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7 Nutritional, endocrine, and social influences on reproductive physiology at the origins of social behavior

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13

14 **Abstract**

15 Understanding the evolutionary origins of social behavior in insects requires understanding the
16 physiological basis for reproductive plasticity. Solitary bees and wasps, or those living in small, flexible
17 societies, will be key to understanding how conserved pathways have evolved to give rise to
18 reproductive castes. Nutrient-sensing and endocrine pathways are decoupled from reproduction in
19 some life stages of social insects. Heterochrony, particularly as it is related to diapause physiology, may
20 be an important mechanism by which this decoupling occurs. Additional research is needed to
21 understand how these pathways became sensitive to cues from the social environment. Future research
22 targeting species with a diversity of social behaviors and diapause strategies will be key to
23 understanding the physiological basis of social evolution.

24

25 **Highlights**

- 26 • Nutrient-sensing, endocrine pathways decoupled from reproduction in social insects
- 27 • Diapause requirements may shape the evolution of these relationships
- 28 • Heterochronic shifts in these pathways may facilitate the origins of castes
- 29 • Sensitivity to the social environment evolves with eusociality

30 **Introduction**

31 Variation in reproductive success is one of the defining features of sociality amongst insects, whereby
32 some individuals lay eggs and others care for eggs laid by their nestmates. Yet surprisingly little is known
33 about the physiology underlying this reproductive variation in most social insect species. This is
34 particularly true for species representative of the solitary ancestors from which sociality was derived or
35 species that represent the earliest stages of this transition, in which behavior and reproduction are
36 flexible among castes. Understanding the factors that influence reproductive physiology in these groups

37 will provide a foundation upon which to build our understanding of how changes in these relationships
38 have given rise to fixed reproductive castes found among the most advanced social insect species.

39

40 Here I review recent insights into the nutritional, endocrine, and social influences on reproductive
41 physiology in the Hymenoptera (bees, ants, wasps), with particular focus on those species that are
42 solitary or with social organization representative of the early stages of social evolution. The
43 physiological basis of reproductive development in ants (Family Formicidae), honey bees (*Apis*
44 *mellifera*), and bumble bees (*Bombus* sp.) has been thoroughly reviewed elsewhere [1-4]. I present the
45 highlights of this research primarily to establish the foundation for comparison to reproductive
46 physiology in those species more closely aligned with conditions at the origins of eusociality.

47

48 **Overview of reproductive physiology in non-Hymenopteran insects**

49 Egg maturation (i.e., oogenesis) involves synthesis and incorporation of essential compounds, such as
50 hormones and nutrients necessary for development, into developing oocytes [5]. The target-of-
51 rapamycin (TOR) and insulin/insulin-like signaling (IIS) pathways are the two most highly conserved
52 nutrient sensors involved in regulating this process, while juvenile hormones (JH) and ecdysteroids are
53 classes of highly conserved lipophilic hormones that interact with these nutrient sensing pathways [6].
54 These pathways play an important role in reproductive development in insects, but the details of how
55 they influence each other and their relative positions within reproductive regulatory networks are highly
56 variable across species [6].

57

58 In most insects, activation of the IIS and TOR stimulates the synthesis of JH and ecdysteroids [5,6]. The
59 TOR pathway can be activated directly when free amino acids bind to receptors on the cellular
60 membrane [5]. IIS is a systemic nutrient sensor, because insulin like peptides (ILPs) are released

61 primarily from the brain and bind to insulin receptors (IRs) in the periphery (e.g., ovary, fat body) in
62 response to glucose [5,7]. The IIS also stimulates the TOR pathway via the binding of ILPs to IRs on the
63 cellular membrane, so TOR participates in both a direct and indirect response to nutrients [6]. JH is
64 synthesized in the corpus allatum (CA), and circulates in the hemolymph. When detected by the fat
65 body, it triggers transcription of the *Vg* gene responsible for transcribing the yolk precursor protein
66 vitellogenin [8]. JH is the sole regulator of *Vg* in most insects, but in some lineages (e.g., Diptera),
67 ecdysteroids are more important for egg development [6,8]. Ecdysteroids are a class of cholesterol-
68 derived hormones that includes ecdysone (E) and 20-hydroxy-ecdysone (20E), all of which are
69 synthesized in the ovary, and stimulate the uptake of yolk by developing oocytes [5,9]. Activation of the
70 IIS and TOR pathways also triggers the transcription of *Vg*, and thus stimulates vitellogenesis both
71 directly and indirectly through endocrine pathways [6,10-13].

