



Queen control of a key life-history event in a eusocial insect

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3 **Queen control of a key life-history event in a eusocial insect**

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16 In eusocial insects, inclusive fitness theory predicts potential queen-worker conflict over
17 the timing of events in colony life history. Whether queens or workers control the
18 timing of these events is poorly understood. In the bumblebee *Bombus terrestris*, queens
19 exhibit a 'switch point' in which they switch from laying diploid eggs yielding females
20 (workers and new queens) to laying haploid eggs yielding males. By rearing foundress
21 queens whose worker offspring were removed as pupae and sexing their eggs using
22 microsatellite genotyping, we found that queens kept in the complete absence of adult
23 workers still exhibit a switch point. Moreover, the timing of their switch points relative
24 to the start of egg-laying did not differ significantly from that of queens allowed to
25 produce normal colonies. The finding that bumblebee queens can express the switch
26 point in the absence of workers experimentally demonstrates queen control of a key life-
27 history event in eusocial insects. In addition, we found no evidence that workers affect
28 the timing of the switch point either directly or indirectly via providing cues to queens,
29 suggesting that workers do not fully express their interests in queen-worker conflicts
30 over colony life history.

31

32 **Keywords:** *Bombus*; eusocial insect; life history; queen control; switch point

33

34

35 1. INTRODUCTION

36 The evolution of eusocial societies represents a prime example of a major transition in
37 evolution leading to a new level of individuality [1, 2]. For this reason, colonies of eusocial
38 insects undergo a life history analogous in some respects to that of individual organisms [3].
39 For example, in annual eusocial Hymenoptera, the change from colony growth (worker
40 production) to reproduction (production of new queens and/or males) is a key life-history
41 event because it represents sexual maturation at the colony level. Inclusive fitness theory
42 predicts potential queen-worker conflict over both sex allocation [4] and the timing of the
43 colony's sexual maturation [5, 6]. The outcomes of such conflicts depend on which party, or
44 parties, within the colony 'control' the relevant trait. Control here refers to any processes,
45 either behavioural or physiological, which allow a given party to affect the trait, including
46 responses to the external environment. By determining the primary sex ratio [7, 8], i.e. the
47 ratio of haploid to diploid eggs laid, queens in the eusocial Hymenoptera potentially exert
48 considerable control in queen-worker conflicts. However, workers may also exert control,
49 through differential rearing of offspring or, as in worker matricide, differential treatment of
50 queens [8, 9]. Additionally, the timing of colony sexual maturation may depend on queens
51 responding to cues provided by workers. Such cues might provide information either on the
52 colony's growth stage or on external environmental conditions such as resource availability
53 [e.g. 10]. They might also provide a means by which workers could indirectly manipulate the
54 timing of colony sexual maturation in their own interests. There has been considerable focus
55 on queen control of the primary sex ratio with respect to sex allocation [8]. But whether such
56 control extends to colony sexual maturation, and whether workers can influence this event,
57 either directly or indirectly, has not been experimentally tested.

58

59 The bumblebee *Bombus terrestris* is an annual eusocial insect in which colonies are founded
60 by single queens in spring and produce first workers and then sexuals (new queens and
61 males) before dying out in late summer [11]. Queens exhibit a well-characterized 'switch
62 point' in which, over approximately 8 days, they change from laying diploid eggs yielding
63 females (workers or new queens) to laying haploid eggs yielding males [11, 12]. The switch
64 point typically occurs 2–4 weeks after the eclosion of the first worker (emergence from pupa)
65 [11, 13]. Along with the laying of diploid eggs yielding new queens, which tends to happen
66 shortly beforehand, the switch point marks the colony's sexual maturation [11, 13]. Since
67 potential queen-worker conflict over sex allocation and colony sexual maturation are both
68 present [5, 6], and since queen and workers might benefit from facultatively adjusting the
69 timing of male production to match local conditions [12, 13], it has been hypothesized that
70 the social (colony) environment should affect the switch point. However, previous work has
71 shown little evidence for this, since experimental manipulations of *Bombus* colonies,
72 including doubling worker number in *B. terrestris*, had no significant effect on the timing of
73 male production [14-16]. Queens do not switch to laying haploid eggs through having
74 exhausted their supplies of stored sperm, since post-switch queens retain plentiful, viable
75 sperm [17].

76

77 We therefore hypothesized: (1) that *B. terrestris* queens can control the occurrence of the
78 switch point endogenously [cf. ref. 11], i.e. in the complete absence of workers; and (2) that
79 workers do not influence the timing of the switch point directly or indirectly. We tested these
80 hypotheses in a single experiment in which we manipulated the presence of workers within
81 incipient colonies and recorded the occurrence and relative timing of switch points.

