

Development of parasitic *Maculinea teleius* (Lepidoptera, Lycaenidae) larvae in laboratory nests of four *Myrmica* ant host species

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Abstract *Maculinea* butterflies are social parasites of *Myrmica* ants. Methods to study the strength of host ant specificity in the *Maculinea*–*Myrmica* association include research on chemical and acoustic mimicry as well as experiments on ant adoption and rearing behaviour of *Maculinea* larvae. Here we present results of laboratory experiments on adoption, survival, development and integration of *M. teleius* larvae within the nests of different

Myrmica host species, with the objective of quantifying the degree of specialization of this *Maculinea* species. In the laboratory, a total of 94 nests of four *Myrmica* species: *M. scabrinodis*, *M. rubra*, *M. ruginodis* and *M. rugulosa* were used. Nests of *M. rubra* and *M. rugulosa* adopted *M. teleius* larvae more readily and quickly than *M. ruginodis* colonies. No significant differences were found in the survival rates of *M. teleius* larvae reared by different ant species. Early larval growth of *M. teleius* larvae differed slightly among nests of four *Myrmica* host species. Larvae reared by colonies of *M. rugulosa* which were the heaviest at the beginning of larval development had the lowest mean larval body mass after 18 weeks compared to those reared by other *Myrmica* species. None of the *M. teleius* larvae was carried by *M. scabrinodis* or *M. rubra* workers after ant nests were destroyed, which suggests a lack of integration with host colonies. Results indicate that *Myrmica* species coming from the same site differ in their ability to adopt and rear *M. teleius* larvae but there was no obvious adaptation of this butterfly species to one of the host ant species. This may explain why, under natural conditions, all four ants can be used as hosts of this butterfly species. Slight advantages of particular *Myrmica* species as hosts at certain points in butterfly larval development can be explained by the ant species biology and colony structure rather than by specialization of *M. teleius*.

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Introduction

Colonies of social insects, such as ants, bees or termites are used by numerous similar-sized organisms as hosts, provi-

ding shelter and food resources (Hölldobler and Wilson, 1990; Thomas et al., 2005). Social parasitism requires adaptations to infiltrate and integrate into host colonies (Hölldobler and Wilson, 1990) where more efficient exploitation of host resources is generally connected with increasing host specificity (Thomas and Elmes, 1998; Elmes et al., 1999). Thomas et al. (2005) proposed two hypotheses to explain variation in the strength of host specificity in social parasites. The first hypothesis states that host specificity depends on the level of penetration of a social parasite towards the most protected and resource-rich niches within a social colony. The second hypothesis suggests that host specificity can also depend on the developmental stage of the social parasite when entering the social colony; the earlier the stage the higher the host specificity.

One of the best known interactions in which the level of host specificity has been investigated is between the butterfly genus *Maculinea* van Eecke, 1915 and their *Myrmica* (Latreille, 1804) host ants (Thomas and Settele, 2004; Thomas et al., 2005). *Maculinea* larvae first feed on specific food plants and in their final instar they are adopted by *Myrmica* workers and taken into ant colonies where they spend 11–23 months (Thomas et al., 1998; Schönrogge et al., 2000; Witek et al., 2006). It is known that *Maculinea* larvae are picked up and carried to nests by workers of any *Myrmica* species that encounter them (Elmes et al., 1991a, 2002; Akino et al., 1999; Thomas, 2002). The next and more important step is ‘initial integration’ into the ant society which takes place within the first 24–48 h inside the colony. During this time some larvae are accepted but others are killed or neglected (Wardlaw et al., 2000; Elmes et al., 2004; Schönrogge et al., 2004). The final step termed ‘full integration’ determines survival and development of *Maculinea* larvae, including overwintering, inside host colonies (Elmes et al., 2004; Schönrogge et al., 2004). Both ‘initial’ and ‘full’ integration are much more successful in colonies of the main host although sometime colonies of other *Myrmica* species can rear a small fraction of a butterfly population (Thomas et al., 1989).

