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1 **Evidence of a fixed polymorphism of one-year and two-year larval growth in the**
2 **myrmecophilous butterfly *Maculinea rebeli***

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Running title: polymorphic growth in *Maculinea rebeli*

Keywords: larval development, fixed polymorphism, social parasites, *Myrmica* ants,
Maculinea rebeli, *alcon X*, *Phengaris*

Abstract

1. *Maculinea* (= *Phengaris*) species of butterfly have complex life-styles in which the larvae feed briefly on specific foodplants before entering the final instar, after which they live as social parasites within *Myrmica* ant colonies and acquire 98%-99% of their ultimate biomass by either preying on ant brood (predatory species) or being fed by worker ants (cuckoo species). Cuckoo species demonstrate two growth forms, whereby some individuals develop to pupate in 11 months and others remain 23 months in the nest, a strategy that in theory represents the most efficient exploitation of resources within *Myrmica* societies. Early studies suggested that development rates in *Maculinea rebeli* (referred to by other authors as the xerophilous form of *Maculinea alcon*- or *M. alcon X*) were not plastic but represented an unusual fixed polymorphism, in which eggs were genetically predetermined as 1- or 2-year larvae.
2. We tested this idea in lab experiments using *M. rebeli* from the French Hautes-Alpes, with these results: (i) The growth strategy of every individual larva is pre-determined by the mother either genetically or, less probably, during oogenesis. (ii) Intra-nest competition and host fitness affects survival and growth but does not influence the inherent growth strategy. (iii) The ratio of 1- and 2-year developing larvae is initially about 50:50, although differential survival in crowded nests may alter the ratio of surviving individuals. (iv) All females tested laid eggs that hatched into both 1- or 2-year developers. (v) Growth patterns vary between different regions. Larvae from the Spanish Pyrenees initially grow less than those from the Alps, with a smaller initial differentiation between the size classes.

58 **Introduction**

59 *Maculinea* (= *Phengaris*) van Eecke, 1915 species oviposit on specific food plants where
60 their larvae develop quickly to the fourth and final instar but achieve little growth (Elmes *et*
61 *al.*, 2001). They then drop to the ground and employ mimetic behaviours, semio-chemicals
62 and acoustics to trick foraging ants of the genus *Myrmica* Latreille, 1804 into treating them as
63 members of their society and carry them into the nest, where they are placed with the ant
64 brood (Thomas, 1984, 2002; Elmes *et al.*, 1991a; Akino, 1999; Als *et al.*, 2001; Thomas *et*
65 *al.*, 2005, 2013; Nash *et al.*, 2008; Barbero *et al.*, 2009). Caterpillars remain underground for
66 a minimum of 10 months, gaining >98% of their pupal weights in the final instar, an
67 exceptional growth pattern for any insect (Elmes *et al.*, 2001). All live as social parasites
68 (*sensu* Wilson & Hölldobler, 1990) of their ant hosts, preying solely on ant larvae and eggs
69 (predatory species) or being fed directly by the worker ants, often combined with low level
70 predation during the first pre-hibernation period (cuckoo species) (Elmes *et al.*, 1991a;
71 Thomas & Elmes 1998; Thomas *et al.*, 2005).

72
73 Although certain ants, including *Myrmica* species, produce larvae that develop in two or more
74 cohorts at different rates (Elmes *et al.* 1998), fixed polymorphisms in growth that span >1
75 year are exceedingly rare in the animal kingdom, having previously been reported from
76 natural populations – as distinct from laboratory manipulations (Miyatake & Yamagishi 1999;
77 Lewis 2018) - only from salmonid fish (Gross, 1985) outside the genus *Maculinea* (Thomas
78 *et al.*, 1998a). Nevertheless, we found a clear-cut polymorphism in caterpillar growth in both
79 species of cuckoo *Maculinea*, *M. rebeli* (Hirschke, 1904) and *M. alcon* (Denis and
80 Schiffermüller, 1775), whereby a proportion of individuals develop to pupate in one year and
81 the remainder stay two years in the nest (Thomas *et al.*, 1998a; Schönrogge *et al.*, 2000; Als

82 *et al.*, 2002). The same apparently applies to some populations of predatory species, but here
83 the polymorphism may be phenotypically plastic (Schönrogge *et al.*, 2000; Witek *et al.*,
84 2006).

85

86 In early studies, that included a range of densities of *M. rebeli* (Elmes *et al.*, 1991b; Thomas
87 *et al.*, 1998a), we found that ~20%-40% of 4th instar caterpillars grew rapidly after being
88 carried into their host ant nest in August, gaining 50%-80% of their final body weight in the
89 first 10-12 weeks (FDL - fast developing larvae). These then lost up to 50% of body mass
90 over winter, itself exceptional for a butterfly larva, before growing rapidly in spring to pupate
91 and eclose in June-July (Fig. 1a from Thomas *et al.*, 1998a). In most populations strong
92 contest competition occurred between caterpillars for ant attention, and at high densities many
93 individuals were neglected and died until the cohort was reduced to a sustainable number
94 (Thomas *et al.*, 1993). The other 60%-80% of caterpillars grew much more slowly (SDL -
95 slow developing larvae) and were kept by the nurse ants in a different part of the nest where
96 they were not in direct competition with the FDL. Slow developers grew throughout the
97 following summer after the FDL had pupated, and by the second winter were 33% heavier
98 than the weight that their FDL siblings had achieved by the previous winter. They resumed
99 growth in their second spring and summer, and metamorphosed following a similar trajectory
100 to the FDL in the previous year, achieving a slightly heavier final body weight than the latter
101 (Fig. 1a).

102

103 We originally suggested that the polymorphism was probably genetically based, although it
104 might be triggered as a phenotypic response early in the 4th instar. Like Witek *et al.* (2006),
105 we cited bet-hedging as a possible (non-exclusive) selective force engendering this

106 polymorphism. However, we considered ergonomic benefits a more likely driver, being an
107 efficient way to exploit the regular, but seldom abundant, supply of food that occurs over a
108 long period of the year within temperate ant colonies, which contrasts with the brief pulse of
109 larval food available to most herbivorous insects, or with the need for plasticity for species
110 that have very short growing seasons or innutritious foods (Thomas & Elmes 1993; Thomas
111 *et al.*, 1998a; Schönrogge *et al.*, 2000).

112
113 In an ESS model exploring the costs and benefits of evolving polymorphic growth in
114 *Maculinea*, Hovestadt *et al.*, (2007) assumed a genetically fixed polymorphism in which all
115 individual mothers were capable of laying a mixture of FDL and SDL eggs. They concluded:
116 (i) If two or more larvae are competing for resources within a nest, a genotype for delayed
117 development (SDL) could become fixed in a population that has the FDL strategy as the norm
118 (in practice, we had estimated an average of 24 larvae entered each host nest on study sites in
119 Spain and France, with none sampled [N = 270] receiving a solitary larva: Hochberg *et al.*,
120 1991; Thomas *et al.*, 1998b). (ii) Interacting factors favouring a SDL strategy include low
121 mortality in the 2nd winter; competition among siblings in the same host nest; and the
122 frequency with which a nest moves, taking its cuckoo parasites with it, into gentian-free space
123 at the start of year 2, i.e. it recruits no caterpillars to compete with the resident SDL in the
124 second year (Thomas *et al.*, 1997, 1998b). These parameters were important in determining
125 the overall proportion of two-year developers in a population, but in theory the most this
126 could be fixed at was 50% (Hovestadt *et al.*, 2007), not the 75% observed surviving to
127 adulthood by Thomas *et al.*, (1998a). We and others have watched the same female lay
128 several eggs on the same gentian, and since these reach the 4th instar at about the same time,
129 all are likely to be adopted by the same host nest. (iii) The only parameter studied that might

