

## New information on the biology of *Maculinea nausithous* and *M. teleius* (Lepidoptera : Lycaenidae)

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### Summary

Fourth instar larvae of the two obligately ant-associated lycaenid species *Maculinea nausithous* and *M. teleius* are adopted by their host ants in different ways. While larvae of *M. nausithous* are carried into the ant nest within a few minutes after their first contact with *Myrmica* ants, caterpillars of *M. teleius* have to perform a complex adoption behaviour first (including secretions from the dorsal nectary organ), lasting 30-90 min or more. Third instars of *M. nausithous* are adopted in the same way as fourth instars under laboratory conditions. Trail following behaviour of the larvae of both lycaenid species along artificial as well as natural pheromone trails of *Myrmica rubra* could not be demonstrated. The releasing factors for adoption (pheromone copies, behavioural mimicry) are discussed. Site-specific differences in the use of the foodplant, *Sanguisorba officinalis*, as oviposition substrate are documented. In habitats with a low number of foodplants, the flowerheads are sometimes severely overcrowded with eggs, resulting in a considerable loss of reproductive potential. The significance of the findings are discussed with respect to conservation efforts for these two highly endangered lycaenid species.

### Introduction

The 5 species of the genus *Maculinea* VAN EECKE are the only European lycaenids that are obligately dependent on ants : their larvae complete their development in ant nests as kleptoparasites, either feeding on ant brood or being fed directly by the ants (review : COTTRELL 1984). Because of this striking biology the genus *Maculinea* has been rather intensively studied throughout this century. The best-investigated species is undoubtedly *M. arion* LINNAEUS, 1758 (e.g. THOMAS 1977, ELMES & THOMAS 1987). While the two sibling species that feed on *Sanguisorba officinalis*, i.e. *M. nausithous* BERGSTRÄSSER, 1779 and *M. teleius* BERGSTRÄSSER, 1779, have also been the subject of several thorough investigations (CHAPMAN 1920, VAN SCHEPDAEL 1958, MALICKY 1968, THOMAS 1982, 1984), there is still considerable

disagreement on some aspects of their biology. This controversy applies, in particular, to the manner in which the larvae of both species reach the nests of their host ants. SCHROTH & MASCHWITZ (1984) presented experimental evidence that the larvae of *M. teleius* are able to follow pheromone trails of their host ant, *Myrmica rubra* LINNAEUS, 1758 (= *laevinodis* NYLANDER, see KUTTER, 1977); they failed to observe any carrying of the caterpillars by worker ants. In contrast, several other authors have reported that the larvae are carried by the ants into their nests following a specific adoption behaviour (THOMAS 1984, ELMES & THOMAS 1987). In the present article, I shall describe the results of trail-following and adoption experiments with larvae of both, *M. teleius* and *M. nausithous*. In addition, further observations on the biology of these two lycaenid species are reported.

## Material and methods

Flowerheads of *Sanguisorba officinalis* LINNAEUS were collected in late August and early September from several habitats with substantial *Maculinea* populations in the Spessart region in Hesse. At that time, most of the *Maculinea* larvae had reached the 2nd or 3rd instar and were to leave the flowerheads soon. The flowerheads were kept in closed plastic containers until the final instar larvae ( $L_4$ ) left them. These  $L_4$  were used for experiments with ants. Third instars were successfully reimplanted into fresh *Sanguisorba* heads. After all larvae had left the flowers, the flowerheads were dissected under a stereo microscope and the egg-shells were counted.

A large nest of *Myrmica rubra* (> 500 worker ants) was taken from a moist meadow where both *Maculinea* species occurred. This earth nest was kept in a plastic terrarium and the colony did well for more than 6 months. The following experiments were conducted :

### a) Trail experiments with artificial trails :

The gasters of 5 worker ants were squeezed and dissolved in 0.1 ml water (the method used by SCHROTH & MASCHWITZ, 1984). The gaster contains the poison gland which is known to produce the trail substance of *Myrmica* ants (CAMMAERTS-TRICOT 1974, CAMMAERTS-TRICOT *et al.* 1977). To obtain extracts for control experiments, the thoraces of the same 5 ants as well as one ant pupa were squeezed and extracted in the same manner. These latter extracts contained high amounts of hemolymph, but no trail substance of *M. rubra*. In addition, pure water was tested. 20-75  $\mu$ l of the respective solutions were applied onto white cardboard in form of an S-shaped "trail" (length 20 cm). In each experiment, one gaster extract was tested against one type of control (water, thorax or pupa extract). The experimental and control "trails" crossed each other twice. Immediately after displaying the extracts, final instar larvae of both *Maculinea* species were put on the "trails" and their behaviour was observed. No larvae were used that had already been adopted by the ants before.