72

73 Variation in how these pathways regulate reproductive physiology within and among species likely
74 promotes and enables the immense diversity of reproductive strategies found among insects. This is
75 likely a prerequisite for the origin of social insect castes, among which nutrient-sensing and endocrine
76 pathways can function independently of each other and are decoupled from reproduction in some
77 individuals.

78

79 **Nutritional influences on caste-related reproductive physiology may be constrained by diapause**

80 Among the Hymenoptera, the nutritional influences on reproductive development are best understood
81 in two species: honey bees that live in highly eusocial societies and bumble bees that are obligately
82 eusocial, but go through a solitary phase during nest initiation each year. In these species, nutrition
83 plays an important role in caste determination, and in parallel, reproductive development, but with
84 some important differences. In the earliest stages of honey bee development, the relationship between

85 the IIS, TOR and the lipophilic endocrine pathways are mostly similar to patterns observed in other
86 insects. Early stage larvae destined to become queens receive more of the highly proteinaceous royal
87 jelly, which leads to elevated JH titers via activation of the IIS and TOR pathways [4,14-16]. However, the
88 regulatory relationship between nutrition, IIS, and reproduction is drastically altered among later larval
89 stages and adults. Among fourth and fifth instar larvae, expression of both insulin receptor genes and
90 *tor* are down-regulated in queen-destined larvae [4,17]. Although adult queens continue to have better
91 access to nutritious royal jelly, and thus more nutrient stores than workers, they have lower expression
92 of IIS genes [18].

93

94 In contrast, bumble bee reproduction seems to be mediated by a conserved relationship between
95 nutrition and physiology. In the European buff-tailed bumble bee (*B. terrestris*), *ILP*, *insulin-like growth*
96 *factor-1 (IGF-1)*, and several hexamerin protein storage transcripts are more abundant in reproductive
97 queens than in workers or virgin and diapausing queens [19,20]. Conversely, insulin receptors *InR-1* and
98 *InR-2* are down-regulated in reproductive queens, which is consistent with known feedback responses
99 within an activated IIS [20].

100

101 This difference in the relationship between nutrient-signaling and reproductive physiology in honey bees
102 and most other insects, including bumble bees, is generally assumed to be a product of advanced stages
103 of eusocial evolution [1,21]. While this is likely true, there is another difference between honey bees and
104 most other insects in which reproductive physiology has been studied – diapause (Figure 1). Unlike
105 bumble bees and many other insects, honey bees are able to survive the winter on food stores in the
106 hive, and thus avoid diapause. In most other temperate insects, however, nutrient signaling also plays a
107 major role in diapause [22,23]. In fact, genes involved in diapause regulation in bumble bee queens also
108 tend to be differentially expressed between queens and workers [23]. This suggests the effects of

109 nutrition on reproductive physiology and the evolution of social castes is likely constrained by diapause
110 requirements.

111

112 Clues regarding how the influence of diapause has shaped the relationship between nutrition and
113 reproduction over the course of social evolution come from studies in species with more flexible social
114 organization. Paper wasps in the vespid subfamily Polistinae live in small groups with a reproductive
115 division of labor and cooperative brood care. Unlike for obligately eusocial species, polistine castes are
116 not morphologically specialized, and they retain totipotency throughout their lifetimes. This flexibility
117 provides insight into physiological changes that accompany the earliest stages of caste evolution. Among
118 new nest foundresses, workers, and queens of *Polistes metricus*, lipid stores and ovary development are
119 positively correlated, and are also correlated with expression of IIS genes [24]. Consistent with this,
120 *hexamerins* and several genes in the IIS are up-regulated in queen-destined larvae, as compared to
121 worker-destined larvae [25,26]. Nutrient restriction of lab-reared larvae led to significant up-regulation
122 of genes involved in lipid metabolism, though the resulting transcriptional profile was only partially
123 similar to that of workers [27]. A separate study with a similar experimental design found significant
124 effects of larval diet on reproductive development at emergence [28]. When protein was restricted for
125 larvae-rearing foundresses, offspring emerged with traits very similar to that of workers, including more
126 developed ovaries, than when protein was unrestricted or supplemented. *P. metricus* gynes (i.e.,
127 females that will become nest foundresses the following spring) emerge from development with
128 inactivate ovaries, ready to overwinter in diapause, while worker ovaries are somewhat activated at
129 emergence, since they do not overwinter [29]. Indeed, ovarian development among *P. dominula*
130 workers has been shown to depend on diet upon emergence [29]. Collectively, these results suggest
131 larval diet has more direct influence over diapause requirements than reproductive development in
132 *Polistes* wasps, but diet directly influences reproductive physiology in adults.