130 permit the proportion of SDL to be fixed at values of >50% was a ‘priority effect’ - the extent
131 to which resident SDL larvae outcompete newly recruited FDL in late summer of year-1 and
132 spring year-2. Unfortunately, this was the parameter for which there is least empirical data.
133
134 Since the oviposition period for *M. rebeli* is restricted to 3-4 weeks in June-July in our study
135 regions, coinciding with optimum flower-bud production by *Gentiana cruciata* L. (Thomas &
136 Elmes, 2001), it follows that the range of “successful” growth rates must be strongly
137 constrained if larval development is genetically pre-determined. Clearly, if growth rate is
138 determined at conception, there should be strong disruptive selection to fix rates around the
139 optimum for FDL and SDL under average levels of competition, providing it can be shown
140 that the SDL strategy might in some circumstances be advantageous. Here we present
141 experimental evidence to test key assumptions for which there was anecdotal, weak or no
142 evidence in Hovestadt *et al.*’s (2007) model. (i) Is the growth strategy of each individual larva
143 pre-determined by its mother? (ii) Does intra-nest larval competition influence growth
144 strategy? (iii) Is the initial ratio of SDLs $\leq 50\%$ as predicted by Hovestadt *et al.*, (2007)? (iv)
145 Do individual females lay a mixture of eggs that hatch into 1- and 2-year developing larvae,
146 or into one form only? (v) Do larval growth patterns vary between different regions?
147 Although we and colleagues have demonstrated the existence of the growth polymorphism in
148 *M. rebeli* larvae from the Gap region of the Hautes-Alpes and the Rhône-Alpes in France, the
149 Spanish Pyrenees, and the Black Forest, Germany, it is apparently absent from north Italian
150 (Casacci *et al.*, 2019) and Hungarian populations (A. Tartally pers. comm.) and appeared,
151 anecdotally, to vary by region in the amount of growth achieved in the first summer and
152 autumn. In a final experiment, we compared growth rates from Spain and France.

153

154

155 **Materials and methods**

156 *Nomenclature*

157 The scientific names ascribed to our study species have oscillated over the past 25 years
158 (Tartally et al., 2019) as new studies emerge supporting one view over another and *vice versa*:
159 further change is likely in the light of, as yet unpublished, completed studies of the larval
160 semio-chemicals. Here we follow the most recent comprehensive review of the biology of
161 genus (Tartally et al., 2019) and advice of Balletto *et al.* (2010) in using the familiar (1915)
162 name *Maculinea* for the genus. However, we recognise that certain authors prefer *Phengaris*
163 according to Opinion 2399 (2017) (a third name, *Glaucopsyche*, deemed by many to be
164 correct in the 1990s, is now abandoned). The specific name, *rebeli*, is equally contentious,
165 with some authors considering it simply a distinctive xerophylic ecotype of *Maculineaalcon*
166 Denis & Schiff. (e.g. Koubínová *et al.* 2017; Bereczki *et al.* 2018), tentatively termed *M.*
167 *alcon X* by Tartally et al. (2019) after Tartally et. al. (2014) and Bereczki et al. (2018)
168 concluded that the original *Maculinea rebeli*, named by Hirschke in 1904, is a third, high
169 altitude ecotype of *M.alcon*. Here, for clarity and consistency, we follow Cassaci *et al.*
170 (2019) in retaining the specific name *rebeli*, which also follows the nomenclature used in
171 twenty-two of the twenty-three references cited here that describe earlier studies of the same
172 ecotype, as well as in most cases the same populations, used in this study of polymorphism.

173 *Materials*

174 *Maculinea rebeli* caterpillars and *Myrmica schencki* Emery, 1895 host ants were collected
175 from the Hautes-Alpes near Gap, south-west France (~1200-1500 m a.s.l) and, for the
176 comparison between regions, from the Spanish Pyrenees near Panticosa (~1400 a.s.l).
177 *Myrmica rubra* L., which successfully rears larvae under benign laboratory conditions
178 (Wardlaw *et al.*, 1998; Elmes *et al.*, 2004), were collected from Dorset, UK. *M. rubra* was

179 used twice to rear larvae for 7 and 28 days in ‘buckets’ before setting up experiments with the
180 natural host, *M. schencki*. All other experiments were established with *M. schencki*, using
181 rearing techniques described by Wardlaw *et al.* (1998), on the same day that the larvae
182 emerged. Rearing conditions were matched for all treatments, including type of artificial nest,
183 the number, age and origin of host workers, standardised numbers of ant larvae but no
184 queens, food supply and temperature regime. Although many individuals were reared through
185 to pupation in their second year (Fig. 1), most experiments were terminated in September-
186 December of their second year, when classification between FDL and SDL was unequivocal
187 but before their captive host ant nests had unduly declined in fitness (Wardlaw *et al.*, 1998).
188 Analysis was by Minitab or R. We set up nine experiments or studies:

189
190 *Are bimodal growth rates set or apparent before contact with ants? Is growth strategy*
191 *determined by an attribute that is independent of size at the start of the 4th instar?*

192 We measured the head-widths of 48 full-grown 3rd instar larvae extracted from gentian seed
193 pods to investigate modality, and counted the number of pore cupola glands on their bodies.
194 We also weighed 71 freshly emerged 4th instar larvae to investigate putative bimodality prior
195 to rearing each individual in a separate culture containing 30-40 *M. schencki* nurse workers.
196 Just before introduction to ants, we measured the respiration rate, as evidenced by CO₂
197 production, of each individual as a possible indicator of metabolic variation, using a flow-
198 through analyser model LI-6251 connected to a data acquisition and analysis system (Sable
199 System, Salt Lake, Utah using Datcan V software); see Nielsen *et al.* (1999) for further
200 details. When each caterpillar was actively growing we recorded its behaviour and
201 interactions with host workers for 100 sec on twelve occasions per larva (Σ 20 min per larva).
202 The behaviours included the average number of ants actively attending or guarding a larva,

203 the number of ants ‘licking’ its cuticle or posterior gland, and the frequency that the
204 caterpillar crawled or begged for food (see Elmes *et al.*, 1991a for detailed methods and
205 definitions). We reared 40 cultures on beyond the first summer to confirm their classification
206 of individuals as FDL or SDL.

207

208 *Does competition in ant nests or the fitness of nurse ants determine growth strategy?*

209 We explored this in four experiments in which we varied the density of workers above and
210 below the average of 30 workers per larva found, in earlier studies, to be needed to rear an
211 individual *M. rebelei* to adulthood in one year (Elmes *et al.* 1991b; Thomas *et al.* 1993;
212 Thomas & Elmes 1998). We also varied the age of nurse workers, thereby altering their
213 propensity to tend *M. rebelei* (or their own larvae), for like many social insects, the workers of
214 *Myrmica* species display temporal polyethism, in which individual adults progress from
215 behaving as nurses to becoming foragers as they age (Brian 1979; Hölldobler & Wilson
216 1991). (i) 134 larvae were reared singly from the outset, each with 30-40 freshly hatched *M.*
217 *schrencki* nurse workers, to determine the size and proportion of FDL to SDL under conditions
218 of surplus food, optimum nursing, and no intra-specific competition. (ii) Thirty eight larvae
219 were reared singly as in (i) but with 40 older, less fit *M. schrencki* nurse workers each. (iii)
220 Ninety eight larvae were reared as in (i) but with two larvae per ant nest; i.e. under moderate
221 competition of 15-20 ants per social parasite. This number would usually be adequate to rear
222 both larvae but might result in one of a pair being neglected if the other could attract more
223 than its fair share of worker attention (Thomas *et al.* 1993; Thomas & Elmes 1998). (iv) 143
224 larvae were reared singly as in (i) but with just 5 old worker ants per larva. (v) To study
225 whether growth rates were plastic under more intense competition, thirty-three larvae were
226 reared for 4 weeks post-adoption in large stock nests of *Myrmica rubra*, then weighed and

227 ranked by size. They were then transferred in groups of three into 11 cultures, each containing
228 30 fresh *M. schencki* workers. Culture 1 received the 3 smallest caterpillars, culture 2 the next
229 3 smallest, and so on to culture 11 which had the largest three larvae. Each culture was then
230 reared under identical conditions of food and temperature regime until the onset of winter
231 hibernation when each individual was again weighed (Weight_A), and later classed as FDL or
232 SDL.