b) Trail experiments with natural trails :

In a separate plastic box ("arena") the ants were offered a carbohydrate food source (solution of honey in water). This box was connected with the ants' nest by a small strip of cardboard. After the first ants had detected the food source, they started feeding and, when returning to their nest, laid recruitment pheromone trails. Nestmates readily followed these trails, and after 25 min. more than 100 ants were in the arena. Then the paper strip (which had been marked with dozens of pheromone trails by the ants) was separated and *Maculinea* larvae were put onto it. Subsequently their behaviour was observed.

c) Adoption experiments :

In the same way as above an arena was connected with the ants' nest, either containing an additional food source or not. Larvae of both *Maculinea* species were put into the arena and the reactions of both ants and caterpillars were observed.

## Results

a) TRAIL FOLLOWING

In 74 trials with larvae of *M. teleius* not a single complete trail following was observed. On 13 occasions a larva followed an artificial trail for 1-5 cm, but 5 of these partial following responses were on thorax or pupal extracts lacking the trail pheromone of *M. rubra*. The complete quantitative results are given in Table 1. The ants, on the other hand, accepted the artificial trail with the gaster extract and followed it regularly, at least for 10 cm. Thus, although certainly no optimal artificial trails were offered, these trails did contain the trail substance of *Myrmica rubra*. Thorax and pupal extract trails were not followed by the ants.

In nine trials with *M. nausithous*, not even a partial following response was observed (trials made with 20 or 40 µl extract).

Three larvae of *M. teleius* and one of *M. nausithous* were placed on a cardboard strip on which dozens of natural ant trails had been laid previously. No larva was able to find these trails. Instead, all caterpillars followed the edge of the paper readily with no respect to the direction. Two of the *M. teleius* larvae first crawled towards the ant nest but after 15 cm they returned into the arena. The other *M. teleius* larva and the one of *M. nausithous* soon left the cardboard and crawled about in the arena. No evidence for trail following was seen.

When larvae were put on the trail while ants were present, they were adopted soon. They showed no trail following response.

Table 1

Results of trail following experiments with fourth instar larvae of *Maculinea teleius* using artificial trails from *Myrmica rubra*. + : complete following response,  $\pm$  : partial following response ( $< 5$  cm), - : no following response ; \* : not tested

| Extract amount ( $\mu$ l) | Following response | Gaster extract | Control extract |
|---------------------------|--------------------|----------------|-----------------|
| 20                        | +<br>$\pm$<br>-    | 0<br>1<br>3    | 0<br>0<br>11    |
| 30                        | +<br>$\pm$<br>-    | 0<br>5<br>10   | 0<br>4<br>8     |
| 40                        | +<br>$\pm$<br>-    | 0<br>0<br>2    | 0<br>1<br>14    |
| 60                        | +<br>$\pm$<br>-    | 0<br>1<br>16   | *<br>*<br>*     |
| 75                        | +<br>$\pm$<br>-    | 0<br>1<br>8    | *<br>*<br>*     |

#### b) ADOPTION

The adoption behaviour differs between the two *Maculinea* species (THOMAS 1984). When a worker ant detected a larva of *M. teleius* it palpated the caterpillar. The larva soon offered a secretion droplet from its dorsal nectary organ (DNO). Often the ant left the larva several times, but returned after some seconds. Then the larva continued secretion from the DNO. Finally, it showed the "humping" behaviour (see THOMAS 1977 for terminology) after some time of palpation, and suddenly the ant picked up the larva with its mandibles and carried it into the nest. The whole adoption from the first encountering to the final carrying took on average more than 44 min. (22-85 min.,  $n = 21$ ). Many of the larvae remained without motion where they had been placed initially and showed no searching behaviour (such as crawling).

In contrast, the larvae of *M. nausithous* were palpated much more intensively than those of *M. teleius*, and on no occasion a secretion from the DNO could be observed with certainty. Instead, the larvae were often picked up immediately after the first contact and carried into the nest, with a mean adoption time of only 5.40 min. (0.30-13 min.,  $n = 40$ ). This difference, in comparison to *M. teleius* is highly significant (MANN-WHITNEY test,  $U = 1292$ ,  $p < 0.001$ ). *M. nausithous* does not show the "humping" behaviour. Instead,

the larvae contract the central body segments (“dumb-bell shape”, THOMAS 1984), whereupon they are picked up by the ants.

