nesting bee (*Nomia melanderi*) sustainably managed to pollinate alfalfa across an intensively agricultural landscape. Apidol 39, 315-323.

374 Cane, J.H., 2016. Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. J Insect Physiol
375 95, 105-109.
376 Cane, J.H., Dobson, H.E.M., Boyer, B., 2016. Timing and size of daily pollen meals eaten by adult females
377 of a solitary bee (*Nomia melanderi*) (Apiformes: Halictidae). Apidol 48, 17-30.
378 Danforth, B.N., Eardley, C., Packer, L., Walker, K., Pauly, A., Randrianambinintsoa, F.J., 2008. Phylogeny
379 of Halictidae with an emphasis on endemic African Halictinae. Apidol 39, 86-101.
380 Fox, J., Weisberg, S., 2011. An R companion to applied regression, 2nd ed. Sage, Thousand Oaks, CA.
381 Gibbs, J., Brady, S.G., Kanda, K., Danforth, B.N., 2012. Phylogeny of halictine bees supports a shared
382 origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea: Anthophila: Halictidae). Molecular
383 Phylogenetics and Evolution 65, 926-939.
384 Giray, T., Giovanetti, M., West-Eberhard, M.J., 2005. Juvenile hormone, reproduction, and worker
385 behavior in the neotropical social wasp *Polistes canadensis*. Proc. Natl. Acad. Sci. U. S. A. 102, 3330-
386 3335.
387 Gross, J., Ligges, U., 2015. nortest: Tests for Normality, R package version 1.0-4.
388 Hahn, D.A., Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: nutrient storage
389 and utilization. J Insect Physiol 53, 760-773.
390 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models.
391 Biometrical J 50, 346 - 363.
392 Kapheim, K.M., in review. Nutritional, endocrine, and social influences on reproductive physiology at the
393 origins of social behavior. Curr Op Insect Sci.
394 Kapheim, K.M., Chan, T.Y., Smith, A.R., Wcislo, W.T., Nonacs, P., 2016. Ontogeny of division of labor in a
395 facultatively eusocial sweat bee *Megalopta genalis*. Insect Soc 63, 185-191.
396 Kapheim, K.M., Johnson, M.M., 2017. Support for the reproductive ground plan hypothesis in a solitary
397 bee: links between sucrose response and reproductive status. Proc. R. Soc. Lond., B, Biol. Sci. 284,

20162406.

Kapheim, K.M., Smith, A.R., Ihle, K.E., Amdam, G.V., Nonacs, P., Wcislo, W.T., 2012. Physiological variation as a mechanism for developmental caste-biasing in a facultatively eusocial sweat bee. Proc. R. Soc. Lond., B, Biol. Sci. 279, 1437-1446.

Lawson, S.P., Ciaccio, K.N., Rehan, S.M., 2016. Maternal manipulation of pollen provisions affects worker production in a small carpenter bee. Behav Ecol Sociobiol 70, 1891-1900.

Michener, C.D., 1974. The social behavior of the bees. Harvard University Press, Cambridge, MA.

Mitra, A., 2013. Function of the Dufour's gland in solitary and social Hymenoptera. J Hymenopt Res 35, 33-58.

Padilla, M., Amsalem, E., Altman, N., Hefetz, A., Grozinger, C.M., 2016. Chemical communication is not sufficient to explain reproductive inhibition in the bumblebee *Bombus impatiens*. R Soc Open Sci 3, 160576.

Richards, K.W., 1994. Ovarian development in the alfalfa leafcutter bee, *Megachile rotunda*. J Apicult Res 33, 199-203.

Roulston, T.H., Cane, J.H., 2000. Pollen nutritional content and digestibility for animals. Plant Syst Evol 222, 187-209.

Schwarz, M.P., Richards, M.H., Danforth, B.N., 2007. Changing paradigms in insect social evolution: insights from halictine and allodapine bees. Annu Rev Entomol 52, 127-150.

Shpigler, H., Amsalem, E., Huang, Z.Y., Cohen, M., Siegel, A.J., Hefetz, A., Bloch, G., 2014. Gonadotropic and physiological functions of juvenile hormone in bumblebee (*Bombus terrestris*) workers. PLoS ONE 9, e100650.

Smith, A.R., Kapheim, K.M., O'Donnell, S., Wcislo, W.T., 2009. Social competition but not subfertility leads to a division of labour in the facultatively social sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). Anim Behav 78, 1043-1050.

422 Smith, A.R., Kapheim, K.M., Perez-Ortega, B., Brent, C.S., Wcislo, W.T., 2013. Juvenile hormone levels
423 reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera:
424 Halictidae). Horm Behav 63, 1-4.
425 Team, R.C., 2016. R: A language and environment for statistical computing. R Foundation for Statistical
426 Computing, Vienna, Austria.
427 Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with S, 4th ed. Springer, New York.
428 Wasielewski, O., Giejdasz, K., Wojciechowicz, T., Skrzypski, M., 2011. Ovary growth and protein levels in
429 ovary and fat body during adult-wintering period in the red mason bee, *Osmia rufa*. Apidol 42, 749-
430 758.
431 Willis, J.H., Brunet, P.C.J., 1966. The hormonal control of colleterial gland secretion. J Exp Biol 44, 363-
432 378.
433 Winston, M.L., 1987. The biology of the honey bee. Harvard University Press, Cambridge, MA.
434 Wuellner, C.T., 1999. Alternative reproductive strategies of a gregarious ground-nesting bee, *Dieunomia*
435 *triangulifera* (Hymenoptera: Halictidae). J Insect Behav 12, 845-863.
436 Yanega, D., 1989. Caste determination and differential diapause within the first brood of *Halictus*
437 *rubicundus* in New York (Hymenoptera, Halictidae). Behav Ecol Sociobiol 24, 97 - 107.
438 Yanega, D., 1992. Does mating determine caste in sweat bees - (Hymenoptera, Halictidae). J Kansas
439 Entomol Soc 65, 231-237.