233

234 *Is there a maternal effect on growth strategy?*

235 To determine whether an individual adult laid FDL, SDL or a mixture of eggs, we caught
236 nine newly emerged, copulating female *M. rebeli* in the Hautes-Alpes, and caged each singly
237 in a net placed over a suitable *G. cruciata* plant for oviposition (Thomas & Elmes, 2001)
238 having ensured that no previous *M. rebeli* eggs had been laid on it (cuckoo *Maculinea* have
239 the most conspicuous eggs and most durable shells of all European butterfly species; Thomas
240 *et al.*, 1991). Most females started to lay on the same day, and were kept caged on their plants
241 for 1-3 full days. Each gentian's flower-shoots were then cut and kept separately in the
242 laboratory after Wardlaw *et al.* (1998), until their *M. rebeli* emerged as 4th instar larvae. Each
243 newly moulted larva was weighed and then reared either singly with a culture of 40 *M.*
244 *schencki* workers collected from the same site or, for those from the most fecund parents, in
245 groups of up to 10 larvae per nest after a maximum of seven singletons had been established
246 from each parent. Their development as FDL or SDL was monitored

247

248 *Does growth pattern vary between populations?*

249 We obtained sufficient *M. rebeli* to compare with populations from the French Alpes from the
250 Pyrenees, near Panticosa, Spain. As in the previous study (Thomas *et al.*, 1998a), the

251 trajectory for FDL is calculated only from larvae that survived to pupation and that for SDL
252 from larvae that survived to hibernate a second winter combined with data for SDL taken
253 from the field when the FDL were pre-pupal and reared a further year to pupation in the
254 laboratory.

255

256 **Results**

257

258 *Is the growth pattern of an individual larva set or apparent in larvae prior to interacting with*
259 *ants?*

260 We found no evidence that any experience or obvious attribute of larvae prior to their
261 adoption by ants induced them to become FDL or SDL morphs. Neither the respiration rate
262 prior to adoption (Table 1) nor third-instar larval head-width was correlated with the winter
263 body weight or subsequent growth path of the final instar. Nor did the initial density of larvae
264 on *G. cruciata* influence the weight of pre-adoption larvae leaving the plant (mean larval
265 weight from <5 eggs laid per gentian stem = 1.644 mg \pm 0.099 mg s.e.m., N = 26; mean
266 larval weight from >30 eggs per stem = 1.830 mg \pm 0.093 mg s.e.m., N = 122; $T_{80df} = -1.44$, P
267 = 0.153) or the ratio of individuals that developed along either pathway. For example in Table
268 2, larvae growing at densities ranging from three to 51 individuals per plant showed no trend
269 in the ratio of morphs produced across the nine plants, P = 0.664, $R^2 = 2.9\%$. In addition, the
270 weight distribution of larvae entering the final instar was invariably unimodal (Fig 2a), and
271 the initial weight on entering a *M. schencki* nest did not differ between individuals that
272 subsequently grew as FDL or SDL (Fig 2b). Hence pre-adoption larval weight was neither a
273 predictor of mass at the onset of the first winter's hibernation nor of its eventual development
274 path (Fig 2c). By contrast, in another experiment where 65 larvae were reared to winter under
275 similar conditions of surplus resources, we were unable to weigh the larvae until a week after

276 their adoption, when they had integrated with their host *M. schencki* society for 4-7 days and
277 had started growing, roughly doubling in weight to 3.237 ± 0.961 mg and later achieving an
278 average hibernation Weight_A of 27.78 ± 8.82 mg. For this cohort, even 4-7 days with ants
279 were sufficient to establish a clear correlation between early growth and Weight_A (Spearman
280 Rank correlation $R_{df=63} = 0.262$, $P = 0.035$), suggesting that final instar larvae embark on a
281 fixed growth strategy within a week of their 11-23 months as social parasites.

282

283 *Can growth trajectories with ants be altered by the density of larvae in nests or the fitness of*
284 *ants?*

285 We detected no plasticity for larvae to switch between FDL and SDL pathways in response to
286 conditions in their local nest environment, although a higher proportion of FDL individuals
287 died under unfavourable conditions. When reared singly under optimum conditions of surplus
288 (30-40) young nurse ants per larva, the overall weight distribution was unimodal at the onset
289 of the first winter (Weight_A) but there was a clear-cut separation later with the larger half of
290 the cohort at hibernation growing on to pupate in their first summer (FDL) while all the
291 smaller individuals were 2-year SDLs (Fig. 3a). The small number of mortalities recorded in
292 this experiment was independent of size or growth strategy, and the overall ratio of FDL to
293 SDL was 54% to 46% ($N = 117$). A similar ratio of 49% FDL to 51% SDL was obtained in a
294 separate experiment when 39 larvae were reared singly, each with 40 older *M. schencki*
295 workers that were less fit to provide nursing activities: under these suboptimal conditions, the
296 familiar bimodal distribution of weights was clear by the time of the first winter's hibernation
297 (Fig 3b). Under moderate competition of two social parasites per nest at densities of 20 young
298 nurse ants per larva, a bimodal distribution in growth is again apparent (Fig. 3c) but, given
299 reduced resources, many of the faster-growing larvae died before their first winter leaving a

300 realised ratio of 74% SDL to 26% FDL. Finally, when *M. rebeli* were tended by old nurses
301 and at very high densities of five ants per larva, all but five of the largest individuals died in
302 the first few weeks, leaving a highly skewed ratio of 93% SDL to 7% FDL by the first winter
303 (although the five FDL all survived to adulthood the following July) (Fig. 3d). Further
304 evidence of a lack of plasticity between growth strategies was provided by raising 33 *M.*
305 *rebeli* larvae in a large stock *Myrmica* nest for 4 weeks, then dividing them into triplets
306 ranked by size, with the three smallest individuals (mean weight 1.9 mg) thereafter reared
307 together with 90 young *M. schencki* to tend them; the three next smallest larvae reared in a
308 second identical culture, and so on up to the eleventh culture which contained the three
309 largest larvae (mean weight 31.1 mg). If larvae could switch between morphs, we might
310 expect some of the small individuals to win and grow rapidly under the reduced competition
311 and that some of the largest larvae might lose and grow less well under strong competition
312 with large peers. However, the results showed that hibernation Weight_A depended mostly on
313 the size attained by each 4 week-old fourth instar at the start of the experiment (Fig 3e,
314 ranked correlation $R_{df=30} = 0.712$, $P < 0.001$), with no evidence that larvae can switch from 2-
315 year developers to 1-year developers or *vice versa*.

316

317 *Do different females lay eggs that produce exclusively 1-year, 2-year, or a mixture of larvae?*

318 All nine female *M. rebeli* whose eggs were reared separately produced a mix of 1-year and 2-
319 year larvae (Table 2). Although some individuals laid many eggs, we show results only for
320 the larvae that were reared singly with abundant resources, using 2-6 solitary larvae from
321 each female, since many that were reared at high densities in *M. schencki* nests died
322 prematurely, exacerbated by the comparatively old age – and hence the low fitness – of nurse
323 worker ants in nests sampled that year, which contained few pale callows and had shorter

324 than usual lifespans. The singletons are the same larvae whose growth patterns are shown in
325 Fig. 3b.

326

327 *What is the mechanism leading to fast or slow larval development?*

328 As with the earlier instars of *M. rebeli* larvae (above), we detected no obvious attribute to
329 indicate that a final instar larva was a 1-year or 2-year developer. However, some behaviours
330 were indicative (Table 1). Although we had previously found no difference between the
331 density of secretory pore cupola glands on the bodies of FDL and SDL, the fast developers
332 showed significantly enhanced instances of the average number of ants attending or guarding
333 a larva, the number of ants licking its cuticle (but not its anal gland), and the frequency when
334 the caterpillar moved or begged for food (Table 1). Of these, the strongest interaction was
335 with nurse ants 'licking' the surface of its tended larva (Fig 4), suggesting that the pore
336 cupola of fast developers may be more active than those of SDL, even if their densities were
337 the same.