A higher integration with *Myrmica* colonies is observed in *M. alcon* ([Denis and Schiffermüller], 1775) and *M. ‘rebeli’* (Hirschke, 1904), which both follow a cuckoo strategy, where they are treated as ant brood and are fed directly by ant workers with even higher priority than the ant larvae (Elmes et al., 1991b; Thomas et al., 1998). Integration is lower in predatory species such as *M. arion* (Linnaeus, 1758) and *M. teleius* (Bergsträsser, 1779), which prey upon ant larvae (Thomas and Wardlaw, 1990, 1992). Numerous studies have been carried out on host ant specificity between *Maculinea* butterflies and their *Myrmica* host ants in recent years. Most of them are based on recorded frequency distribution of *Maculinea* pupae in relation to the host ant species they were found with (Elmes et al., 1994,

1998; Stankiewicz and Sielezniew, 2002; Als et al., 2004; Tartally and Csősz, 2004; Woyciechowski et al., 2006; Tartally and Varga, 2008; Witek et al., 2008, Patricelli et al., 2010). A more mechanistic understanding of host specificity in *Maculinea*–*Myrmica* association has been achieved by studying chemical and acoustic mimicry (Schönrogge et al., 2004; Nash et al., 2008; Barbero et al., 2009) and through the investigation of ant adoption and rearing behaviour (Als et al., 2001; Elmes et al., 2004; Sielezniew and Stankiewicz, 2007, Nash et al., 2011). The latter studies were restricted to the cuckoo feeding *M. ‘rebeli’* and *M. alcon* and they showed that caterpillars were more readily adopted by the main host species and that after adoption they achieved a high social rank within the ant society, while with other *Myrmica* species they appeared to survive only because they were merely tolerated (Thomas et al., 1989; Als et al., 2001; Elmes et al., 2004; Schönrogge et al., 2004; Sielezniew and Stankiewicz, 2007).

However, there is surprisingly little information about adoption and rearing behaviour of predatory *Maculinea* species such as *M. teleius* (Fiedler, 1990; Figurny, 1999). The first detailed studies on the host specificity of *M. teleius* showed that *M. scabrinodis* (Nylander, 1864) was its main host species (Thomas et al., 1989) while recent studies that recorded the frequency distribution of *M. teleius* pupae across the host ant species have shown that its larvae can successfully develop and survive inside nests of other *Myrmica* species from the same site (Stankiewicz and Sielezniew, 2002; Tartally and Varga, 2008; Witek et al., 2008).

To gain a better basic understanding of the interactions between this *Maculinea* species and its *Myrmica* hosts we studied the adoption, survival, growth and integration of *M. teleius* larvae inside ant colonies in laboratory experiments. The life cycle of *M. teleius* was completed within nests of four *Myrmica* species previously recorded as the hosts in our study site in the Kraków region (Witek et al., 2010), where it was found that *M. ruginodis* (Nylander, 1864) nests can provide slightly better conditions than colonies of *M. scabrinodis*. Nevertheless, it was difficult to detect whether *M. teleius* has a main host in the investigated area. We expected that a comparison of the timing of adoption of *M. teleius* larvae, survival rate and integration within colony hierarchy among different *Myrmica* species would be a good measurement of host ant specificity of *M. teleius* in our study area.

Materials and methods

Collection of *Myrmica* ants and *Maculinea* larvae

Myrmica colonies of four species [*M. scabrinodis*, *M. rubra* (Linnaeus, 1758), *M. ruginodis* and *M. rugulosa* (Nylander,

1849)] and *M. teleius* caterpillars were collected in August 2004 at three meadows belonging to one metapopulation system of *M. teleius* (Nowicki et al., 2007) located in the Vistula river valley in southern Poland (50°01'N; 19°54'E), four km south-west of Kraków centre. The total number of collected colonies was 94 (meadow 1: 12 colonies of *M. scabrinodis* and 12 of *M. rubra*; meadow 2: 12 colonies of *M. scabrinodis*, 12 *M. rubra* and 12 *M. rugulosa*; and meadow 3: 12 nests of *M. scabrinodis*, 10 *M. rubra* and 12 *M. ruginodis*). For each collected nest we established single *Myrmica* sub-colony consisting of one queen, 250 workers and 300 mg of ant brood (ant larvae and pupae). Artificial nests for each sub-colony were constructed from a transparent plastic box (28 × 17 × 6 cm) with walls coated with fluon to prevent ants from escaping. Part of the box floor was covered with fine plaster, which was moistened with water to maintain a suitable humidity. The plaster was covered by a flowerpot saucer with an entrance notch which was treated by ants as the suitable nest (ant brood was kept there). A small box with food was situated at the opposite end of the artificial colony space. Once per week *Myrmica* ants were fed with glucose and frozen fly larvae.