133

134 Larval nutrition may also influence the reproductive fate of females that function similarly to workers in
135 the small carpenter bee, *Ceratina calcarata* [30]. As larvae, these so-called eldest dwarf daughters
136 receive a smaller pollen mass, composed of different floral resources, than the pollen provided to her
137 reproductive siblings. Upon emergence, the queen physically coerces this daughter into foraging for the
138 colony [31]. Presumably as a result of her small size and energy expenditure during foraging, this
139 daughter is unable to diapause, and thus never becomes reproductive. This influence of larval diet on
140 diapause outcome in a temperate species further suggests that nutritional influences on reproduction,
141 and its correlated social behavior, may have evolved under constraints related to the physiology of
142 diapause.

143

144 Tropical species that do not diapause thus make for an interesting comparison, because the influence of
145 diet on reproductive physiology is likely to be more congruent across life stages. *Megalopta genalis*
146 (Halictidae) is a tropical sweat bee with flexible social behavior [32]. A recent study found that several
147 genes in the glycolysis pathway are up-regulated in worker abdominal tissue in this species, as
148 compared to reproductive females [33]. The glycolysis pathway is activated primarily in the fat body,
149 and is responsive to the nutrient-sensing IIS and TOR pathways [34]. This up-regulation could thus
150 indicate that *M. genalis* workers are storing energy in response to low nutrient levels, which is known to
151 inhibit reproductive development in other insect orders [5,6]. The finding that *M. genalis* workers have
152 significantly lower vitellogenin titers than queens is also consistent with a conserved pattern of IIS/TOR
153 regulation on reproductive physiology in this species [35]. *M. genalis* workers perform the majority of
154 the foraging for their colony, and donate food to nestmates through trophallaxis [36,37]. It is therefore
155 likely that they are undernourished, and that this may play a role in reproductive suppression.

156

157 Larval nutrition is also likely to influence reproductive physiology in this species. Among cells collected
158 from *M. genalis* nests, the quantity and quality of larval provisions found with female-destined eggs was
159 significantly more variable than those found with male-destined eggs, which is what one would expect if
160 nutrition was an important factor in reproductive caste determination [38]. Moreover, when newly
161 emerged females are kept in the lab under standard environmental and nutritional conditions, some
162 females reach reproductive maturity, while others do not develop ovaries at all [35]. The primary source
163 of this variance is likely to be the amount of nutrient stores these females have carried over from
164 development, and thus variation in larval diet.

165

166 An effect of size and quality of larval diets on reproductive caste determination has also been observed
167 in temperate halictid bees that diapause, including *Halictus ligatus* [39] and *H. scabiosae* [40].

168 Interestingly, worker-destined and queen-destined diets in these species differed in sugar content,
169 which contributes to lipid stores [41], and is thus likely to have an indirect effect on reproduction
170 mediated through diapause survival [22]. In the non-diapausing *M. genalis*, protein content, but not
171 sugar content, varied among female larval diets [38]. Protein stores are more likely to influence
172 oogenesis, as amino acids stimulate the TOR pathway and are necessary for vitellogenesis [5,6]. This
173 may reflect a direct influence of larval diet on reproductive physiology, independent of diapause. This
174 provides additional support for the hypothesis that evolutionary changes in the role of nutrient-signaling
175 on reproductive maturation related to caste determination may be different for diapausing and non-
176 diapausing species. Additional comparisons of dietary influence on reproductive maturation in closely
177 related species that differ in social behavior, but also with different diapause patterns, will be necessary
178 to test this hypothesis.