338

339 *Does growth strategy vary between regions?*

340 As expected from previously finding many similar-sized small larvae when sampling nests
341 for pupae in June in the Pyrenees, we measured a comparable polymorphism in populations
342 from the Spanish Pyrenees to that in the Hautes-Alpes (Figs. 1b and 1a respectively).
343 However, growth paths were not identical. A considerable overlap between morphs existed in
344 initial pre-winter growth in Pyrenean butterfly larvae (for clarity only the inner quartiles are
345 shown in Fig. 1b). For this population, $Weight_A$ is therefore a poor predictor of post-winter
346 growth, although the 1-year individuals tended to be heavier ($Weight_A$ FDL = 21.4 ± 5.68 mg,
347 $Weight_A$ SDL = 16.7 ± 5.61 mg., N = 17). Relatively more growth occurred post-winter, and

348 FDL appeared to produce smaller (~65-70 mg.) pupae relative to SDL (~80-85 mg.). Our
349 small sample of individuals produced 21 FDL and 17 SDL - not significantly different from a
350 50% split between the two morphs ($\chi^2_1=0.421$, P = ns).

351

352 **Discussion and conclusions**

353 A few species of slow-growing insects possess juvenile periods that can vary phenotypically
354 by one or more years within the same population, in response to resource availability or
355 unusual weather (Wigglesworth, 1964; Denno & Dingle, 1981; Williams & Simon, 1995).
356 However, none was suspected of possessing a fixed polymorphism of annual and biennial
357 growth paths prior to studies of the two cuckoo species of *Maculinea* (Elmes *et al.*, 1991b;
358 Thomas *et al.*, 1998a; Schönrogge *et al.*, 2000). Indeed, among animals, the existence of two
359 genetically-fixed developmental morphs spanning different years in the wild was known only
360 from salmonid fish (Gross, 1985). However, artificial polymorphisms in growth rates can be
361 achieved among Diptera after strong selection in the laboratory (Miyatake & Yamagishi
362 1999; Lewis 2018).

363

364 Our original suggestion (Thomas *et al.*, 1998a) that growth rates for 4th instar *M. rebeli* larvae
365 were fixed was based on the observations that (i) a proportion of SDL were always found in
366 wild (French Hautes-Alpes) populations and this appeared unrelated to host nest size or
367 condition; (ii) larvae reared in the laboratory under a wide range of conditions with several
368 species of host ant, and with <30 ants per larva, generally produced about the same ratio of
369 75% SDL: 25% FDL; (iii) although larval competition (crowding) could reduce growth
370 (Elmes *et al.*, 1991b), caterpillars reared in very crowded conditions nevertheless still showed

371 bimodal distributions of caterpillar size after several weeks' growth in the host nest; in fact
372 bimodality was often amplified in these circumstances. Here, having described the basic
373 growth strategies and learnt how to rear the caterpillars over a two year period, we made
374 experiments designed to answer questions more conclusively. From the results of Figs 1-4
375 and Tables 1 and 2, we draw five conclusions with reasonable confidence.

376
377 *The SDL growth strategy is pre-determined by the mother, either genetically or during*
378 *oogenesis.* The initial Weight_I of final instar larvae is affected by the mother, and perhaps by
379 other environmental factors in the wild, but we have never found any link between Weight_I
380 and subsequent growth strategy of larvae (Figs. 2b,c and 3e). However, a higher Weight_I is
381 important in determining whether a larva survives the one or two day integration period when
382 adopted by a host nest (Elmes *et al.*, 1991b, Schönrogge *et al.* 2004) especially when intra-
383 nest competition is high. We have never been able to force an initially SDL larva to grow
384 more quickly and, while starvation or other stress can cause a FDL to grow more slowly, we
385 conclude that a small FDL can never successfully hibernate a second winter. When larvae
386 were grown in pairs (moderate intra-nest competition) the proportion of SDL pairs and FDL
387 pairs was similar to that predicted by a binomial allocation based on the overall ratio of
388 SDL:FDL seen in meta-analysis of experiments (Thomas *et al.*, 1998a). The only 'plasticity'
389 we have induced is occasionally to rear a FDL individual through to pupation in its initial
390 autumn instead of entering hibernation, given surplus food and continuing artificial warmth in
391 the laboratory, as reported also by Tartally *et al.* (2005).

392
393 *Intra-nest competition has a major effect on survival and growth but does not influence the*
394 *inherent growth strategy.* When larvae were reared as singletons with a surplus of fit young

395 nurse workers, we obtained an essentially normal distribution of $Weight_A$ centred on about
396 25mg, with almost all the > 25mg individuals being successful FDL and all the smaller ones
397 entering a second winter (Fig 3a). The ratio was about 50:50. Singletons from known
398 mothers (Table 1) reared by older workers, which would mostly have been relegated to
399 foraging duties in a wild *Myrmica* society (Brian 1979; Hölldobler & Wilson 1991), had a
400 bimodal distribution of $Weight_A$, though the ratio of SDL:FDL was still 50:50 (Fig 3b). We
401 suggest that this difference was due to a paucity of fit young nurse workers that stressed the
402 latter colonies, and that under these conditions SDL are less attractive to the workers, receive
403 less attention, and do not grow to their maximum potential $Weight_A$, whereas the FDL, which
404 consume far less food, grow fairly normally. As colony stress is increased by reducing the
405 worker: larva ratio (as in Figs 3c and d) or perhaps in the field due to external fluctuations in
406 colony food supply, individual larvae receive less attention and only the fastest growing FDL
407 exceed the target $Weight_A$ of ~25 mg and fewer of the slowest SDL do not attain enough
408 weight to survive the first winter, resulting a preponderance of surviving SDL and an
409 amplified bimodal pattern of weights among survivors (Elmes *et al.*, 2004).

410
411 *The inherent ratio of SDL:FDL morphs is 50:50.* In an ESS model exploring this system,
412 Hovestadt *et al.* (2007) predicted that selection for 1-year and 2-year morphs of larvae could
413 become fixed as an ergonomically efficient way of exploiting resources within a *Myrmica*
414 society, but that it was highly unlikely for more than 50% of individuals to be SDL. However,
415 our original meta-analysis (Thomas *et al.*, 1998a) indicated a 75:25 ratio in favour of SDL, a
416 ratio that we now realise was skewed by the net effects of high competition in several
417 experiments rather than the underlying genetic effect. In all three of our new experiments
418 where larvae were reared singly with surplus ants, we obtained ratios of the two morphs that

419 were close Hovestadt's maximum of 50:50, both when sourced from the French Alps (Figs
420 3a, b) and the Spanish Pyrenees (Fig 1b). It is also clear that this ratio is inherent when eggs
421 are laid, since the ratio was also about 50:50 between individuals in cohorts that occurred at
422 low densities on *Gentiana cruciata* and which experienced 100% survival from oviposition to
423 the final instar, indicating that there was no differential mortality of FDL or SDL during
424 either the egg stage or larval instars 1-3 (e.g Table 2, females A, C, D, E, F, H).

425
426 *The polymorphism is individual-based rather than population-based.* The progeny of nine
427 females from one population indicated that all parents in this population produce both SDL
428 and FDL (Table 2). The data are not sufficient to determine whether each female averages a
429 50:50 ratio, but they indicate that the population does not comprise two types of female
430 specialising either in a FDL or SDL strategy (Fig 3b). We can also exclude mating with more
431 than one male as an explanation, since all females in Table 2 were collected as fresh
432 individuals above the ant nests from which they had just emerged <1 hour earlier, some with
433 unhardened wings.