In the above experiments only larvae within 12 h after the final moult were used. Three larvae of each *Maculinea* species were used in an adoption experiment more than 36 h after the final moult. In these cases the adoption took considerably longer and the larvae were much less attractive, in particular releasing less intensive palpation. In *M. nausithous* the adoption times were 27, 27, and 37 min., respectively, i.e. twice as long as the maximum value observed with young caterpillars. In *M. teleius*, 2 of the older larvae were not adopted within 90 min.

In addition, 5 prefinal instars (L<sub>3</sub>) of *M. nausithous* were offered to the ants. These larvae were taken up immediately after an ant had found them and were carried into the nest (mean adoption time 2.12 min.). The 3rd instars showed no dumb-bell shape contraction. Nevertheless the ants did not treat them as a prey, and the whole behavioural sequence looked like an adoption.

### c) DISTRIBUTION OF EGGS OF *M. NAUSITHOUS*

Randomly selected terminal flowerheads were inspected for the presence of eggs and eggshells at 3 sites with large *M. nausithous* populations in the Spessart region. In addition, one meadow with dense growth of tall *Sanguisorba officinalis* where I had never seen any *Maculinea* adults was also sampled. The four sites were: 1) a moist meadow of only 500 m<sup>2</sup> along a creek (200 m above sea level) together with the edge of a moist meadow along an agricultural road with only rather few tall, well developed *Sanguisorba* plants; 2) a moderately moist meadow (about 200 m above sea level), and 3) a moist meadow (300 m above sea level), both with good populations of *S. officinalis*; and 4) a south-exposed, rather dry meadow. Site 1)-3) are regularly grazed by sheep (several times a year), and site 4) is mown once a year and, in addition, grazed by sheep.

The quantitative results are given in Figure 1. They clearly show differences among the study sites in the use of flowerheads of *S. officinalis* as oviposition substrate. On site 1 with rather few adequate plants but high *M. nausithous* population density, the flowerheads received an average of 7.65 eggs, while at site 2 and 3 the figures were 5.02 and 3.68, respectively. A similar pattern shows the relative use of flowerheads: at site 1, 94.7% of the *Sanguisorba* inflorescences contained eggs, while at site 2 and 3 the respective values were 85.4 and 75%.

The number of eggs per flowerhead (clumped in classes of 0, 1-3, 4-6 etc.) fits well to a Poisson-distribution. Thus, the choice among suitable flowerheads is rather random and apparently does not depend on further factors,

such as presence of host ants. If we assume that each female lays an average of 3 eggs per suitable flowerhead at one visit (which is in accordance with my direct observations), the quantitative figures indicate that at site 1 each flowerhead is visited about three times (2.91), while the average number of oviposition visits at the sites 2 and 3 is 1.95 and 1.5, respectively. Surprisingly, also at site 4 a few eggs were found in the flowerheads.

## Discussion

### a) ADOPTION

The above trail experiments do not confirm the observations of SCHROTH & MASCHWITZ (1984) : I found no evidence for trail following in the larvae of *Maculinea teleius* and *M. nausithous*. In contrast, all larvae were actively adopted by the ants and carried into their nest as it has been reported by THOMAS (1984). This contradiction needs further explanation. I suggest that in the experiments of SCHROTH & MASCHWITZ a following response undoubtedly occurred, but this was probably due to the presence of ant hemolymph instead of recognition of the trail substance. The control experiments in the study of SCHROTH & MASCHWITZ were performed using water only and were thus not adequate to discriminate between pheromone and hemolymph effects. Because last instar larvae of *M. teleius* feed on ant brood they should be attracted by the adequate *Myrmica* hemolymph to some extent.

*Maculinea* larvae which are freshly moulted into the final instar remain rather passive when placed in an arena. Similarly THOMAS (1984) observed that the larvae of *M. teleius* do not crawl about very much after having left the *Sanguisorba* flower-head but instead hide on the ground and wait for a *Myrmica* ant to adopt them. It is not known whether older (and hungry) larvae try to search for potential host ants actively which would in part explain the observations of SCHROTH and MASCHWITZ. My experiments with 36 h old L<sub>4</sub> do not support such a hypothesis.