179

180 Solitary bees and wasps will be especially important in these comparisons, as they most closely
181 represent the ancestors that gave rise to eusociality. However, the requirements for reproductive
182 development have not been studied in most solitary Hymenoptera, and especially those that vary in
183 diapause patterns. Progress in this area was recently made with a greenhouse study of the blue orchard
184 bee, *Osmia californica*. All emerging females had small, undeveloped terminal oocytes, and only those
185 females that consumed pollen (the primary dietary source of protein for bees) were able to complete
186 oogenesis and commence egg-laying after 10 days [42]. It is not clear how far this result extends to
187 other solitary Hymenoptera, because *Osmia* overwinter as adults, and reproductive development is
188 initiated prior to breaking diapause [43]. Another member of the solitary family Megachilidae,
189 *Megachile rotundata*, overwinters as prepupae and can develop mature oocytes when reared in the
190 laboratory with access to pollen and mates [44]. Whether either or both of these is required has not
191 been tested.

192

193 Conversely, protein consumption did not have a significant effect on ovary activation in newly emerged
194 females of the solitary alkali bee (*Nomia melanderi*) [45]. These halictid bees diapause as prepupae, and
195 activate reproductive maturation upon emergence. Lab-reared 10-day-old females had significantly
196 longer terminal oocytes than newly emerged bees, whether they were given access to protein or not.
197 However, the lab-reared bees did not develop fully mature oocytes, which could suggest that mating or
198 ecological cues are necessary to complete oogenesis in this species, as for many insects [46].

199 Nevertheless, protein is likely required for continued egg production throughout the season, because it
200 was recently discovered that alkali bees consume pollen on a daily basis [47]. Similar experiments
201 involving additional species of solitary bees and wasps are needed to determine how nutrition is linked
202 to reproductive physiology across the Hymenoptera. These studies will also need to address the effects
203 of larval nutrition on diapause outcomes and reproductive maturation in order to test the hypothesis

204 that the link between nutrition and reproduction has been shaped by diapause physiology, in addition to
205 social evolution.

206

207 **Endocrine influences on reproductive physiology may evolve via heterochrony**

208 The evolutionary origins of the queen and worker caste must have necessarily accompanied functional
209 changes in endocrine pathways, but the nature of these changes is unknown. The ancestor that gave rise
210 to social insect castes is presumed to have had endocrine-mediated ovarian cycles coupled with
211 maternal care behavior, such that periods of foraging and brood defense occurred during a period of
212 ovary inactivation following egg-laying [48,49]. This hypothesis, called the ovarian ground plan
213 hypothesis (OGPH), posits that changes in endocrine cycles that bridge these correlated suites of
214 behavior and physiology enabled decoupling of discreet stages of the ancestral cycle. This decoupling
215 gave rise to queens specialized on egg-laying with activated ovaries and workers specialized on brood
216 care and foraging with inactivated ovaries [48,49]. In support of this hypothesis, there is substantial
217 evidence that JH and ecdysteroids have attained novel functions in highly social species (e.g., honey
218 bees [50,51], ants [52,53], swarm-founding wasps [54]), and species with flexible social behavior (e.g.,
219 *Polistes* wasps [55,56]). This indicates that there have been evolutionary changes in the endocrine
220 pathways mediating the association between behavior and reproductive physiology, but the ancestral
221 pathways from which these changes evolved are unknown.

222

223 One of the most significant of these changes has been disruption of the regulatory relationship between
224 JH and vitellogenesis in adult honey bees [51] and some ants [57,58]. At least for honey bees, however,
225 the conserved gonadotropic effects of JH are conserved during the final stages of pre-imaginal
226 development [4,59,60]. This temporal shift in function of JH led Rodrigues & Flatt [61] to hypothesize

227 that heterochrony is a key axis of endocrine flexibility that may have allowed for the decoupling, and
228 subsequent neofunctionalization, of endocrine pathways in social evolution.