Caterpillars of *M. teleius* were obtained from the flowers of its food plant *Sanguisorba officinalis* L. (Dicotyledonopsida: Rosaceae). From each meadow, 500 single stems of *S. officinalis* were collected and in the laboratory all plants from one meadow were gathered into bunches of 25 stems with the base of the stems placed in water. Each bunch was enclosed in a plastic bag to prevent caterpillars from escaping. Bunches were shaken every morning and evening to obtain the larvae. Only the fourth-instar larvae (determined according to Śliwińska et al., 2006) collected in the morning were used for the experiment (so as to use only fresh fourth-instar larvae). Each caterpillar was immediately weighed to 1 mg precision using a Cole-Parmer (Acculab, USA) balance.

Adoption of *M. teleius* larvae

Caterpillars obtained from *S. officinalis* were introduced into ant colonies collected from the same meadow. The larvae were put in small boxes which were placed within the ants' living space at the furthest distance from the ant nest entrance. Only a single larva was introduced into each laboratory nest. The adoption time was measured as the time (min) from the first contact between the *M. teleius* caterpillar and a *Myrmica* worker until the larva was brought into the ant nest; continuous observation was conducted during the first 8 h (= 480 min). When adoption did not occur within this time, the next observation was carried out after 20 h (= 1,200 min). If the larva was adopted between 8 and 20 h, the exact time of adoption was unknown and in such cases we used the mean time of 14 h (= 840 min). When the caterpillar was not adopted within 20 h it was immediately

replaced by a fresh one placed directly into the ant nest. Results from these colonies were not used for the analysis of the "adoption" experiment but combined with data from other colonies were used for the analysis of the survival and development of *M. teleius* larvae.

Survival and development of *M. teleius* larvae

Each *Myrmica* colony (94 artificial nests) contained one *M. teleius* larva. Survival and growth (body mass changes) of *M. teleius* larvae were assessed at 7-day intervals throughout the first 8 weeks after adoption. During this time the temperature in the ant colonies was maintained at 20°C. In mid-October all ant nests were placed into climate controlled cabinets with a 10-h light and 14-h darkness cycle. The temperature in the cabinets approximated 18, 12, 10, 7, 5°C in October, November, December, January and the beginning of February, respectively. Then, in February, the temperature was raised to 8°C, in March to 10°C and in April to 15°C. This temperature cycle (with low winter temperatures) was similar to those described by Wardlaw et al. (1998). From mid-October to mid-April all measurements of larval growth and survival were made once per month. At the end of April all artificial nests with remaining larvae were moved to the lab with a temperature of 20°C and in a natural diurnal cycle, and subsequent survival and body mass changes were assessed at 14-day intervals. Observations continued until the beginning of July 2005. During each assessment every *Myrmica* colony was provided with the same amount of sugar and number of fly larvae. The initial amount of ant brood (as food for *M. teleius* larvae) was the same in each colony (300 mg). This amount was not artificially supplemented later in the experiment in order to keep the system as natural as possible (however, additional eggs were laid by *Myrmica* queen and workers, so that some brood was usually present as food for the butterfly caterpillars). Eleven *Myrmica* colonies lost their queens during the experiment, particularly in its early phase.

Integration experiment

Eight nests of *M. rubra*, nine nests of *M. scabrinodis* and *S. officinalis* stems (as a source of *M. teleius* caterpillars) were collected in August 2005 in the Kraków region. Each of the nests provided 150 workers, 1 queen and 13 ant larvae. Ants were kept in the laboratory for one week prior to the start of experiment. The collection and introduction of *M. teleius* larvae as well as the construction of artificial nests were conducted as described above for the adoption experiment.

Larvae of *M. teleius* obtained from *S. officinalis* were immediately placed into ant nests, one larva per nest. The experiment was carried out 48 h after the caterpillars were placed into *Myrmica* colonies. Ant nests were destroyed by

moving the humid plasters and flowerpot saucers to the opposite side of the box (i.e. about 20 cm away). Observation started from this moment and finished 20 min after the last *Myrmica* larva was moved by workers into the new ant nest. It was recorded whether the *Maculinea* larvae were also taken to the new nests.

Statistical analysis

Most statistical analyses were conducted with Statistica 6.0 software (StatSoft, 2003), with the exception of the Log-rank test for which the SAS statistical software package was used (SAS Institute, 2004). Differences in the proportion of successful adoptions of *M. teleius* larvae among different *Myrmica* species were tested using Fisher's exact tests, generalized to more than two compared categories (Carr, 1980, as implemented at <http://www.quantitativeskills.com/sisa/>). There were no differences among the three meadows in the proportion of adoptions made by either *M. scabrinodis* or *M. rubra* colonies. Thus, data from all *M. scabrinodis* and *M. rubra* nests were pooled separately. The time of adoption of *M. teleius* larvae by, and their survival inside nests of, particular *Myrmica* species were analysed by Log-rank tests. To assess the median adoption time, censored data were also included as without them this value would be underestimated. To compare differences in body mass changes of *M. teleius* larvae reared by *Myrmica* hosts, repeated measures ANOVA was performed. To estimate the general growth pattern of *M. teleius* larvae inside ant colonies and to compare this with other *Maculinea* species, the body mass data of *M. teleius* larvae from nests of *M. scabrinodis*, *M. rubra* and *M. ruginodis* were pooled. Data coming from *M. rugulosa* nests were not included since larvae reared by this host species appeared to follow a slightly different growth path. Bonferroni correction was used for each post hoc comparison.