82

83 2. MATERIALS AND METHODS

84 Post-diapause, mated *Bombus terrestris terrestris* queens ($n = 328$) were obtained from a
85 commercial supplier in three cohorts (groups received on successive dates) and housed singly
86 in boxes in standard conditions (see electronic supplementary material). The date on which
87 each queen laid her first egg was noted ($n = 138$ queens). Immediately after each queen had
88 produced her first pupa, i.e. her oldest larva had pupated, she was assigned to a 'social' or
89 'asocial' treatment ($n = 41$ queens). The social treatment allowed queens to raise a colony in
90 the normal way. In the asocial treatment, any pupal cocoons were removed before eclosion
91 and discarded. The asocial treatment therefore allowed queens to lay eggs but prevented
92 them from being exposed to any adult offspring. To control for effects of disturbance, equal
93 numbers of cocoons were removed from social queens and then returned.

94

95 Every new egg-cell produced by queens in both treatments was removed, censused, sampled
96 for some of its eggs (1–2 eggs removed per egg-cell), resealed and replaced. All removed
97 eggs ($n = 1352$) were frozen for sexing. Colonies were terminated following either the death
98 of the queen or male eclosion, or, if these events had not occurred, 4–6 months after the
99 beginning of the experiment.

100

101 Sampled eggs were sexed using genotyping at five polymorphic microsatellite loci (see
102 electronic supplementary material). Queens were also genotyped. Egg genotypes were
103 scored blindly with respect to sampling date and treatment, and then reconciled with the
104 genotypes of each queen \times mate combination, as deduced from the queen and egg genotypes.

105 A queen's switch point was defined as the number of days between her first egg and her first
106 observed haploid egg (as inferred from the egg genotypes). A two-way ANOVA was used to
107 test whether switch point was affected by social versus asocial conditions. All means are
108 expressed ± 1 SD.

109

110 **3. RESULTS**

111 Of 41 queens producing pupae, 17 were assigned to the social treatment and 24 to the asocial
112 treatment. Of these, 10 and 7 queens, respectively, produced sufficient eggs (>5) that could
113 be sexed (see electronic supplementary material) and exhibited a switch point. Among these
114 'switching queens', the mean switch points of social and asocial queens were 53.0 ± 8.4 and
115 56.0 ± 14.3 days, respectively (figures 1a, 2). These switch points did not differ significantly
116 (two-way ANOVA, $F_{1, 15} = 0.16$, $p = 0.697$). Furthermore, there was no significant effect of
117 cohort ($F_{2, 14} = 1.71$, $p = 0.216$) on switch point, or significant treatment-cohort interaction
118 ($F_{2, 11} = 3.17$, $p = 0.082$). Switch points of the asocial queens did not differ significantly
119 (Wilcoxon rank sum continuity test, $W = 775$, $n = 177$, $p = 0.239$) from simulated switch
120 points calculated by re-sampling eggs from social queens at sample sizes equivalent to those
121 obtained in asocial queens (see electronic supplementary material). This showed that lower
122 sample sizes of eggs for asocial queens (figure 2) did not bias the switch point estimates.
123 However, a power analysis showed that the minimum detectable difference in the switch
124 points of our samples was 11–12 days (see electronic supplementary material).

125

126 Queens that failed to exhibit a switch point laid their last diploid egg significantly earlier than
127 the switch point of switching queens (43.0 ± 13.5 v. 54.2 ± 10.9 days after first egg,
128 respectively; Welch's t -test, $t_{15} = 2.36$, $p = 0.032$), suggesting that non-switching queens
129 failed to switch because they had stopped laying eggs before the switch point was reached.
130 Among switching queens, social queens laid significantly more eggs (over 3 times more)
131 before the switch point than asocial queens (Welch's t -test, $t_{11} = 4.71$, $p < 0.001$; figure 1b).
132 Asocial queens almost certainly laid fewer eggs through lacking resources and aid supplied
133 by workers, and this, combined with earlier cessation of egg-laying leading to a lower

134 probability of switching, would account for the smaller proportion of asocial queens that
135 exhibited a switch point relative to social queens.