434
435 *Growth patterns vary between different populations.* We recorded a similar pattern of an
436 apparently fixed 50:50 polymorphism in the final larval instar growth of *M. rebeli* from the
437 Pyrenees to that in France, although the former grew less than the latter prior to winter under
438 standardised lab conditions (Fig 1). This might have evolved in response to different temporal
439 patterns of food supply available to ant nests. Late summers at the Spanish site tend to be
440 hotter and drier than on the French sites, and there may be less insect prey available for ants:
441 on the other hand, winter snow melts earlier on the Spanish site and spring/summer is longer
442 and earlier. Thus, more food may be available in spring and less in autumn on southern sites

443 compared to more northern ones. A similar trend in growth patterns was noted between
444 populations of *M. alcon* where populations at different European latitudes are associated with
445 different ant species (Tartally *et al.*, 2019), with northern populations growing more in
446 autumn than southern populations (Schönrogge *et al.*, 2000). However, other selective forces
447 may be decisive. For example, Cassaci *et al.* (2019) recently found an almost complete
448 absence of small unequivocally SDL larvae in summer (one individual of 136 prepupal larvae
449 or pupae) when sampling seven populations in Italy from the central Apennines to the Alps.
450 Clearly, further studies of the distribution and pattern of a polymorphism in *Maculinea rebeli*
451 are required, and whether the two morphs differ from a 50:50 ratio in populations where it
452 does exist.

453

454 We made little progress in identifying any underlying mechanism by which some larvae grow
455 quickly and others slowly, beyond finding that FDL move and beg more, and receive more
456 attention from workers. This again is an area for future research, for example into whether the
457 pore cupola of FDL produce chemically different (Elmes *et al.*, 2002) or greater quantities of
458 myrmecophilous secretions. Nevertheless, our results confirm several assumptions made in
459 Hovestadt *et al.*'s (2007) ESS model exploring the evolution of dimorphic growth rates in a
460 social parasite that exploits its host's trophallactic feeding system. Hovestadt concluded that
461 ergonomic efficiency was a much more likely agent than bet-hedging in the original selection
462 and fixing of the polymorphism, but that the two putative benefits are not mutually exclusive.
463 There is little doubt that under modern practices of land-use, bet-hedging enhances the
464 persistence of small isolated populations that are not part of a meta-population (Witek *et al.*
465 2006; Van Swaay *et al.* 2009). For example, it may enable this rare and threatened butterfly to
466 survive single years when no flowers of its initial food plant, *G. cruciata*, are available for

467 oviposition, due to intensive hay-cuts or excessive trampling by cattle (which find *G. cruciata*
468 unpalatable), events that we have occasionally witnessed on study sites in the Hautes-Alpes.

469

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473 rigorous methodology, and hugely enjoyable company were an irreplaceable pillar in 40
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480

481 **References**

482 Akino, T., Knapp, J.J., Thomas, J.A. & Elmes, G.W. (1999) Chemical mimicry and host specificity in
483 the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proceedings of the Royal*
484 *Society of London B*, **266**, 1419-1426.

485

486 Als, T.D., Nash, D.R., & Boomsma, J.J. (2001) Adoption of parasitic *Maculineaalcon* caterpillars
487 (Lepidoptera : Lycaenidae) by three *Myrmica* ant species. *Animal Behaviour*, **62**, 99-106.

488

489 Balletto E., Bonelli S., Settele J., Thomas J.A., Verovnik R., Wahlberg N. (2010) *Maculinea* Van
490 Eecke, 1915 (Lepidoptera: Lycaenidae): proposed precedence over I Doherty, 1891. *Bulletin of*

491 *zoological Nomenclature*, **67**, 129-132

492

493 Barbero, F., Thomas, J.A., Bonelli, S., Balletto, E. & Schönrogge, K. (2009) Queen ants make
494 distinctive sounds that are mimicked by a butterfly social parasite. *Science*, **323**, 782-785.

495

496 Bereczki, J., Pecsénye, K., Varga, Z., Tartally, A. & Tóth, J. P. (2018) *Maculinea rebeli* (Hirschke) –
497 a phantom or reality? Novel contribution to a long-standing debate over the taxonomic status of an
498 enigmatic Lycaenidae butterfly. *Systematic Entomology*, **43**, 166–182 DOI: 10.1111/syen.12259

499

500 Brian, M.V. (1979) Caste differentiation and division of labour. *Social Insects vol. 1* (ed. Herman, H.)
501 pp 121-222. Academic Press, London.

502

503 Casacci, L.P., Schönrogge, K., Thomas, J.A., Balletto, E., Bonelli, S. & Barbero, F. (2019)
504 Host specificity pattern and chemical deception in a social parasite of ants. *Science Reports*, **9**, 1619.

505

506 Denno, R. F. & Dingle, H. (1981) *Insect life history patterns*. Springer, New York.

507

508 Elmes, G.W., Thomas, J.A. & Wardlaw, J.C. (1991a) Larvae of *Maculinea rebeli*, a Large Blue
509 butterfly, and their *Myrmica* host ants: wild adoption and behaviour in ant nests. *Journal of Zoology*,
510 **223**, 447-460.

511

512 Elmes, G.W., Wardlaw, J.C. & Thomas, J.A. (1991b) Larvae of *Maculinea rebeli*, a Large Blue
513 butterfly and their *Myrmica* ant hosts: patterns of caterpillar growth and survival. *Journal of Zoology*,
514 **224**, 79-92.

515

516 Elmes, G.W., Thomas, J.A., Wardlaw, J.C., Hochberg, M.E., Clarke, R.T. & Simcox, D.J. (1998) The

517 ecology of *Myrmica* ants in relation to the conservation of *Maculinea* butterflies. *Journal of Insect*
518 *Conservation*, **2**, 67-78.

519

520 Elmes, G.W., Thomas, J.A., Munguira, M.L. & Fiedler, K. (2001) Larvae of lycaenid butterflies that
521 parasitise ant colonies provide exceptions to normal insect growth rules. *Biological Journal of the*
522 *Linnean Society*, **73**, 259-278.

523

524 Elmes, G.W., Akino, T., Thomas, J.A., Clarke, R.T. & Knapp, J.J. (2002) Interspecific differences in
525 cuticular hydrocarbon profiles of *Myrmica* ant species are sufficiently consistent to explain host
526 specificity in *Maculinea* (Large blue) butterflies. *Oecologia* **130**: 525-535.

527

528 Elmes, G.W., Wardlaw, J.C. Schönrogge, K. & Thomas, J.A. (2004) Food stress causes differential
529 survival of socially parasitic larvae of *Maculinea rebeli* (Lepidoptera, Lycaenidae) integrated in
530 colonies of host and non-host *Myrmica* species (Hymenoptera, Formicidae). *Entomologia*
531 *Experimentalis et Applicata*, **110**, 53-63.

532

533 Gross, M.R. (1985) Disruptive selection for alternative life histories in salmon. *Nature*, **313**, 47-48.

534

535 Hochberg, M., Thomas, J.A. & Elmes, G.W. (1992) The population dynamics of a Large Blue
536 Butterfly, *Maculinea rebeli*, a parasite of red ant nests. *Journal of Animal Ecology*, **61**, 397-409.

537

538 Hölldobler, B. & Wilson, E.O. (1990) *The Ants*, Springer, Berlin.

539

540 Hovestadt, T., Mitesser, O., Elmes, G.W., Thomas, J.A., Hochberg, M.E. (2007) An ESS model for
541 the evolution of dimorphic development strategies in the social parasite *Maculinea rebeli*. *The*
542 *American Naturalist*, **169**, 466-480

543

544 Koubínová, D., Dincă, V., Dapporto, L., Vodá, R., Suchan, T., Vila, R. & Alvarez, N. (2017)

545 Genomics of extreme ecological specialists: multiple convergent evolution but no genetic divergence

546 between ecotypes of *Maculinea alcon* butterflies *Scientific Reports* **7**, no. 13752. DOI:

547 10.1038/s41598-017-12938-8.