Results

Adoption of *M. teleius* larvae

In 65 *Myrmica* colonies (69%) adoption was carried out successfully. There was a significant difference between the four *Myrmica* species in the proportion of successful adoptions of *M. teleius* larvae (Fisher exact test, $p = 0.005$). *M. teleius* larvae were adopted by 83% (10 nests) of *M. rugulosa*, 76% (26 nests) of *M. rubra*, 58% (21 nests) of *M. scabrinodis* and 25% (3 nests) of *M. ruginodis* colonies. Post hoc pairwise comparisons among species showed that adoption by colonies of *M. ruginodis* was significantly lower than by nests of *M. rubra* and *M. rugulosa* (Table 1).

Adoption was measured up to a maximum of 20 h (1,200 min) after the first contact and the median adoption

Table 1 Results of post hoc pairwise-comparison in the proportions of *M. teleius* larvae successfully adopted by different *Myrmica* species

	<i>M. rubra</i>	<i>M. ruginodis</i>	<i>M. rugulosa</i>
<i>M. scabrinodis</i>	0.056	0.037	0.08
<i>M. rubra</i>		0.002*	0.2
<i>M. ruginodis</i>			0.005*

p values for Fisher exact test

* Significant results

Table 2 Results of post hoc pairwise-comparison in the adoption time of *M. teleius* larvae by different *Myrmica* species

	<i>M. rubra</i>	<i>M. ruginodis</i>	<i>M. rugulosa</i>
<i>M. scabrinodis</i>	0.04	0.39	0.06
<i>M. rubra</i>		0.006*	0.15
<i>M. ruginodis</i>			0.018

p values are given

* Significant results

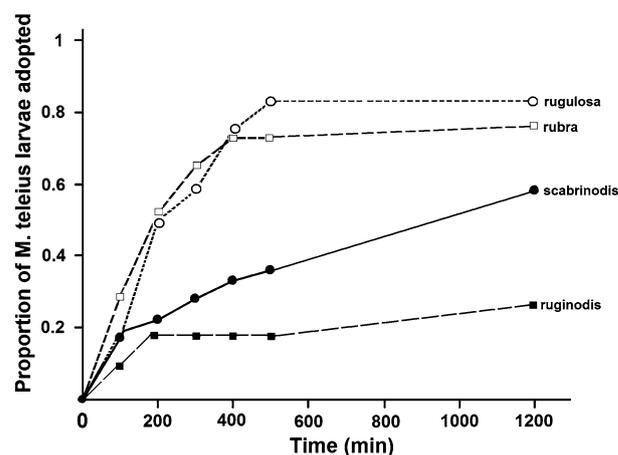


Fig. 1 Proportion of *M. teleius* larvae adopted by four host *Myrmica* species: *M. scabrinodis* (36 nests), *M. rubra* (34 nests), *M. ruginodis* (12 nests), *M. rugulosa* (12 nests)

times were: 166, 176, 840 and 1,191 min for *M. rubra*, *M. rugulosa*, *M. scabrinodis* and *M. ruginodis*, respectively. The adoption time was significantly different among the four *Myrmica* species (Log-Rank test, $\chi^2 = 12.63$, $df = 3$, $p = 0.0055$) and post hoc pairwise comparisons among species showed that colonies of *M. rubra* adopted *M. teleius* larvae significantly faster than colonies of *M. ruginodis* (Table 2; Fig. 1).

