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Food stress causes differential survival of socially parasitic caterpillars of *Maculinea rebeli* integrated in colonies of host and non-host *Myrmica* ant species

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Abstract

Final instar larvae of *Maculinea rebeli* Hirschke (Lepidoptera, Lycaenidae) are social parasites of *Myrmica* Latreille (Hymenoptera, Formicidae) nests. In the populations of the southern French Alps and Spanish Pyrenees, >95% adult *M. rebeli* emerge from colonies of *Myrmica schencki* Emery, despite >60% caterpillars being adopted by other *Myrmica* species (non-hosts). However, in laboratory culture caterpillars can be reared successfully by many of the non-host species. This contradiction, which has led some to question the existence of host specificity, has been explained by the lack of stress, particularly food stress, in laboratory cultures compared to wild conditions. Here, we report the results of an experiment that tested the survival of *M. rebeli* caterpillars that had been growing well, after being socially integrated into a series of host and non-host cultures, and were then subjected to a 4-week period of stress induced by a 'starvation diet' estimated to be less than the minimum for ant survival. Significantly more *M. rebeli* survived in *M. schencki* cultures than with any of the other *Myrmica* species (all died in most non-host cultures). Under a starvation diet, caterpillars are killed and eaten along with dead workers – this never happens under an ample diet – rather than simply starving to death. It was noted that the proportion of young *M. rebeli* caterpillars that survived initial integration into an ant colony (including some *M. schencki* colonies) was a good predictor of subsequent survival under starvation conditions. We concluded that two key phases of host specificity exist in the life of this social parasite: initial integration, in which the caterpillar simply has to be accepted into a host society, followed by full integration, when a relatively high hierarchical status within the host society becomes essential for a caterpillar's survival during periods when the host colony is stressed, e.g., by food shortage. This experimental regime provides a useful bioassay for testing host specificity in other populations of *Maculinea*.

Introduction

There is both practical and theoretical interest in the biology of *Maculinea* butterflies, because the five European species all have high international conservation status (e.g., IUCN, 1990; Elmes & Thomas, 1992; Van Swaay & Warren, 1999) and their highly specialised life histories (review, Thomas et al., 1998a; Elmes et al., 1998) provide many examples of the processes predicted by theoretical ecology, such as the population level consequences of contest vs. scramble competition (Thomas & Wardlaw, 1992; Thomas et al., 1993; Thomas & Elmes, 1998), apparent competition and mutualism between an ant and a plant (Thomas et al., 1997), the influence of spatial patterns in habitat quality on population dynamics (Clarke et al., 1997), a polymorphism in larval growth (Thomas et al., 1998b), the first unambiguous example of the production by a caterpillar of chemicals that mimic the recognition chemicals of ants (Akino et al., 1999), and the first example of a parasitoid that produces analogues of ant semiochemicals in order to penetrate ant-nests to parasitize its caterpillar hosts (Thomas & Elmes, 1993; Thomas et al., 2002).

Field observations of populations of *Maculinea rebeli* Hirschke (Lepidoptera, Lycaenidae) have shown that the

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final, 4th instar caterpillars are social parasites, having a cuckoo relationship with *Myrmica* Latreille (Hymenoptera, Formicidae) red ant colonies (see Materials and methods for details of the general *Maculinea* life cycle). In the Pyrenees and Southern Alps of Europe, *M. rebeli* is specific to one host species, *Myrmica schencki* Emery (Thomas et al., 1989). Caterpillars are taken back to the nest (retrieved – see below) by foraging ants of any species of *Myrmica*, with only 10–40% of populations being taken into *M. schencki* nests. However, more than 95% of all adult butterflies emerge from *M. schencki* nests (Elmes et al., 1996, 1998; Thomas & Elmes, 1998). In contrast, when caterpillars are reared in the laboratory, their survival is only slightly higher (6–11% more) with *M. schencki* during the first days of integration following retrieval, and thereafter Elmes et al. (1991a) showed that it is possible to rear caterpillars to maturity equally well with most of the common *Myrmica* species (referred to throughout this paper as non-hosts, see Methods). These contradictions have puzzled researchers, and have led some to question whether host-specificity is the important constraint on the population dynamics of *Maculinea* species predicted by modelling studies (Hochberg et al., 1992, 1994; Clarke et al., 1997; Thomas et al., 1998a). Apart from theoretical interest, understanding how and when host specificity operates is important because it may also reassure practical nature conservationists that determining the specific ant host of any *Maculinea* population remains a vital first step before undertaking any active management programme (Elmes et al., 1998).

We predict the consequences for *M. rebeli* caterpillars that are integrated into wild colonies of *M. schencki* and non-host species, such as *Myrmica sabuleti* Meinert, by analogy with the behaviour of *Myrmica* ants towards their own and alien ant larvae. After a few days' integration, *Myrmica* will treat conspecific non-kin larvae equally with their own kin larvae, although initially they prefer (retrieve more quickly) their own kin larvae (Gerrish, 1994). In the laboratory it is possible to persuade *Myrmica* ants to rear the larvae of other *Myrmica* species (see Elmes & Wardlaw, 1983), unless they are in competition with conspecific larvae, in which instance the alien larvae are usually killed or neglected (G.W. Elmes, unpubl.). Discrimination increases when the ants are disturbed or stressed; in the wild the most common cause of colony stress is temporary food shortage. When laboratory cultures of *Myrmica* workers and brood are starved (e.g., see the experiments of Brian & Abbott, 1977; Elmes, 1989; Gerrish, 1994), the workers first kill and recycle the eggs and small larvae, and then their large larvae and pupae; this sequence is followed in several different genera of ants (Gerrish, 1994).