My experiments confirmed the marked differences in the adoption sequence between the larvae of *M. teleius* and *M. nausithous* found by THOMAS (1984). The adoption of *M. nausithous* caterpillars is a rather rapid process. Several larvae were carried into the nest after a few seconds of being palpated by a worker ant. On average the adoption of *M. nausithous* larvae took less than 6 min. compared with more than 44 min. in *M. teleius*. In *M. nausithous* larvae usually no secretions from the DNO preceeded the final transport. Instead, the larvae were immediately recognized as highly attractive. The most likely explanation is that the larvae of *M. nausithous* effectively mimic the brood pheromone of *Myrmica* ants and are thus carried into the nests at

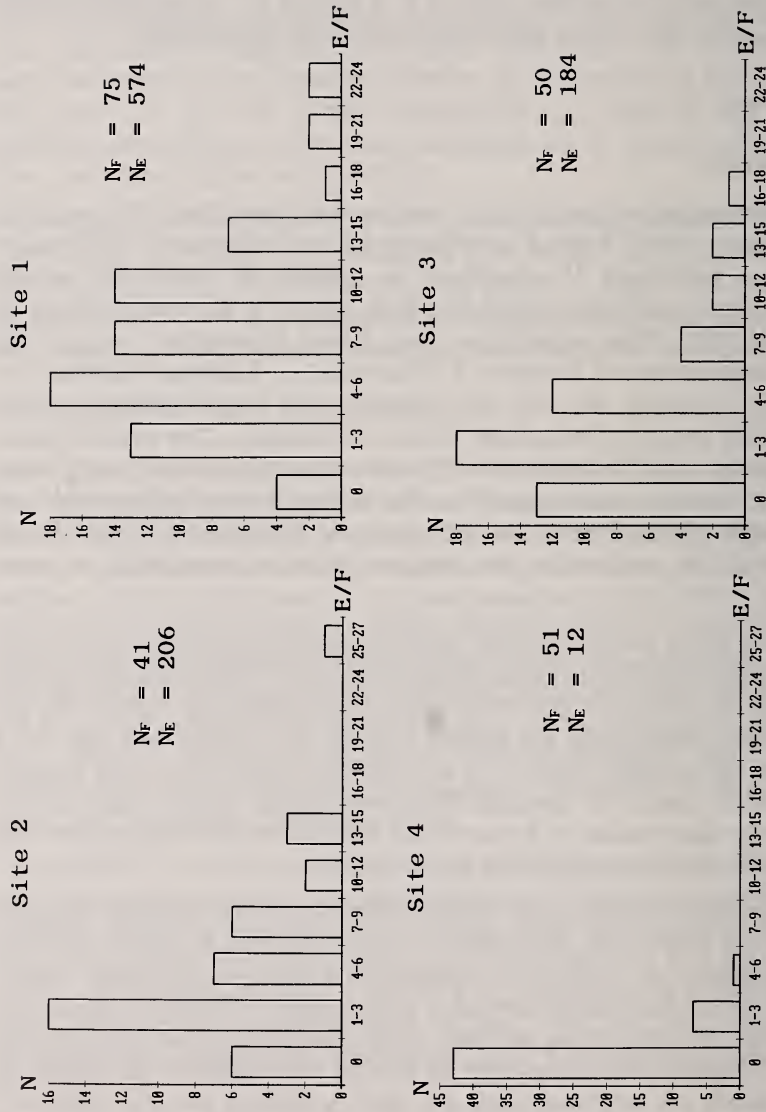


Fig. 1. Distribution of *M. nausithous* eggs at study sites 1-4 in the Spessart region (West Germany). E/F : number of eggs per flowerhead, N : number of respective flowerheads,  $N_F$  : number of flowerheads examined,  $N_E$  : total number of eggs in the sample. Further details see text.

once as is the case with ant brood offered outside the nest. The dumb-bell shape of the *M. nausithous* larva further stimulates the ants to carry it into the nest. Possibly this contraction of the central body segments is another element of ant-brood mimic and enhances the carrying ability of the ants. The pheromone copy might be released from the pore cupola organs (PCOs, see HENNING 1983) which occur densely scattered over the whole body in the larvae of *Maculinea* species in unusually high numbers (KITCHING & LUKE 1985, FIEDLER, pers. observ.).

Interestingly, third instars of *M. nausithous* were also readily adopted by the ants without any aggressiveness. The third instars do not show the dumb-bell shape contraction, this behaviour obviously being confined to the last instar. Nevertheless adoption was possible and this further strengthens the hypothesis that chemical stimuli are responsible for the release of carrying behaviour in the ants. The PCOs are already present on the third instars in large numbers.