548 Lewis, S.A. (2018) *The genomics of development rate variation in Cochliomyia macellaria (Diptera:*

549 *Calliphoridae)*. MSc thesis, 106pp. Purdue University, Indianapolis

550

551 Nash, D.R., Als, T.D., Maile, R., Jones, G.R. & Boomsma J.J. (2008) A mosaic of chemical

552 coevolution in a large blue butterfly. *Science*, **319**, 88-90.

553

554 Nielsen, M.G., Elmes, G.W. & Kipyatkov, V.E. (1999) Respiratory Q10 varies between populations

555 of two species of *Myrmica* ants according to the latitude of their sites. *Journal of Insect Physiology*,

556 **45**, 559-564.

557

558 Opinion 2399 (2017): Case 3508 *Maculinea* Van Eecke, 1915 (Lepidoptera: Lycaenidae): precedence

559 over *Phengaris* Doherty, 1891 not granted. *Bulletin of Zoological Nomenclature*, **74**, 117-119.

560

561 Schönrogge, K., Wardlaw, J.C., Thomas, J.A. & Elmes, G.W. (2000) Polymorphic growth rates in

562 myrmecophilous insects. *Proceedings of the Royal Society of London B*, **267**, 771-777.

563

564 Schönrogge, K., Wardlaw, J.C., Peters, A.J., Everett, S., Thomas, J.A. & Elmes, G.W. (2004)

565 Changes in chemical signature and host specificity from larval retrieval to full social integration in the

566 myrmecophilous butterfly *Maculinea rebeli*. *Journal of Chemical Ecology*, **30**, 91-107.

567

568 Miyatake, T. & Yamagishi, M. (1999) Rapid evolution of larval development time during mass-
569 rearing in the melon fly, *Bactrocera cucurbitae*. *Researches on Population Ecology* , **41**, 291-297.
570 DOI:10.1007/s101440050034
571
572 Tartally, A. (2005) Accelerated development of *Maculinea rebeli* larvae under artificial conditions
573 (Lycaenidae). *Nota Lepidopterologica*, **27**, 303-308.
574
575 Tartally, A., Koschuh, A. & Varga, Z. (2014) The re-discovered *Maculinea rebeli* (Hirschke, 1904):
576 Host ant usage, parasitoid and initial food plant around the type locality with taxonomical aspects
577 (Lepidoptera, Lycaenidae). *Zookeys*, **406**, 25-40.
578
579 Tartally, A. *et al.* (2019) Patterns of host use by brood-parasitic *Maculinea* butterflies across Europe.
580 *Philosophical Transactions of the Royal Society B*,
581 20180202.<http://dx.doi.org/10.1098/rstb.2018.0202>.
582
583 Thomas, J.A. (1984) The behaviour and habitat requirements of *Maculinea nausithous* (the dusky
584 large blue butterfly) and *M. teleius* (the scarce large blue) in France. *Biological Conservation*, **28**, 325-
585 347.
586
587 Thomas, J.A. (2002) Larval niche selection and evening exposure enhance adoption of a predacious
588 social parasite, *Maculinea arion* (large blue butterfly), by *Myrmica* ants. *Oecologia*, **122**, 531-537.
589
590 Thomas, J.A., Munguira, M.L., Martin, J. & Elmes, G.W. (1991) Basal hatching by *Maculinea*
591 butterfly eggs: a consequence of advanced myrmecophily? *Biological Journal of the Linnean Society*,
592 **44**, 175-184.
593

594 Thomas, J.A., Elmes, G.W. & Wardlaw, J.C. (1993) Contest competition among *Maculinea rebeli*
595 larvae in ant nests. *Ecological Entomology*, **18**, 73-76.
596
597 Thomas, J.A., Elmes, G.W., Clarke, R.T., Kim, K.G., Munguira, M.L. & Hochberg, M.E. (1997)
598 Field evidence and model predictions of butterfly-mediated Apparent Competition between Gentian
599 plants and Red ants. *Acta Oecologica*, **18**, 671-684.
600
601 Thomas, J.A. & Elmes, G.W. (1998) Higher productivity at the cost of increased host-specificity when
602 *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation.
603 *Ecological Entomology*, **23**, 457-464.
604
605 Thomas, J.A., Elmes, G.W. & Wardlaw, J.C. (1998a) Polymorphic growth in larvae of the butterfly
606 *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proceedings of the Royal Society of*
607 *London B*, **265**, 1895-1901
608
609 Thomas, J.A., Clarke, R.T., Elmes, G.W. & Hochberg, M.E. (1998b). Population dynamics in the
610 genus *Maculinea* (Lepidoptera: Lycaenidae). *Insect population dynamics: in theory and practice* (ed.
611 by Dempster, J.P. & McLean, I.F.G.) pp. 261-290, Chapman & Hall, London.
612
613 Thomas, J.A. & Elmes, G.W. (2001) Foodplant niche selection rather than the presence of ant nests
614 explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proceedings of the*
615 *Royal Society of London B*, **268**, 471-477.
616
617 Thomas, J.A., Schönrogge, K. & Elmes, G.W. (2005) Specialisations and host associations of social
618 parasites of ants. *Insect Evolutionary Ecology* (ed. Fellowes, M. D. E., Holloway, G. J. & Rolff, J.) pp.
619 479-518, CABI Publishing, Wallingford, UK.

620

621 Thomas J.A., Elmes, G.W., Sielezniew, M., Stankiewicz-Fiedurek, A., Simcox, D.J., Settele, J. &
622 Schönrogge, K. (2013) Mimetic host shifts in an endangered cuckoo-species of butterfly social
623 parasite. *Proceedings of the Royal Society B*, **280**, 711-719.

624

625 Van Swaay, C.A.M., Maes, D. & Warren, M.S. (2009) Conservation status of European butterflies.
626 Ecology of Butterflies in Europe, eds. Settele, J., Shreeve, T., Konvička, M. & Van Dyck, H. pp. 322–
627 338, Cambridge University Press, Cambridge.

628

629 Wardlaw, J.C., Elmes, G.W. & Thomas, J.A. (1998) Techniques for studying *Maculinea* butterflies: I.
630 rearing *Maculinea* caterpillars with *Myrmica* ants in the laboratory. *Journal of Insect Conservation*, **2**,
631 79-84.

632

633 Wigglesworth, V.B. (1964) *The life of insects*. Weidenfeld & Nicolson, London.

634

635 Williams, K.S. & Simon, C. (1995) The ecology, behavior and evolution of Periodical Cicadas.
636 *Annual Review of Entomology* **40**, 269.

637

638 Witek, M., Sliwinska, E.B., Skórka, P., Nowicki, P., Settele, J. & Woyciechowski, M. (2006)
639 Polymorphic growth in larvae of *Maculinea* butterflies, as an example of biennialism in
640 myrmecophilous insects. *Oecologia*, **148**, 729-733.