Against this background, we explained the apparent contradiction in our earlier studies between field and laboratory survival, by hypothesising that *M. rebeli* caterpillars mimic *M. schencki* ant larvae, probably chemically (Elmes et al., 1991a,b), with a social status equal or superior to large *M. schencki* larvae. Subsequently, Thomas et al. (1998b) confirmed that within 1 week of being integrated into a *M. schencki* colony, *M. rebeli* caterpillars have a social status (measured by brood retrieval tests) which is superior or similar to that of large *M. schencki* larvae, while chemical mimicry was confirmed by Akino et al. (1999) and Elmes et al. (2002).

Based on these observations, we predict that when wild *M. schencki* host colonies containing *M. rebeli* caterpillars (from south-western European populations), suffer food shortages, the ants should recycle most or all of their own larvae before the high-status caterpillars. Conversely, when an *M. rebeli* caterpillar is reared by non-host *Myrmica*, the workers of a non-host *Myrmica* colony should prefer their own brood to the caterpillar, which would be emitting an *M. schencki* analogue signal. In the laboratory, the deduction from the observations of ant behaviour, now tested here, is that caterpillars integrated into non-host *Myrmica* colonies should be neglected and perhaps killed, far sooner, when the colony is stressed by food shortage, than caterpillars living in similarly stressed *M. schencki* host colonies.

We now recognise that the process of adoption (see Elmes et al., 1991a) comprises three overlapping phases of increasing interaction between the caterpillars and their host ants, when different intensities of host specificity could (co)evolve: (i) 'Retrieval' of the caterpillar from outside the nest by foragers; this is specific to *Myrmica*, but indiscriminate between *Myrmica* species, most probably the caterpillar is emitting a generic *Myrmica* larval signal (Akino et al., 1999). (ii) 'Initial-integration' into the society during the first 24–48 h inside the nest; some caterpillars are accepted almost immediately, a few are killed but most are neglected before eventually becoming fully integrated; when neglected, many caterpillars will prey upon ant larvae if available (Wardlaw et al., 2000). Generally, the period of neglect is shortest in colonies of the main host species, but its duration not only varies between host species, but also between colonies of *M. schencki* and individual caterpillars. Initial integration probably has a chemical basis (Elmes et al., 2002). Some *Myrmica* colonies appear more benign than others, more readily accepting foreign brood and queens (noted over many years of laboratory experiments). The benign nature of any colony (or population) is unpredictable, probably due to a range of genotypic, social, and environmental factors experienced in its recent history such as food supply, local competition, microclimate, and

the number and condition of any queens (Elmes, 1987; Elmes, 1989; Elmes & Keller, 1993). (iii) 'Full-integration', or long-term acceptance, which determines subsequent survival and growth, and is possibly related to the initial integration; this is investigated here.

The experiment reported here was designed to test our hypothesis that food shortage causes the differential survival (of fully integrated caterpillars) between host and non-host nests observed in the field for south-western European populations of *M. rebeli*. However, a secondary aim was to establish a laboratory protocol that might form the basis of a simple bioassay for predicting the host ant species for other populations of *M. alcon* Denis and Schiff., and *M. rebeli*, without resorting to laborious field studies that might damage smaller more fragile sites than those previously studied. This is important, because it is already known that *M. alcon*, a close relative of *M. rebeli*, uses three different host species of *Myrmica* in different parts of its European range (Elmes et al., 1994) and in the Northern Alps (Germany, Austria, Poland) there is increasing evidence that *M. rebeli* uses *M. sabuleti* as its host species (Stettmer et al., 2001; Steiner et al., 2003). Consequently, the working hypothesis (Elmes et al., 1994), current among scientists studying the cuckoo species of *Maculinea*, is that *M. alcon* and *M. rebeli* are part of a complex of genetically discrete cryptic species (or subspecies) each having a discrete geographical range in Europe within which it specialises on one (from up to 10 possible) *Myrmica* host species. The species of gentian used for oviposition is secondary to discriminating between the various forms. Populations in which host specificity appears to be less acute (Als et al., 2001, 2002; Steiner et al., 2003) might occur when one or more cryptic species exist sympatrically. Thus, the populations of '*M. rebeli*' from Germany and Austria which use *M. sabuleti* ants might be a distinct species or subspecies from the south European *M. rebeli* used in our experiment. We would predict that a bioassay of the northern populations, based on the protocol used here, would show a better survival with *M. sabuleti* than with *M. schencki*.