136

137 4. DISCUSSION

138 We found that queens of the bumblebee *Bombus terrestris* reared in asocial conditions, in
139 which they were never exposed to their adult worker offspring, switched to laying haploid,
140 male eggs as did social, control queens allowed to produce adult worker offspring in the
141 normal way. This result experimentally demonstrates that the switch point, which represents
142 a key life-history event in colony development, can be controlled by queens endogenously.
143 Moreover, we found no significant difference in the timing of the switch point between social
144 queens and asocial queens. However, our data do not preclude an influence of workers on the
145 timing of the switch point within the limits specified by the power analysis. Nonetheless, we
146 found no evidence that workers exert a large influence over colony sexual maturation either
147 directly or indirectly, suggesting that workers do not express their interests fully in queen-
148 worker conflicts over colony life history.

149

150 An endogenous mechanism of determining the switch point permits *B. terrestris* queens to
151 exercise control in kin-selected conflicts with workers over sex allocation [18] and colony
152 sexual maturation [5, 6]. However, the switch point may still be responsive to external cues.
153 For example, Duchateau *et al.* [12] found that *B. terrestris* queens undergoing longer periods
154 of diapause exhibited earlier switch points, suggesting that queens use 'personal' cues
155 stemming from their pre-founding or founding experience and/or their own quality to
156 modulate the timing of the switch point.

157

158 We hypothesize that queens use such personal cues to initiate an internal interval timer [19]
159 that 'counts down' to the switch point. This hypothetical timer appears to be independent of
160 periodism in light levels and temperature, since queens were kept in darkness at constant
161 temperature. Queens do not lay a standard number of diploid eggs before switching to laying
162 haploid eggs, since we found that social queens laid significantly more eggs than asocial
163 queens before switching even though they switched at the same relative date. Overall, the
164 proximate mechanism underlying endogenous queen control of the switch point in *B.*
165 *terrestris* is unknown and deserves future investigation.

166

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216

217 Figure legends

218

219 Figure 1. (a) Time of the switch point (days from queen's first egg) and (b) number of eggs
220 laid before the switch point in *Bombus terrestris* queens in social ($n = 10$) and asocial ($n = 7$)
221 treatments. Diamonds, means; thick horizontal bars, medians; boxes, interquartile range;
222 whiskers, range. (a) NS, not significant (ANOVA); (b), ***, $p < 0.001$ (Welch's t -test).

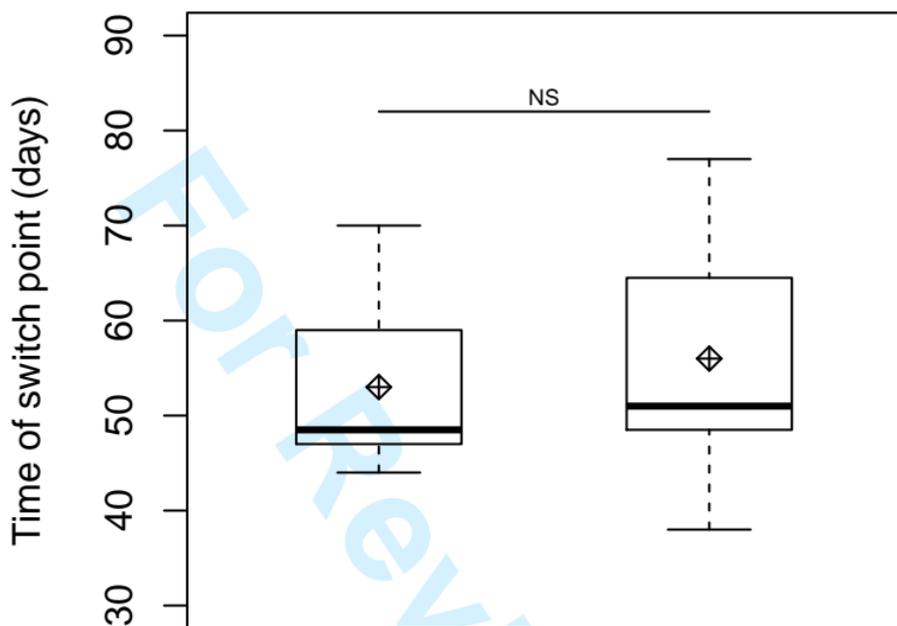
223

224 Figure 2. The numbers of sampled diploid and haploid eggs laid by *Bombus terrestris* queens
225 over time in (a) social and (b) asocial treatments ($n = 10$ and 7 queens, respectively). Each
226 plot represents a separate queen (id code in upper left corner). White shading, diploid eggs;
227 black shading, haploid eggs; total heights of bars, number of eggs genotyped in each time
228 bloc.

229

230

a)



b)