Survival and development of *M. teleius* larvae

Median survival time of *M. teleius* larvae in nests of host ant species was: 11.5 (0.5, 27 lower and upper quartiles) weeks

for *M. ruginodis*, 10.0 (0, 29) for *M. rubra*, 7.0 (3, 13) for *M. rugulosa* and 3.0 (0, 29) for *M. scabrinodis*. There were no significant differences in the survival rate among *M. telei* larvae from different *Myrmica* species (Log-Rank test, $\chi^2 = 3.43$, $df = 3$, $p = 0.32$) although it should be noted that no larvae reared by *M. rugulosa* colonies survived beyond 20 weeks of observation (mid-November). The highest mortality rate was observed in the first week after adoption and towards the end of larval development (Fig. 2). At the end of the experiment only one caterpillar reared by a colony of *M. rubra* pupated successfully and later eclosed as an adult. There was no significant difference in *M. telei* larvae surviving their first week after adoption between nests of the four *Myrmica* species ($\chi^2 = 3.48$, $p = 0.32$), although the lowest survival was observed in colonies of *M. scabrinodis* where 44% of the larvae died. In the other three species it ranged between 25 and 29%.

In four of the 11 colonies without a queen, *M. telei* larvae survived until the middle of the experiment (22 weeks) and this proportion was not different from those nests that contained a queen (in 31 nests with queen *M. telei* larvae survived until 22 weeks, Fisher exact test, $p = 0.25$).

The repeated measures ANOVA of body mass changes of caterpillars which survived until the 18th week (126 days) showed a significant effect of time ($F = 38.87$, $df = 9$, 306, $p < 0.001$) and of the interaction between time and the host ant species ($F = 2.26$, $df = 27$, 306, $p < 0.001$) but no significant effect of only the host ant species ($F = 1.76$, $df = 3$, 34, $p = 0.17$). The main observed difference concerns larvae reared within nests of *M. rugulosa*, where *M. telei* larvae were the heaviest at the beginning of the larval development compared to larvae from other *Myrmica* ant nests, but then larval weight gain decreased and after 18 weeks and caterpillars from *M. rugulosa* nests were the smallest among all host ant species (Fig. 3).

The initial mean body mass (\pm SD) of *M. telei* larvae was 3.5 ± 0.79 mg. After the first week of adoption this changed to 10.1 ± 1.89 mg. In the seventh week after adoption, before winter, the mean body mass of larvae reached 13.1 ± 1.83 mg and then during the winter months a very slight decrease was observed. The second increase of *M. telei* larval body mass was detected in the consecutive spring (Fig. 4). Interestingly, in the 38th week three of the six larvae that were still alive grew rapidly while the other three changed their body mass very slowly, leading to a high standard deviation in caterpillar mass (Fig. 4).

Integration of *M. telei* larvae

In none of 17 *Myrmica* nests (nine nests of *M. scabrinodis* and eight of *M. rubra*) did worker ants carry *M. telei* larvae to the new nests after destruction of the old one. In

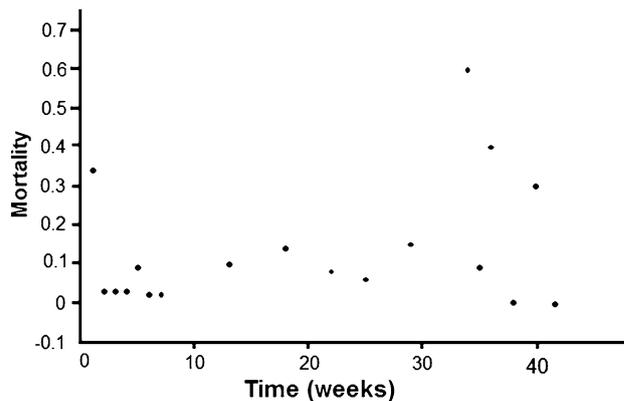


Fig. 2 Mortality rate of *M. telei* larvae reared in the laboratory nest of four *Myrmica* species (*M. scabrinodis*, *M. rubra*, *M. ruginodis* and *M. rugulosa*)

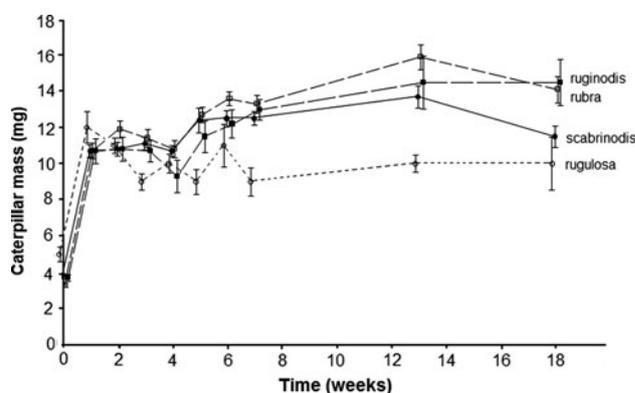


Fig. 3 Body mass changes (mean \pm SE) of *M. telei* larvae reared in nests of four *Myrmica* host species during first 18 weeks after adoption