Materials and methods

The *Maculinea–Myrmica* system

In brief, adult butterflies emerge in early summer and oviposit on specific food plants (Thomas et al., 1998a); in the case of *M. rebeli* these are *Gentiana cruciata* L. (Gentianaceae). On hatching, the caterpillars feed on the flowers and pass through three moults in just a few weeks; the fresh, still very small 4th instar caterpillars drop to the ground and wait to be retrieved and carried back to the ant nest by any species of foraging *Myrmica* ants (Fiedler, 1990; Elmes et al., 1991a; Thomas, 2002). Once initially integrated into the ant colony, the caterpillars of three species grow by preying on the ant larvae, whereas those of the other two species, including *M. rebeli*, are cuckoos, mimicking ant larvae (see Introduction). Full integration only occurs in cuckoo species. Early field studies indicated that each *Maculinea* species specialises on a different species of *Myrmica* ant and only occasionally survives to maturity with other *Myrmica* species (Thomas et al., 1989). However, later studies indicated that the situation in the cuckoo species might be more complex (see Introduction). All *Maculinea* caterpillars have exceptional growth patterns compared to other butterflies, remaining in their final 4th instar, but gaining >95% of their final biomass, during the 10 months, or in some cases 22 months, that they live underground with the ants (Elmes et al., 2001).

Experimental stock

Foodplants with *M. rebeli* eggs were collected in the Hautes-Alpes, France, in a manner that did not damage the natural population of butterflies (Elmes & Thomas, 1992; Wardlaw et al., 1998). They were taken to the laboratory and kept as cut flowers in water until sufficient freshly moulted 4th instar caterpillars had been produced. Colonies of three non-host species that co-occur with *M. rebeli* – *Myrmica rubra* L., *M. sabuleti*, and *M. scabrinodis* Nylander – were collected from south-west England, while colonies of *M. schencki*, which is very rare in the UK, were collected from the same general region and at the same time as the *M. rebeli* eggs, but from a site which did not support a population of *M. rebeli*. It might be argued that the *M. schencki* colonies were sympatric with the *M. rebeli* caterpillars tested, or at least far less allopatric than the non-host colonies, and that this could influence the outcome of our experiment (see also Discussion). We did not consider that this was very probable, because published data indicates that *M. rebeli* caterpillars integrate into host colonies using chemical mimicry (Akino et al., 1999) and that the intraspecific variation in the chemical models (even between geographically dispersed samples) is small compared to the chemical variation between *Myrmica* species (Elmes et al., 2002; K. Schönrogge, unpubl.).

Experimental design

We used four 'benign' colonies of each of the ant species to rear the caterpillars: 2 weeks prior to the experiment all stock colonies were given some *M. rebeli* caterpillars to rear, and only those that had socially integrated two or more caterpillars were considered benign (usually all *M. schencki* colonies from any source behave benignly, but a few non-host colonies can kill all introduced *M. rebeli*). Fifty workers from each colony (four colonies × four species = 16 cultures) were established in small Brian nests (Wardlaw, 1991;

Wardlaw et al., 1998), maintained at 21 °C, and fed on an ample diet of protein (*Drosophila* larvae) and carbohydrate (sugar). We aimed to obtain an equal number of *M. rebeli* caterpillars socially integrated and growing well in every culture. Each morning, for 3 days, the caterpillars were introduced (in rotation between cultures) and after 72 h each had received four caterpillars (the target number). During the next week, we replaced any caterpillars that had failed to integrate and had died, and then continued to feed the cultures well for the next 3 weeks. Thus, by the start of the experiment all the caterpillars were within 10 days of age of each other, similar to the age structure of a wild cohort, and most had been in their host colonies for 4 weeks, and would be expected to survive well for the next 4 weeks (see below). At this time the cultures were put on a near-starvation diet for 4 weeks, and periodically disturbed to create additional stress. Throughout the remainder of this paper, we refer to time as weeks from the start of starvation (time 0 equivalent to the end of week −1), thus the pre-starvation growth period was weeks −4 to −1, the starvation period was weeks 1–4 and the subsequent well fed period was weeks 5–8 (surviving cultures were put into hibernation at the end of week 8). The starvation diet was equivalent to 0.07 full-grown *Drosophila* larva and 0.07 mg of sugar per worker per week (see Brian & Abbott, 1977; Elmes, 1989); extra food was given for the caterpillars in proportion to the total fresh weight of surviving caterpillars, treating every 3 mg of caterpillar biomass as one worker equivalent (on average, a full grown *Myrmica* worker larva weighs about 3 mg).

Ideally, a similar number of non-stressed controls should have been reared, but constraints on obtaining large numbers of caterpillars (imposed by *M. rebeli*'s rarity and high conservation status) within a few days of each other precluded this. However, many previous rearing tests (Elmes et al., 1991a,b and G.W. Elmes, J.C. Wardlaw and J.A. Thomas, unpubl.) provided controls which showed a very high survival in unstressed colonies (both host and non-host species, collected both sympatrically and allopatrically to *M. rebeli*) over an equivalent period to that of the starvation period here (4–8 weeks after initial integration): e.g., 100% survived with *M. sabuleti* (number of caterpillars, n = 49), *M. scabrinodis* (n = 37), *M. ruginodis* (n = 34), *M. sulcinodis* (n = 13), and 99% with *M. schencki* (n = 147). Furthermore, there was no suggestion that the caterpillars survived differently, during this period, when they were reared by sympatric *M. schencki* host colonies [98% (n = 55) of French and 100% (n = 48) of Spanish caterpillars survived] compared with allopatric *M. schencki* [100% (n = 14) of French caterpillars survived when reared by Danish hosts, and 97% (n = 30) when reared by Spanish *M. schencki*].