641

642

643

644 **Table 1** Interactions between worker ants and *Maculinea rebeli* larvae reared singly in *Myrmica*

645 *schencki* nests. Pearson Correlation *R* values, df = 67, *** P <0.001, ** P <0.01

646

	FDL	Respiratory rate	No. ants attending	Larvae crawl	Larvae beg
Larval respiratory rate	-0.126				
No. ants attending	0.311	0.007			
Larvae crawl	-0.003	-0.062	-0.201		
Larvae beg	0.185	0.019	-0.030	0.499***	
Ants lick cuticle	0.330**	-0.100	0.576***	0.345**	0.430***
Lick anal gland	0.033	-0.110	0.092	0.347**	0.165
Ants guard larva	0.051	0.081	0.781***	-0.522***	-0.336**

647

648

649

650

651 **Table 2** The progeny of individual females. Due to differential survival by FDL and SDL when reared
652 at high densities, results are given only for the 3-6 individual larvae per female that were reared singly
653 with 40 *Myrmica schencki* nurses per larva

654

Female number	No. of eggs laid per foodplant	Fast developers	Slow developers
A	13	3	3
B	51	3	2
C	3	2	1
D	3	1	1
E	10	2	2
F	5	3	1
G	21	1	4
H	16	2	4
I	21	2	3

655

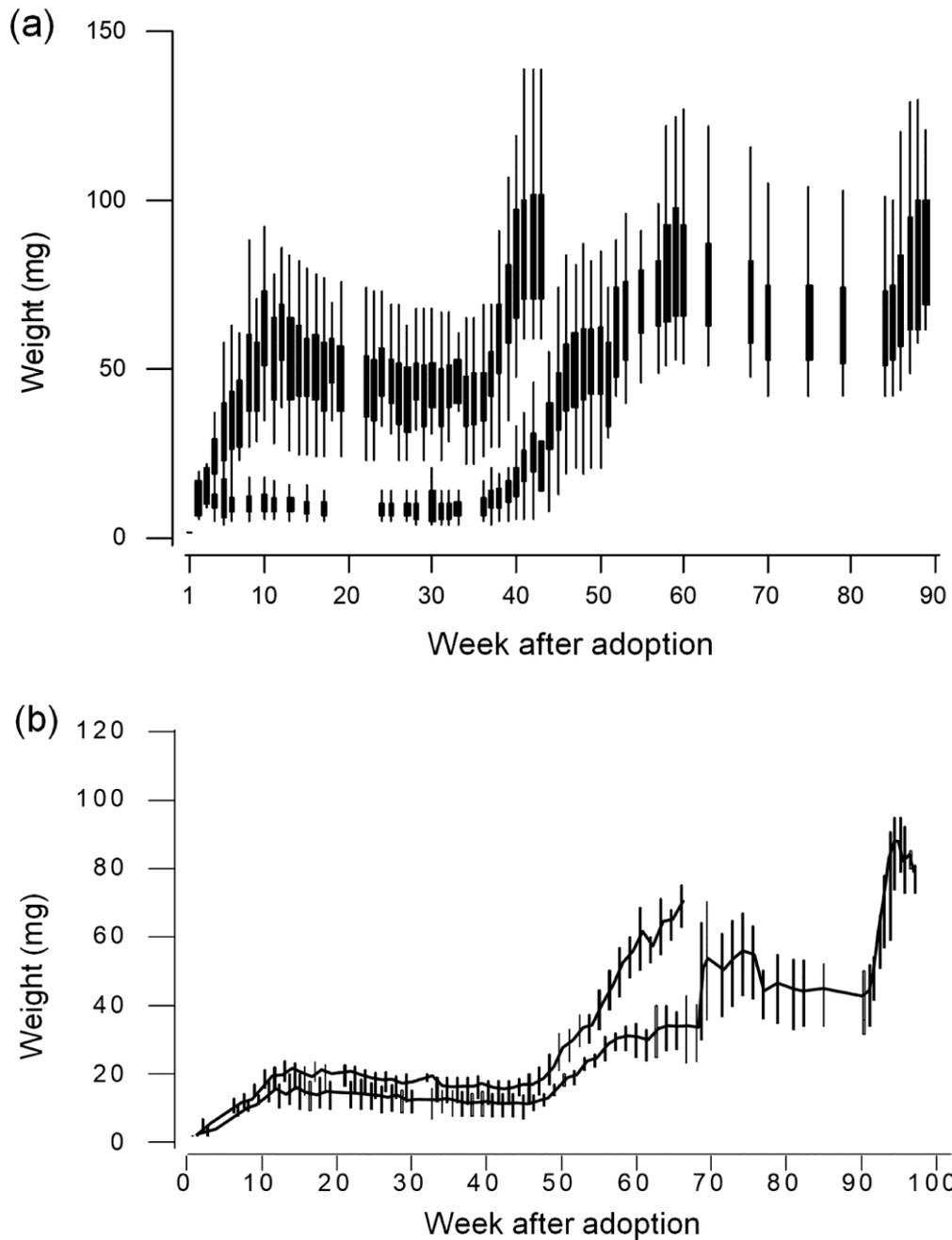
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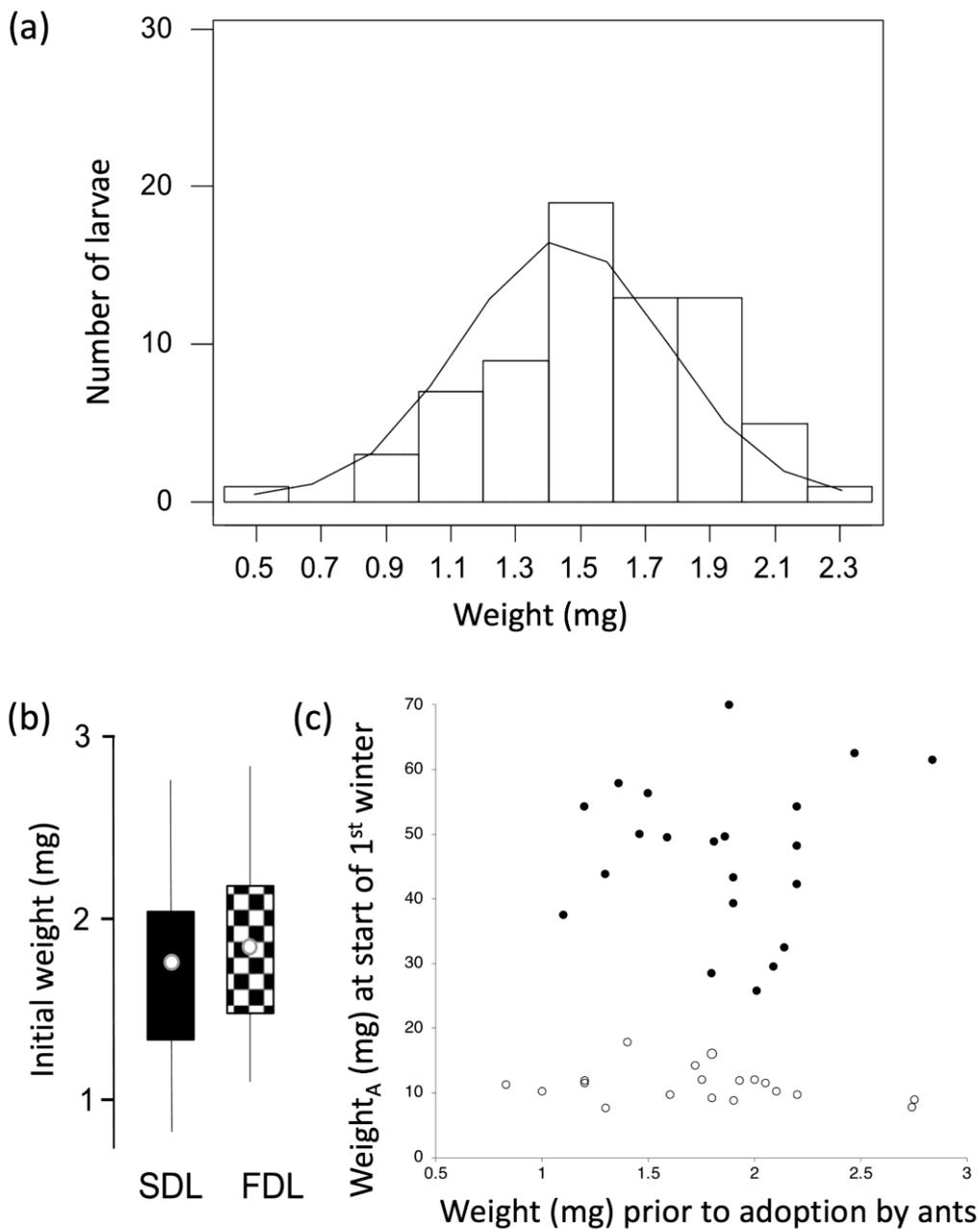
659

660 **Figure 1.** Polymorphic growth by final instar larvae of *Maculinea rebeli* reared at a range of natural
661 densities in *Myrmica schencki* nests from field and lab data combined. Populations from: (a) the
662 Hautes-Alpes, France \pm 50- and 95-percentiles (redrawn from Thomas *et al.*, 1998a, which also
663 contains images of the two growth classes); (b) the Pyrenees, Spain \pm inner quartiles.