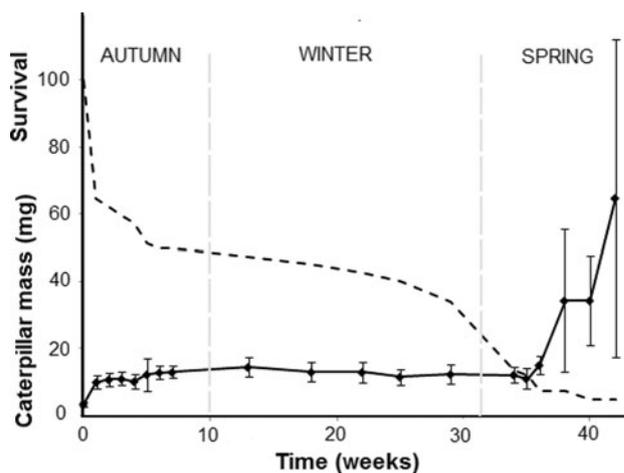


Fig. 4 Growth curve (mean \pm SD) and survival curve of *M. telei* larvae reared in the laboratory nests of three *Myrmica* species (the data for larvae from nests of *M. scabrinodis*, *M. rubra* and *M. ruginodis* were pooled together)

contrast, *Myrmica* larvae were taken to the new nest chamber very quickly (mean time \pm SE, 2.9 ± 0.71 min). In only five nests contact of more than five seconds between worker ants and *M. teleius* larva was observed.

Discussion

Data from our laboratory experiment showed that the four investigated *Myrmica* host species differed in their adoption behaviour towards *M. teleius* larvae. *M. rubra* and *M. rugulosa* adopted *M. teleius* caterpillars more frequently than *M. ruginodis*. Moreover, adoption time was shorter in colonies of *M. rubra* than those of *M. ruginodis* (Fig. 1). It is known that all *Myrmica* species used in the experiment can build polygynous colonies (Czechowski et al., 2002) and *M. rubra* and *M. rugulosa* colonies are very often polygynous, containing a very high number of workers (several thousand individuals; Czechowski et al., 2002). This results in lower relatedness among worker nest mates (Elmes and Petal, 1990; Seppä and Walin, 1996). High genetic variance can be a beneficial for colonies of social insects (Hamilton, 1987; Sundström, 1995; Baer and Schmid-Hempel, 1999; Hughes and Boomsma, 2004), but on the other hand it can increase the likelihood of being infested by social parasites as the result of greater variance in nest-mate recognition cues. Gardner et al. (2007) showed that *Microdon mutabilis* (Linnaeus, 1758) (Diptera: Syrphidae), a social parasite of *Formica lemni* Bondroit, 1917 (Hymenoptera: Formicidae) ants, more often infests host colonies with lower genetic relatedness. A similar situation was found for colonies of *M. rubra* infested by *M. alcon* (Nash and Boomsma, 2008). Thus, the low relatedness inside *M. rubra* and *M. rugulosa* colonies may be an explanation for their higher adoption rate of *M. teleius* larvae.

There was no significant difference in the survival curves of *M. teleius* larvae reared by colonies of the four *Myrmica* host species. However, none of the larvae kept in nests of *M. rugulosa* survived for more than 20 weeks after adoption. We also did not find significant differences for initial integration, although 44% of larvae reared by *M. scabrinodis* colonies died during the first week of adoption, which is a rate almost twice as high as of the other *Myrmica* species. For cuckoo *Maculinea* butterflies (*M. alcon* and *M. 'rebeli'*) it is known that initial integration with ant societies occurs during the first 24–48 h after adoption (Elmes et al., 2004). Additionally, it was shown that some *Myrmica* nests are more benign than others (Elmes et al., 2004) depending on the genotypic and social structure of the colony as well as environmental factors (Elmes et al., 2004). The initial integration of *M. 'rebeli'* larvae was faster by colonies of its main host ant species *M. schencki* (Emery, 1895) (Elmes et al., 2004). In our experiment, the slightly (but not sig-

nificantly) lower initial survival of *M. teleius* larvae inside nests of *M. scabrinodis* is consistent with conclusions from the field that *M. scabrinodis* colonies may be less benign with *M. teleius* larvae in comparison to other *Myrmica* species (Witek et al., 2010). It was shown that *M. scabrinodis* was the most abundant *Myrmica* species in the metapopulation system in the Kraków region but the percentage of infested nests was similar or even lower than those of nests of *M. rubra* or *M. ruginodis*. Moreover, a decrease of infested nests of *M. scabrinodis* has been observed during the last 20 years (Thomas et al., 1989; Witek et al., 2010). This trend could be the consequence of the arms-race between the parasite and its host, which was also observed in other interactions between social parasites and their hosts (Lorenzi and Filippone, 2000; Foitzik et al., 2003; Nash et al., 2008). In this case, *M. scabrinodis* could evolve local resistance if the selection pressure by *M. teleius* was strong.