The number of surviving caterpillars was recorded each week, but they were not weighed until the end of week −1 in order to reduce disturbance to the cultures. Thereafter, they were weighed individually each week and, except in a few cultures which contained individuals of very similar size, we could assume that the rank size of the individuals within the cultures remained the same week-on-week. In addition, the surviving workers were counted and a sample weighed, and the samples of workers from the original well-fed stock nests were weighed as controls.

Statistical analysis

Analysis of the survival of caterpillars needs to be assessed with care, because at the start of the starvation period there are obviously no differences (survival = 1.0 in all cultures) and if starvation was continued for sufficient time there would also be no differences because all *M. rebeli* caterpillars and their nurse workers would have starved to death. We assessed overall differences between ant species in the caterpillar survival rates [based on proportions surviving in each culture (i.e., replicate)] during the four weeks of the starvation regime using non-parametric Kaplan–Meier estimates (Kalbfleisch & Prentice, 1980) of the survival curves for each species, treating survivors at the end of week 4 as having right-censored survival times. The log-rank test statistic (Kalbfleisch & Prentice, 1980), available in MINITAB™ Release 13, was used to test for statistically significant differences in survival curves. However, the assumed χ^2 distribution of this test statistic under the null hypothesis is based on the assumption of independent observations within each ant species. We also performed our own more rigorous randomisation test (Manly, 1997) to correctly allow for the grouping of caterpillars in cultures (which might themselves respond differently). The survival rates for the 16 cultures were randomly assigned, four to each *Myrmica* species, and the log-rank test statistic was calculated for each of 1000 randomisations to provide a valid null frequency distribution of the test statistic (the P-value was then the proportion of values greater than the observed value of the test statistic).

First, we tested the overall null hypothesis H_{0a} of no difference between any of the four *Myrmica* species for the survival rates of socially integrated *M. rebeli* caterpillars. We then tested for differences between the three non-host species (H_{0b}) and then between *M. schencki* and the three non-host species combined (H_{0c}). Statistically significant differences in the overall survival curves would not be expected to necessarily imply statistically significant differences between species every week (see above). It was of interest therefore, to determine when any differences appeared to be greatest. In this experiment we had no a priori view as to when this might be, so at the end of each

week of starvation, we fitted generalised linear models (GLM) with extra-binomial errors to the proportions surviving in each culture to test each of the three null hypotheses H_{0a}, H_{0b}, and H_{0c}. The extra-binomial error model correctly used the variation in survival between cultures of the same species as the error variability to test for differences between species; this is tested using the F-ratio of the between- to within-species mean deviances. The time here when interspecies differences in survival were found to be most significant provides an estimate of the optimal starvation period in future experiments or bioassays for tests, and could be used to set the end of experiment or period for the bioassay. This GLM model was also fitted to the proportions of the 50 workers per culture surviving up to the end of week −1.

Results

Initial integration

Despite adding extra caterpillars to replace those that had failed to integrate by the end of week −4 (start of week −3), we were only able to establish four healthy caterpillars per culture in the *M. schencki* colonies (Table 1). Integration was lowest in *M. sabuleti* cultures, where just two healthy caterpillars per culture was achieved. There was a considerable mortality of workers (average 35%) in all the cultures by the end of week −1 (Table 1); this was expected – colonies suffer high mortalities of old workers in August. Although a greater overall proportion of workers had died in *M. sabuleti* cultures than in the other species (Table 1), the difference amongst species was not statistically significant (GLM, $F_{3,12} = 0.92$, $P = 0.46$). There was no evidence among cultures of the other three species to suggest that the proportion of *M. rebeli* caterpillars that had fully integrated into the culture was influenced by the level of worker mortality (Table 1); therefore, the low integration rate for caterpillars in *M. sabuleti* cultures is unlikely to be related to the poorer survival of workers.

Time to death

There was no mortality of caterpillars in any culture during the 2 weeks of abundant food prior to the start of the starvation diet (weeks −2 and −1, see Figure 1). Some caterpillars died during each week of the starvation period. When ample food was restored, just one more caterpillar died (in a culture of *M. scabrinodis*, in week 5), and there were no further deaths before the end of week 8, when the cultures were put at 8 °C to hibernate (Figure 1).