In contrast to *M. nausithous*, *M. teleius* larvae have to perform a complex adoption behaviour including repeated secretions from the DNO and the final "humping" that induces the ant to pick up the larva and carry it into the nest (see THOMAS 1984 and ELMES & THOMAS 1987 for details). The larvae of *M. teleius* are less attractive for ants and are often abandoned several times during the adoption sequence. This was never observed with *M. nausithous* larvae.

In both species examined the probability of larval adoption by adequate ants drastically decreases with larval age, because older larvae are significantly less attractive to *Myrmica* ants than freshly moulted ones. This is probably due to a loss of the chemical stimuli necessary for adoption. In *M. teleius* there may be an additional effect if the ability to produce DNO secretions (carbohydrates?) should decrease with time.

In summary, the adoption of larvae is apparently more refined in *M. nausithous*. The smaller size of the young fourth instars, the dumb-bell shape, and, in particular, their more effective chemical stimulation of potential host ants make adoption more likely and thus more effective than in *M. teleius*. Furthermore, *M. nausithous* larvae do not need to secrete nutritive liquids from the DNO as *M. teleius* larvae do. Inside the *Myrmica* nest, as well, *M. nausithous* is the more highly adapted species: its larvae, which prey on ant brood, are in addition fed by the ants after hibernation, thus allowing several larvae per ant nest to complete their development (WEIDEMANN, 1986). In contrast, *M. teleius* larvae remain wholly predatory throughout the last instar and, as a consequence (depletion of ant brood), usually only one larva survives in an ant nest (ELMES & THOMAS 1987).

## b) UTILIZATION OF OVIPOSITION RESOURCES

Eggs of *M. nausithous* are sometimes found in considerable numbers on individual *Sanguisorba* flowerheads (THOMAS 1984, GEISSLER, pers. comm.). But the comparison between the three well-populated study sites shows that site-specific differences do occur. At site 1, with a limited number of adequate flower-heads, the mean number of eggs per flowerhead was particularly high. Sites 2 and 3 contained many more *Sanguisorba* heads and these heads had received distinctly fewer eggs. At site 1 more than 25% of the flowerheads contained 10 eggs or more, in site 2 and 3 the respective percentage was only about 10.

The few eggs found at site 4, where I have never observed any *Maculinea* adults, indicate that although both *M. teleius* and *M. nausithous* show a close affinity towards their breeding habitat, to some extent they are able to explore and eventually colonize new habitats. Possibly older females which have already laid most of their eggs perform some kind of dispersal flight as is known from many other Lepidoptera.

Though the caterpillars of *M. nausithous* are not cannibalistic, usually not more than 3-6 larvae of this species are able to develop in one *Sanguisorba* head (ELMES & THOMAS 1987). In my samples, the maximum number of larvae that reached the last instar was 4, but most flower-heads contained 1-3 caterpillars. Thus, if more than 3 eggs are deposited on one head, this surplus has little chance to survive, probably due to the heavy food competition among the young larvae inside such a flower-head. My observations indicate that in some cases no or only one larva out of 10 eggs reaches the last instar. Given the high egg-density per flowerhead found at site 1, 65% of the eggs apparently have *a priori* no chance to survive because of competition inside the flowerheads, regardless of further effects of parasitoids, predators, and availability of sufficient host ant nests to complete larval development. At site 2 and 3, the respective values were 55 and 47%. Certainly egg predators and parasitoids nevertheless play further important roles: I found a number of flower-heads with eggshells, but no significant signs of feeding or even larval exuviae. Further larval mortality occurs inside the ant nests, either due to adoption by inadequate host ants or caused by specialized parasitoids (THOMAS, pers. comm.).

Thus, even in a species like *M. nausithous* which forms large colonies on rather small areas, the availability of oviposition resources may strongly limit the population size and successful reproduction. To maintain long-term conservation of this threatened butterfly species, it is obviously necessary to undertake habitat management yielding sufficient populations of host ants (THOMAS 1984) as well as a large number of flowering *Sanguisorba* plants.

If the latter are too limited, the flowerheads may function as traps excluding a large portion of the reproductive potential from successful growth. Similarly, the foodplants must not be concentrated over too small an area, because that will result in an overexploitation of the host ants present. In *M. teleius* the need for habitat management is even more pronounced because populations of this species depend on a greater number of ant nests and *Sanguisorba* flower-heads for survival: one flower-head together with one ant colony support one individual of *M. teleius* at most. Furthermore, *M. teleius* depends on early transitory succession states in moist grasslands. This may explain why *M. teleius* is even more strongly declining in Germany than *M. nausithous* (e.g. SCHURIAN 1984, SCHADEWALD 1986).

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