Three times higher mortality of *M. arion* larvae was shown inside ant nests with a queen in comparisons with those without a queen (Thomas and Wardlaw, 1990). However, in our study 11 *Myrmica* colonies lost the queen at the beginning of experiment, but there was no effect on the survival of *M. teleius* larvae. Moreover, five of six larvae that survived until late spring were reared inside colonies with the queen and only one came from a colony without a queen.

Comparisons of the initial integration of the predatory *M. teleius* with the cuckoo *M. alcon* and *M. 'rebeli'* (Schönrogge et al., 2004; Sielezniew and Stankiewicz, 2007) generally show a higher survival rate of the latter two cuckoo butterflies, although there are differences depending on the host ant species used. In our experiment, depending on the *Myrmica* ant species, 56–75% of *M. teleius* larvae survived the first week of adoption, whereas survival of *M. alcon* larvae was more than 90% for primary host species and from 5 to 80% for non-host species (Sielezniew and Stankiewicz, 2007). Similar results were obtained for *M. 'rebeli'* where about 80–90% of the larvae survived in colonies of the primary host species (Schönrogge et al., 2004; Sielezniew and Stankiewicz, 2007) but 50% (Schönrogge et al., 2004) or only 10% (Sielezniew and Stankiewicz, 2007) in non-host species. On the other hand, data obtained by Nash et al. (2011) shows that the mortality rate of *M. alcon* larvae was very high at the beginning of the larval development and contrary to other experiments on cuckoo butterflies it did not depend on host ant specificity. The authors demonstrated that the survival rate of *M. alcon* larvae was the highest in the nests of *M. rubra* even when butterflies originated from populations using *M. ruginodis* as their primary host.

It is known that cuticular hydrocarbons play an important role, both during the initial as well as the full integration

phase of cuckoo *Maculinea* larvae with their host ant species (Akino et al., 1999; Elmes et al., 2002; Schönrogge et al., 2004; Nash et al., 2008). Schönrogge et al. (2004) showed that *M. 'rebeli'* larvae reared by colonies of its primary host species use chemical mimicry by an active production of hydrocarbons in addition to those found on pre-adoption larvae (and not necessarily shared with the host), whereas larvae kept in nests of non-host species seem to acquire chemical compounds from ants. Schönrogge et al. (2004) suggested that at least some of the compounds produced in the post-adoption stage by larvae of the cuckoo-feeding *M. 'rebeli'* were related to the high status which these larvae achieve within host colonies. On the other hand, Nash et al. (2011) based on early stage survival of *M. alcon* larvae inside the nests of different host ants, concluded that butterfly caterpillars rely more on the passive acquiring the colony odour rather than on active production of mimetic compounds. Therefore, it is likely that different mechanisms are used during the initial adoption phase and during the later full integration phase. Also other significant factors such as acoustic cues can be involved (Barbero et al., 2009) and it may require a combination of such cues for workers to treat parasitic caterpillars preferentially over their own brood. Little is known about chemical profiles of pre- or post-adoption larvae of predatory *Maculinea* species, but the results from our experiment indicate a lower survival of *M. teleius* larvae during the initial integration in comparison with cuckoo *Maculinea* butterflies. It is important to note that the survival rate of predatory *Maculinea* species is generally lower in laboratory conditions than that of cuckoo ones (Wardlaw et al., 1998). The lack of statistical differences in the survival of *M. teleius* larvae reared by different *Myrmica* species suggests that *M. teleius* larvae may use at best 'chemical camouflage', which means acquisition of chemical signals from its hosts (Howard et al., 1990; Nash and Boomsma, 2008). Additionally, this hypothesis may be supported by the results of the integration experiment of *M. teleius* larvae with colonies of *Myrmica* host ants. In no case were *M. teleius* larvae taken by ant workers to new nests, which indicates a low level, or even absence, of integration with the host ant colony. Similar results were obtained for another predatory species, *M. arion* (Thomas et al., 1998). In the same paper the authors showed that, in the case of the cuckoo *M. 'rebeli'*, the integration rate of larvae with colonies of the primary host was very high. It should be noted that 'initial integration' can take longer in the case of predatory than in cuckoo *Maculinea* butterflies, since the larvae need to obtain chemical compounds from their hosts. In our experiment we measured integration after 48 h from the moment of adoption similar to the experiment performed by Thomas et al. (1998) in order to obtain comparable results.