Our own randomisation test of the Kaplan–Meier log rank statistic assessing overall differences in caterpillar survival curves between the four ant species was marginally significant ($P = 0.076$). [Interestingly, the standard test which ignored the culture grouping structure to the data over-estimated the statistical evidence of the interspecies differences ($\chi^2 = 19.51$, d.f. = 3, $P = 0.0002$)]. The equivalent randomisation test amongst the three non-host species was not significant ($P = 0.387$), suggesting that they had similar survival rates. The randomisation test comparing survival curves in *M. schencki* cultures with those in the three non-host species combined ($P = 0.036$) confirmed that most of the between-species differences were due to different survival curves with *M. schencki*. The GLM tests (Table 2) indicated that the maximal differences between the four species occurred by the end of week 3 (H_{0a} rejected, $F_{3,12} = 3.45$, $P = 0.051$). Differences in week 3 (and in other weeks) were due to higher survival with *M. schencki* than with the other species (H_{0c} rejected; $F_{1,14} = 7.86$; $P = 0.014$); at no stage were there any significant differences in survival amongst the three non-host species (H_{0b}; Table 2).

Table 1 The numbers (n) of *M. rebeli* caterpillars introduced into four cultures of 50 workers of four species of *Myrmica*: n_{-1} = the number apparently fully integrated at end week −1 (the start of starvation) and n_4 = the number of caterpillars surviving starvation diet to end week 4. Wt. = the mean weight of surviving caterpillars at end week −1 (on average grown fourfold since introduction) and Pw = the proportion of workers alive at the end of week −1

Culture #	1	2	3	4	Total
M. schencki					
n	5	5	4	4	18
n_{-1}	4	4	4	4	16
n_4	2	2	3	4	11
Wt.	8.6	7.3	9.3	10.8	9.0
Pw	0.80	0.52	0.82	0.62	0.69
M. sabuleti					
n	6	5	5	6	22
n_{-1}	3	2	2	2	9
n_4	0	0	1	0	1
Wt.	8.4	7.1	10.8	12.8	9.8
Pw	0.36	0.76	0.42	0.58	0.53
M. scabrinodis					
n	4	5	4	4	17
n_{-1}	4	3	3	4	14
n_4	2	0	0	4	6
Wt.	13.0	18.0	11.1	8.9	12.7
Pw	0.90	0.48	0.66	0.88	0.73
M. rubra					
n	4	7	5	5	21
n_{-1}	3	4	3	3	13
n_4	1	0	0	0	1
Wt.	13.5	11.1	10.6	9.7	11.2
Pw	0.76	0.62	0.42	0.80	0.65

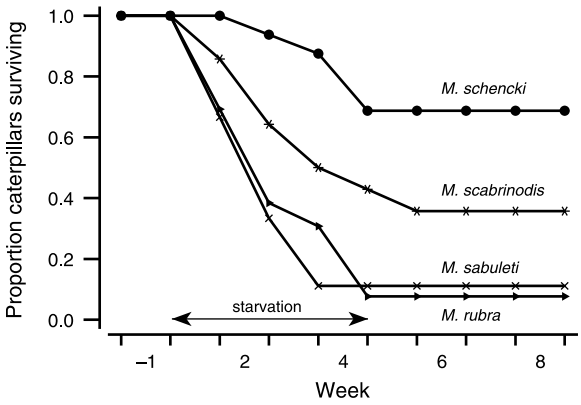

Figure 1 The mean proportion of *M. rebeli* caterpillars that had been integrated into the cultures at the end of week –1 (onset of starvation diet) surviving each week until hibernation (end week 8). Ample diet was resumed at the end of week 4.

Mortality and size of caterpillars

More caterpillars appeared to survive starvation in cultures with a high initial social integration (Table 1): 100% survival of starvation was recorded only in one culture of *M. schencki* (#4) and one of *M. scabrinodis* (#4) both of which had accepted 100% integration of 4th instar caterpillars and, with only one exception (*M. sabuleti* #3), no caterpillar survived 4 weeks of starvation when less than 70% of young caterpillars had originally been socially integrated. The worst starvation survival recorded in *M. schencki* cultures was 50%, in cultures #1 and 2, which had both received a caterpillar that had failed to integrate. A statistically significant correlation between survival at adoption and survival of starvation, ignoring species differences, was apparent by the end of week 1 ($R = 0.53$, d.f. = 14, n = 16 cultures, $P = 0.03$), it increased until week 3 (Figure 2; $R = 0.78$, d.f. = 14, $P < 0.001$) falling slightly in week 4 as more caterpillars died ($R = 0.76$, d.f. = 14, $P = 0.001$). GLM analysis of the survival at the end of week 3 ('end of bioassay' see above) showed that although there was a species effect (in that their mean rank order for initial integration was the same as that for survival of starvation), once the significant effect of initial integration was removed ($F_{1,11} = 6.92$, $P = 0.023$), none of the remaining variation could be attributed to the species of ant ($F_{3,11} = 1.45$, $P = 0.28$). In other words, as far as we can tell from this data set, the same relationship between initial integration success and starvation survival holds both inter- and intraspecifically.

Considering only the cultures in which one or more caterpillars had died in the previous week, and using only those where we could definitely identify (by rank size) which caterpillar had died, it was possible to compare the mean weights (at the start of the week) of individuals that died with those in the same cultures that survived. This showed that, in general, the caterpillars that died were small (n = 31, mean 8.22 mg) whilst survivors were larger (n = 30, mean 11.44 mg) ($F_{1,59} = 9.68$, $P = 0.003$; based on a square root transformation of the fresh weights).