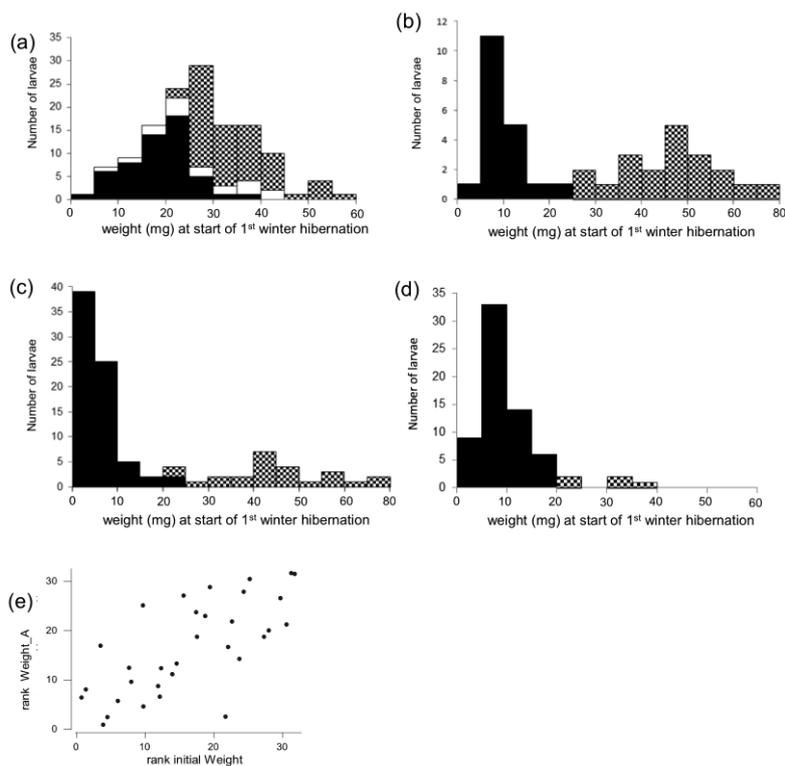
664

665 **Figure 2.** Weight distribution of newly moulted final instar *Maculinea rebeli* larvae before they
 666 encounter ants: (a) Weights are best described by a unimodal curve; (b) Initial weights of larvae that
 667 are later classed as 1-year (FDL) and 2-year (SDL) individuals are the same; circle = mean, two-tailed
 668 $t = -0.50, 37_{df}, P = 0.618$ ns ; Thus (c) initial larval weight shows no correlation to $Weight_{(A)}$ attained
 669 by a larva prior to its 1st winter hibernation ($P = 0.552, R^2 = 0.9\%, N = 39$) and does not indicate
 670 whether an individual becomes a fast (solid circle) or slow (open circle) developer.



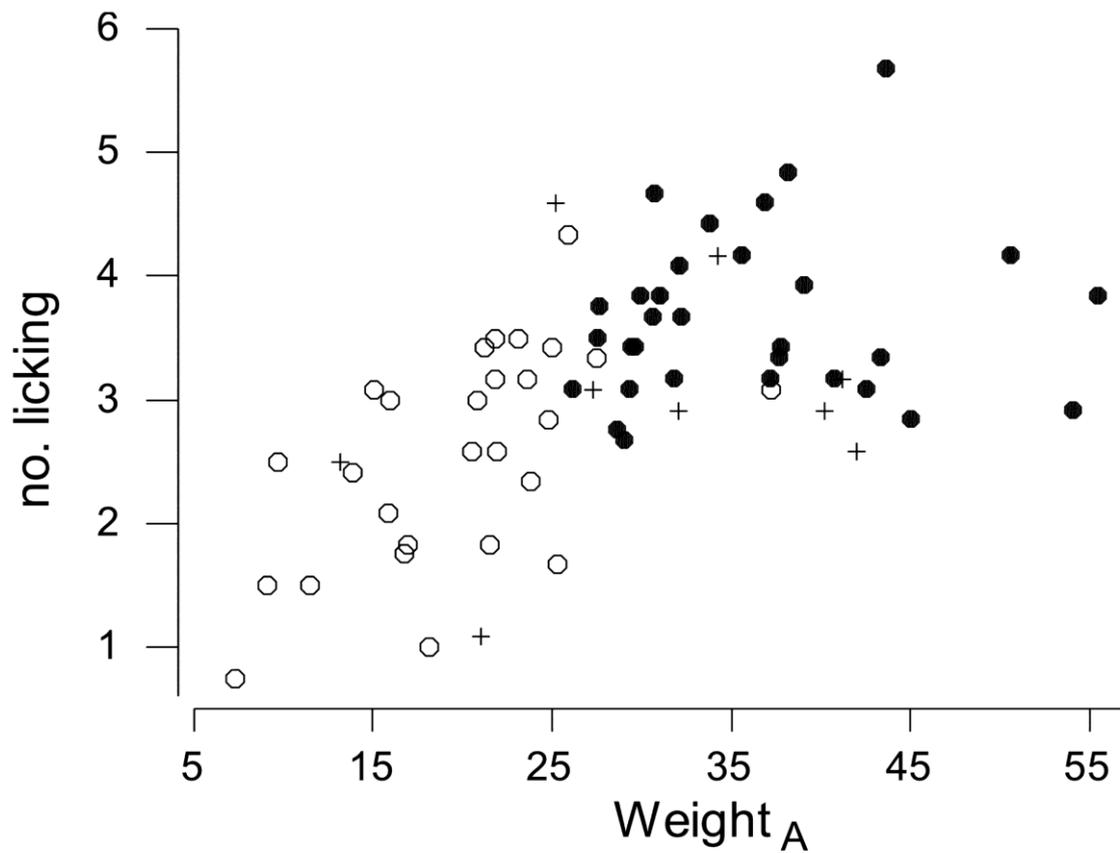
671

672 **Figure 3.** Effects of larval density and host fitness on weight distributions of final instar *Maculinea*
673 *rebeli* larvae prior to their 1st winter hibernation, and their subsequent classification after growing as
674 1-year (hatched) or 2-year (black) developers. All larvae were reared with their natural host *Myrmica*
675 *schencki*: (a) Larvae reared singly with 30-40 young nurse ants, N = 134, white = individuals that died
676 before classification as FDL (chequered columns) or SDL (black). (b) Larvae reared singly, each with
677 40 older nurse ants per larva; N = 39. (c) As (a) but 2 larvae per nest, i.e. 20 ants per larva; N = 98,
678 surviving larvae only are shown. (d) larvae reared at high densities of 6 larvae per worker ant, initial
679 N = 143 larvae of which 76 mainly FDL individuals died before reaching 1st winter hibernation. (e)
680 Competition between larvae ranked by size after living communally for 4 weeks ($Weight_4$) in one
681 large *Myrmica* nest, thereafter reared as eleven triplets (N = 33 larvae) for 6 weeks ($Weight_A$) in
682 standardised cultures containing the 3 smallest individuals together upwards to the three largest
683 together. Despite the wide range of competition for resources, larvae were already fixed on an
684 unchanging development path by week 4 with ants: ranked correlation, $Weight_4$ (4 weeks) cf $Weight_A$
685 (10 weeks): $R_{df=30} = 0.712$, $P < 0.001$.



686

687 **Figure 4.** Ant attention and larval development. The mean number of worker ants ‘licking’ the cuticle
688 of an individual larva at any one time correlated closely with the $Weight_{(A)}$ of the larva prior to
689 hibernation in its first winter. Filled circles indicate (FDL) larvae that later pupated after 1 year; open
690 circles indicate SDL larvae that successfully hibernated a second winter, when the experiment
691 stopped; individuals indicated by a plus died.



692