Despite the random assignment of *M. teleius* larvae to *Myrmica* colonies at the beginning of experiment, larvae put

into nests of *M. rugulosa* turned out to be the heaviest (Fig. 3). On the other hand, higher initial weight did not give the larvae any advantage in survival. Furthermore, although the body mass of *M. teleius* larvae reared by colonies of *M. rugulosa* was the highest during the first week of adoption it then decreased and after 18 weeks it was the lowest among larvae adopted by other *Myrmica* species (Fig. 3). The growth pattern of *M. teleius* larvae inside colonies of *M. scabrinodis*, *M. ruginodis* and *M. rubra* was similar although larvae reared by nests of the latter two species were slightly heavier 18 weeks after adoption. The general larval growth pattern observed in cuckoo *Maculinea* comprises an increase of body mass at the beginning of the larval development during the first week of adoption and again shortly before pupation, as well as a slight decline of body mass during winter (Thomas and Wardlaw, 1992; Thomas et al., 1998; Elmes et al., 1991b; Sielezniew and Stankiewicz, 2007; Schönrogge et al., 2000; Sielezniew and Stankiewicz, 2007; Nash et al., 2011). In contrast to cuckoo *Maculinea* butterflies, *M. teleius* larvae seem to grow very slowly during the early larval development and the mean body mass reached at the beginning of the winter diapause was 14.6 mg (Fig. 4). Data of the experiment of Nash et al. (2011) indicate that at the same time of larval development larvae of *M. alcon* reached a mean body mass from 25 to 55 mg depending on the respective population and the host ant species. Similar results were obtained for *M. 'rebeli'* larvae which on average increased their body mass up to 60 mg before winter (Thomas et al., 1998). In our study, the highest increase in *M. teleius* body mass was observed during the pre-pupation stage in spring (Fig. 4). It is also worth noting that both periods of rapid growth were associated with high mortality rates (Figs. 2, 4). In natural conditions larval survival is potentially further reduced by intraspecific competition. Interestingly, the separation of *M. teleius* larvae into fast and slow developers, which has also been observed in the field (Witek et al., 2006), occurred in May, in contrast to *M. 'rebeli'* and *M. alcon* in which fast and slow developers were already distinguishable in pre-winter larvae (and this trend was particularly evident for larvae of *M. 'rebeli'*; Thomas et al., 1998; Schönrogge et al., 2000). The existence of both strategies (fast and slow developers) under similar laboratory conditions suggests that polymorphic growth has a strong genetic component (Gotthard, 2008).

It should be mentioned that the laboratory conditions such as food regime or temperature cycle, used in our experiments can influence results compared to those under natural conditions (see Tartally, 2004). On the other hand, the most important aim of the study was to compare adoption, survival rate and development of *M. teleius* larvae reared by different *Myrmica* host species with all ant colonies kept in the same laboratory conditions. This allows us

to make among-species comparisons on the assumption that all *Myrmica* species are similarly adapted to laboratory conditions. Moreover, general conclusions from the laboratory experiments agree with those obtained in the field (Witek et al., 2010).

To summarize, our results indicate that particular *Myrmica* species found as hosts in the metapopulation in the Kraków region, differ in their ability to adopt and then rear *M. teleius* larvae, but it is not possible to observe a clear adaptation of this butterfly species to a particular host ant. Some *Myrmica* hosts, such as *M. rugulosa*, adopt larvae very quickly and readily but then the survival of the butterfly larvae is relatively low, whereas colonies of *M. ruginodis* had lower rates of adoption of *M. teleius* larvae, but later survival and increase in body mass for larvae was high. Additionally, it was not possible to show that *M. scabrinodis*, which was considered as the main host of *M. teleius* (Thomas et al., 1989), provided the best conditions for adoption and development of *M. teleius* larvae. Therefore, we have to conclude that some *Myrmica* species are better hosts during adoption and initial integration phases while others provide better conditions for the later development of *M. teleius* larvae. Similar conclusions can be derived from laboratory experiments by Als et al. (2001) and Nash et al. (2011) for larvae of *M. alcon* which used *M. rubra* and *M. ruginodis* as hosts. Results presented in this paper may explain why a multiple host use is observed in our *M. teleius* metapopulation and also in many other populations of this butterfly species.

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