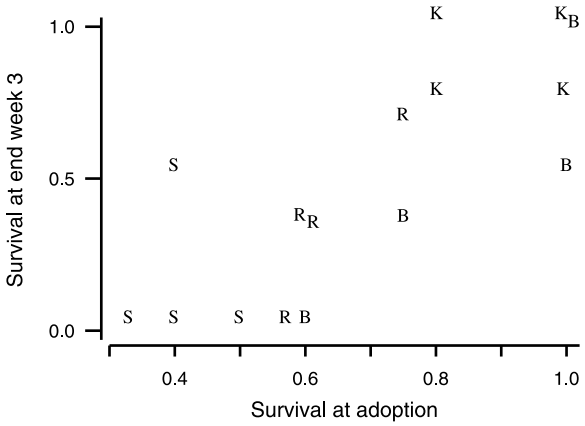

Figure 2 The relationship between the proportions of caterpillars surviving the initial integration and the subsequent proportion of these surviving 3 weeks of starvation. Caterpillars were reared by *M. schencki* (K), *M. scabrinodis* (B), *M. rubra* (R), and *M. sabuleti* (S).

Table 2 Summary of the tests for differences between *Myrmica* species in the survival of *Maculinea rebeli* caterpillars at the end of each week. F ratio tests (with subscripted degrees of freedom) and P-values were based on GLM with extra-binomial errors; see Methods for details. Differences between *M. schencki* and other ants are maximal after 3 weeks starvation

		End of week				
Differences tested		1	2	3	4	5
H_{0a}: between all four species	$F_{3,12}$	2.32	2.10	3.45	2.59	3.25
	P	0.127	0.154	0.051	0.101	0.060
H_{0b}: between three non-*schencki* species	$F_{2,9}$	0.44	0.49	1.02	1.26	1.17
	P	0.658	0.625	0.400	0.329	0.353
H_{0c}: *M. schencki* vs. non-hosts	$F_{1,14}$	6.15	5.44	7.86	4.74	7.11
	P	0.026	0.035	0.014	0.047	0.018

When given ample food, integrated caterpillars and workers which die during an experiment are rarely dismembered and are never eaten, but are discarded whole at the far end of the rubbish heap. In contrast, under starvation conditions we found very few remains of dead caterpillars and workers in the nest; most were chewed to pieces and only caterpillar hairs and skin and ant exo-skeletal fragments were placed on the rubbish heap. In one replicate of *M. rubra* we found the fresh remains of two caterpillars that had been bitten in half, and in one culture of *M. schencki* we saw pieces of one small caterpillar being fed to others. Clearly, dead caterpillars were eaten by hungry ants, but we have no direct observations on whether they were killed and eaten or simply starved to death and then recycled. In one culture of *M. rubra* and two of *M. scabrinodis*, we found caterpillars with fresh lesions, typical of wounds that can be caused by ant bites (Brian et al., 1981), which indirectly suggests that caterpillars were killed.

Nearly all caterpillars lost weight (15–30% of their fresh weight at end week –1) during the starvation period. Those that survived (mostly in *M. schencki* cultures) regained the loss over the next 3 weeks of ample diet (Figure 3). The 11 survivors with *M. schencki* lost on average 23% (12–38%), the single survivors with *M. sabuleti* and *M. rubra* lost 14% and 26%, respectively, whereas the six survivors with *M. scabrinodis* lost on average only 10% (ranging from a 16% loss to a 4% gain). The weight change of individual caterpillars was erratic, especially in cultures where the caterpillars died (Figure 3). In *M. schencki* #4 (in which all survived) the weight change was quite regular; interestingly these caterpillars gained some weight in week 4 when they were probably fed the remains of freshly dead workers. In a culture where two caterpillars died, the surviving caterpillars appeared to gain (or not lose) weight in the weeks when their nest-mates died, suggesting that they were fed the remains of these caterpillars.

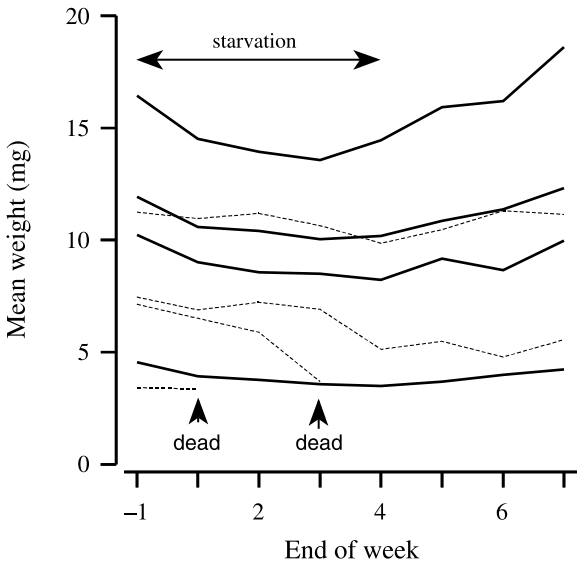

Figure 3 The weight of individual *M. rebeli* caterpillars in *M. schencki* cultures #4 (solid lines), where all four caterpillars survived starvation, and culture #2 (dotted lines), where only two caterpillars survived.

