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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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