229

230 There may be some evidence to support this hypothesis in bumble bees. In gynes preparing for
231 diapause, nutrient sensing pathways normally stimulate lipid storage, rather than JH synthesis and
232 oogenesis. However, JH treatments during this stage leads to ovary activation at the expense of nutrient
233 storage [62]. Upon emergence the next spring, JH titers, ecdysteroids, and *Vg* expression increase and
234 remain high in egg-laying queens [21,63]. This suggests that the conserved gonadotropic role of these
235 hormones is intact among bumble bee queens. These relationships are less clear among queenless
236 workers, however. JH treatment in newly emerged queenless workers leads to an increase in *Vg*
237 expression and subsequent ovary activation by 7 days of age [64], but no JH-associated increase in *Vg*
238 expression was observed after 4 days [21]. One possible explanation for this seemingly inconsistent
239 result is that the role of JH in reproductive development is temporally variable in workers, as suggested
240 by the heterochrony hypothesis of Rodriguez & Flatt [61].

241

242 Temporal plasticity of conserved endocrine pathways may also occur in the paper wasp lineage, but this
243 is most evident at the colony level. A gonadotropic function of JH has been demonstrated in *Ropalidia*
244 *marginata* [65] and among pre-diapause gynes of *Belonogaster longitarus* [66] and *B. petiolata* [67]. *P.*
245 *dominula* queens and queenless workers have higher JH titers than workers in queenright colonies [68],
246 and JH treatments increase fertility in queens [69] and newly emerged workers [70] in this species and
247 the closely related *P. metricus* [71]. However, a recent study performed in the late pre-emergent phase
248 of the colony cycle (e.g., with foundresses, pupae, but no adult offspring) found no association between
249 JH or ecdysteroids with ovary activation in either *P. dominula* or *P. smithii* [72]. This is in contrast to
250 findings from studies that focus on the earliest stages of colony founding in *P. dominula* (e.g., with

251 foundresses, but no brood) [73,74]. Together, these results indicate that JH can become dissociated
252 from its gonadotropic function within very short time periods of an individual lifetime, and this
253 functional shift is likely condition-dependent [69,70].

254

255 Additional insight regarding endocrine-mediated reproductive plasticity comes from recent studies of
256 swarm-founding paper wasps. Queens of the neotropical *Synoeca surinama* have higher ovarian
257 ecdysteroids and JH titers than workers [75]. This species displays advanced features of sociality, such as
258 swarm founding, but has retained caste flexibility, such that queens can transition to workers, and vice
259 versa, depending on colony need and social opportunity. These results thus provide insight into the
260 endocrine aspects of reproductive plasticity, disentangled from other aspects of social behavior.

261

262 The tropical facultatively eusocial bee *M. genalis* provides similar insight. Foundresses in the early stages
263 of nest initiation have higher JH levels than age-matched workers, suggesting that the gonadotropic
264 function of JH is conserved in this species [76]. However, older reproductive females in solitary nests do
265 not have significantly higher JH levels than workers, which could suggest that JH function changes with
266 age or reproductive phase. Along these lines, reproductive queens have significantly higher JH levels
267 than age-matched reproductive solitary females, which suggests that JH has attained novel functions
268 related to social dominance in this species.

269

270 Very little is known about the function of hormones in reproductive physiology of solitary bees and
271 wasps. Repeated treatments with the JH-analog methoprene led to increased oocyte development in
272 fertilized females of the solitary eumenine wasp *Euodynerus foraminatus* [77] and the solitary
273 megachilid bee *O. rufa* [78]. Both of these species overwinter as adults, but the wasp study was
274 performed on newly emerged females prior to diapause, while the bee study was performed on

275 diapausing females. The observed gonadotropic effects of the methoprene treatments were thus
276 coupled with simultaneous effects on diapause termination. This may suggest that JH retains its
277 gonadotropic effects on reproductive development throughout most phases of the early life-stages in
278 solitary Hymenoptera.

279

280 The effects of JH have only been studied in one solitary species that overwinters in a pre-adult stage. JH
281 promotes ovary activation in unmated, newly emerged females of the solitary alkali bee (*N. melanderi*)
282 [45,79]. However, JH does not have any short term effects (< 4 h) on reproductive organs among actively
283 nesting females [79]. This suggests there could be a critical window in which JH is most likely to impact
284 reproductive development in solitary bees. Further research on endocrine function in different life
285 stages of solitary species is imperative for fully testing the heterochrony hypothesis.