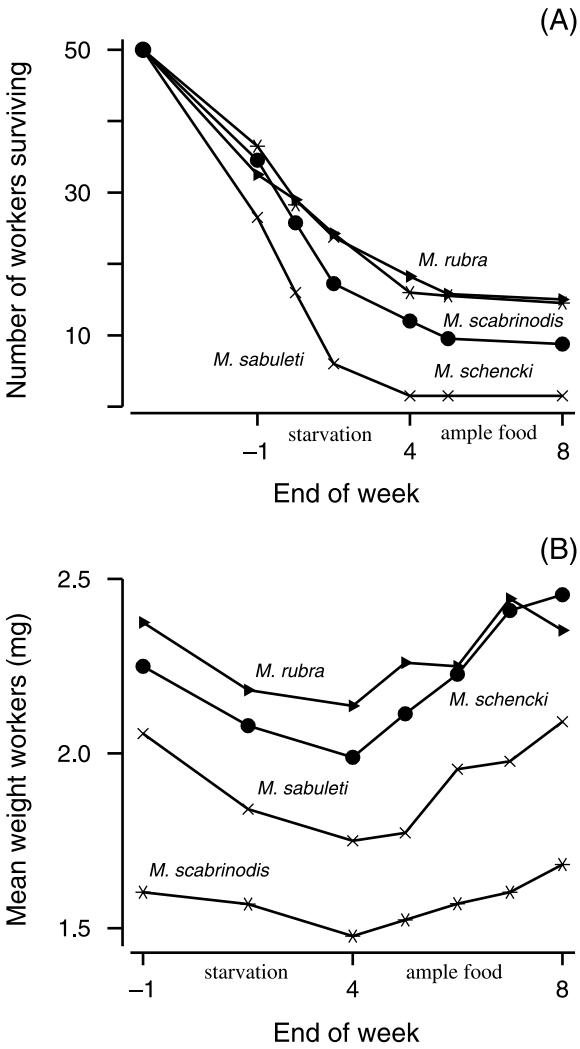

Figure 4 (A) The mean number of workers surviving in the four cultures of each species from the onset of the experiment until hibernation. (B) The mean fresh weight of surviving workers.

Effect of starvation on host workers

Many workers in all four species died during the first 2 weeks of starvation (Figure 4a); fewer died in the last 2 weeks of starvation, and following resumption of ample

food there were no more deaths before hibernation (week 8). Starvation probably simply hastened the death of the oldest (or less fit) workers, which in wild colonies, would have already been replaced by new workers produced in early summer. The workers of all four species lost weight during the starvation period, but soon regained this after 4 weeks of ample food (Figure 4b); by the end of starvation (week 4) the surviving workers were, on average, about 85% of the fresh weight of their control workers. There was no evidence to suggest that the proportion of caterpillars that were killed (or allowed to starve) by the end of week 4 had any effect upon the survival of workers in those cultures ($R = 0.098$, $n = 16$, d.f. = 14, $P = 0.72$) or on the weight lost by the surviving workers ($R = 0.114$, d.f. = 12, $P = 0.70$). No effect could be detected, even when the data was compared as cultures with surviving caterpillars vs. cultures with no surviving caterpillars (Mann–Whitney, $P = 0.50$ and $P = 0.75$, respectively).

Discussion

This experiment provides support for the hypothesis that when well-fed *Myrmica* colonies, which are successfully rearing *M. rebeli* caterpillars, are stressed by a period of food shortage, caterpillars in colonies of their specific host, *M. schencki*, survive longer, and remain in better health, compared to caterpillars reared by other similarly stressed non-host *Myrmica* species. Although unstressed controls were not incorporated into this experiment, the normally very high survival during the same period following initial integration suggests that it was absence of stress in previous laboratory experiments that accounted for the high survival with non-host species (Elmes et al., 1991b; Thomas et al., 1993, see also Methods section). The earlier experiments do not preclude the possibility that we would have observed differential survival between sympatric and allopatric host and non-host species had those cultures been subjected to a period of food stress. This remains to be tested (see below).

In another experiment, when sugar but not protein was withheld from cultures of *M. rubra* containing *M. rebeli* (Wardlaw et al., 2000), the caterpillars continued to survive and grow, even though they appeared to compete with the worker ants for sugar. Combining these results with ours suggests that it is the demand for protein (from caterpillars) when none is available that makes non-host *Myrmica* ant species reject caterpillars. This demand might be flagged by increased caterpillar begging behaviour (Elmes et al., 1991b), or by the caterpillars producing some sort of chemical signal. In either case, caterpillars might be actively drawing more attention to themselves – a dangerous strategy for an imperfect mimic of the host ants.

When kept with their *M. schencki* host, actively growing caterpillars of *M. rebeli* lost 15–40% of their initial weight during 4 weeks of starvation, yet the survivors resumed growing and continued to live and hibernate normally when feeding resumed. The predacious *Maculinea arion* L. can also survive periods of starvation (Thomas & Wardlaw, 1992) when actively growing, indicating that this trait, which is remarkable for lepidopteran larvae, is common to all *Maculinea* species. The weight loss should not be compared directly with the ca. 50% or more weight loss that *M. rebeli* caterpillars can survive during hibernation (Elmes et al., 1991b; Thomas et al., 1998b), because dormancy often involves a considerable dehydration besides a gradual burning-up of fat stores.

It has been suggested that *M. rebeli* caterpillars might have a higher social status than full–grown *M. schencki* larvae or pupae, perhaps similar to that of queens (Thomas & Wardlaw, 1990; Thomas et al., 1998b). If this were the case, other experiments (see Elmes, 1989) would predict that when food is very restricted, *M. schencki* workers should feed caterpillars at the expense of their own survival, and the workers should lose more weight and die more quickly than non-host *Myrmica* workers. We found no evidence to support this prediction. On the other hand, caterpillars survived much better than we would have predicted for *M. schencki* larvae when the workers were losing weight and dying. Therefore, the question of the social status of *M. rebeli* in *M. schencki* colonies remains open.

Workers lost about 11% of their body weight during the 4 weeks of starvation (with no indication of a difference between species, $F_{3,10} = 0.57$, $P = 0.64$); all surviving caterpillars also lost weight. Only caterpillar survival was affected by the species of ants. Indirect evidence suggested that the caterpillars were actively eliminated (recycled) by the stressed workers rather than simply starved to death. In 18 years of rearing *M. rebeli* in well-fed colonies, we had never previously seen caterpillars chopped-up and bitten after successful social integration. The *M. scabrinodis* were slightly anomalous, in that some caterpillars actually gained weight during the first week of starvation, probably because food was rationed per worker and the much smaller *M. scabrinodis* workers (compared to the other three ant species) needed less food for themselves, reducing the initial impact of starvation. Nevertheless, the caterpillars were soon eliminated from most of these cultures and despite the moderately good survival in *M. scabrinodis* colonies (especially #4) only one caterpillar from six survived until temperatures were raised in the following spring (the experiment was terminated at this point), compared with six from 11 survivors in *M. schencki*.

The benign nature of a colony, as measured by the proportion of young 4th instar caterpillars successfully

socially integrated, gives a strong indication of the probability of a caterpillar avoiding execution during any later period of stress (Figure 2). We suggest that intolerant colonies are better at detecting and reacting to chemical recognition signals than benign colonies. Thus, while the general resemblance of cuticular hydrocarbon profiles of young 4th instar *M. rebeli* caterpillars (from this population) to those of *M. schencki* workers and larvae (Akino et al., 1999; Elmes et al., 2002), might be sufficient to explain retrieval by any *Myrmica* and faster initial integration in *M. schencki* colonies, we now consider it insufficient to account for full integration resulting in long-term differential survival and host specificity, even if the mimetic chemicals are supplemented by chemical camouflage (acquired chemicals *sensu* Dettner & Liepert, 1994). If full integration and host specificity has a chemical basis, we believe that the caterpillars must be synthesising additional chemicals that indicate their social status to *M. schencki* workers (K. Schönrogge, unpubl.).

We conclude from this experiment that xenophobia, provoked by food stress, is sufficient to explain the large interspecific asymmetry in adoption and rearing success in wild populations of *M. rebeli*. The occurrence of a few non-host colonies which suffer no stress, neither food nor social, might account for the survival in a small proportion of non-host nests on typical sites in some years (Thomas & Elmes, 1998). For example, the high integration and survival rates in *M. scabrinodis* #4 would indicate that either this colony had a cuticular hydrocarbon profile similar to that of *M. schencki*, or more probably, that these ants were particularly benign. Conversely, one might predict that the few *M. schencki* colonies that are intolerant hosts in the laboratory might be suffering higher than normal social stress. It would be interesting to test this against the hypothesis that these colonies have atypical hydrocarbon signatures (Vander Meer & Morel, 1998; Lenoir et al., 2001), although if queen number and condition were implicated, the two possibilities might not be independent of each other.

It follows that we would expect a proportion of any *Maculinea* population to be reared in non-host colonies. Generally, the proportion should be small although in atypical years and on atypical sites it might be higher. In addition different host–species associations occur in different parts of the ranges of *M. alcon* and *M. rebeli* (Elmes et al., 1994; Als et al., 2001) and two *M. alcon* populations have been identified in which host specificity may be less acutely evolved (Als et al., 2001, 2002). Determining which of these situations prevails in any unknown population of *Maculinea* is the most important first challenge for practical nature conservationists. We suggest that the experimental regime (cross rearing experiment with a 3-week period of starvation) reported here should form the basis of a bioassay to assess the inherent level of host-specificity, including possible confounding effects of allopatry and co-evolution of host ants, in any population of *M. rebeli* or *M. alcon*. The fact that interspecies differences in caterpillar survival were statistically less significant when correctly allowing for any interculture differences (using randomisation tests), illustrates the importance in future bioassays of having sufficient replicates (or test colonies) for each species (or other treatment). Probably four replicates is a minimum.

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