286

287 **Caste-related reproductive physiology evolves via sensitivity to social cues**

288 A notable source of variation in reproductive physiology among ants, bees, and wasps is the role of the
289 social environment on nutritional and endocrine pathways. Exposure to larvae as young adults has
290 significant effects on *Vg* expression, JH levels, ovary activation, and foraging behavior among honey bee
291 workers [80]. Likewise, exposure to queen pheromones suppresses ovarian activation [81] and JH
292 synthesis [82] in worker honey bees, and this is mediated through the Notch signaling pathway in the
293 ovaries [83]. In bumble bees (*B. impatiens*), however, exposure to pheromones of either brood or
294 queens is not enough to suppress oogenesis, *Vg* expression, or JH levels among workers [84], and this is
295 consistent with what has been found for *B. terrestris* [85,86]. However, direct aggression from queens
296 and other workers can inhibit *Vg* expression and ovarian activation in these species [21,84].

297

298 In vespid wasps, the social environment influences reproductive physiology via endocrine pathways. In
299 *P. dominula*, JH levels increased among workers after queen removal [68]. Under queenless conditions,
300 JH is associated with aggression, but no such association exists in queenright colonies. This suggests that
301 endocrine *function* is also responsive to the social environment, and this may be a mechanism by which
302 neofunctionalization occurs during social evolution [70]. The social environment also appears to regulate
303 JH in the epiponine wasp *S. surinama*, as JH titers increase in replacement queens when they are left
304 alone on a nest [75].

305

306 There is some evidence that the social environment also regulates reproductive physiology in *M. genalis*.
307 When workers are left alone in the nest, they become reproductive, and this is associated with down-
308 regulation of genes in the glycolysis pathway in the abdomen [33,87]. This suggests that social inhibition
309 of reproduction could be mediated through nutrient-sensing channels in this species. Associations
310 between behavior and JH levels suggest that aggressive queen-worker interactions may contribute to
311 reproductive suppression in workers [32,37,76], but this hypothesis has not been tested experimentally.
312 Aggression is also associated with fertility in the sub-social carpenter bee, *C. calcarata*, but whether
313 social interactions directly influence reproductive physiology is unknown [88].

314

315 The social environment was found to have no influence on reproductive physiology in the solitary alkali
316 bee (*N. melanderi*). In a recent study, newly emerged females were given JH or control treatments, and
317 reared in cages either alone or with an older, reproductive female [45]. The social treatment did not
318 influence the effects of JH on ovary activation. Comparisons between the nutritional and endocrine
319 pathways regulating reproduction in alkali bees and social halictid bees are likely to reveal the
320 evolutionary mechanisms by which reproductive networks become sensitive to cues from the social
321 environment.

322

323 **Future directions**

324 Filling the gaps in our understanding of reproductive physiology at the origins of sociality will clearly
325 require research involving additional species, particularly those that most closely resemble the
326 ancestors that gave rise to sociality. Basic studies of the nutritional, endocrine, and social influences on
327 reproductive development in solitary species in the families Apidae, Halictidae, and Vespidae will
328 provide the phylogenetic scaffolding required for comparative physiology (Figure 1). Such studies will be
329 most informative if they target species that vary in diapause patterns, and include both larval and adult
330 stages of development. It is likely that plasticity in the coupling of nutrient-sensing and endocrine
331 pathways underlying reproductive development enabled the evolutionary origins of castes.
332 Understanding crosstalk among these signaling pathways in species close to the origins of sociality thus
333 promises to be a fruitful area of future research.

334

335 **Figure Legend**

336

337 Figure 1. Summary of social biology and reproductive physiology in species covered in this review. ✓:
338 experimentally-validated effects on reproductive physiology; +: correlative evidence to suggest effects;
339 X: effects have been tested and were not found; ?: no direct tests have been made. Diapause stage – A:
340 adult, P: prepupae; - : no diapause; Nest-founding strategy – S: solitary, F: facultative cooperation, Sw:
341 swarm-founding; V: variable

342

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