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New facts on the life history of the dusky large blue *Maculinea nausithous* (Lepidoptera: Lycaenidae) obtained by breeding with *Myrmica* ants in plaster nests

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Caterpillars of *Maculinea nausithous* were bred in plaster nests with a colony of *Myrmica ruginodis* ants. Sound recordings were made and the behaviour of the ants and the various stages of the caterpillars was recorded with a video-camera. As for food, the caterpillars were critical and only fed on ant-larvae in a specific stage of development. During all stages (including the pupation) the caterpillars produced vibration signals. Also the butterflies vibrated during the emergence.

Nieuwe feiten over het leven van het donker pimpernelblauwtje Maculinea nausithous *verkregen middels een kweek met* Myrmica-*mieren in gipsnesten* - In kunstmatige gipsnesten met een kolonie *Myrmica ruginodis* mieren werden rupsen van het donker pimpernelblauwtje opgekweekt tot vlinders. Tijdens de hele ontwikkeling van de rups werden geluidsopnamen gemaakt en werd het gedrag van de rupsen en de mieren opgenomen met een video-camera. De rupsen bleken wat hun voedsel betreft zeer kieskeurig: ze aten slechts mierenlarven die in een specifiek ontwikkelingsstadium waren. Tijdens alle ontwikkelingsstadia (inclusief het verpoppen) produceerden de rupsen vibratie signalen. Ook de vlinders vibreerden gedurende het uitkomen.

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INTRODUCTION

In 1962, 1963 and 1967 attempts were made to breed the dusky large blue *Maculinea nausithous* BERGSTR. 1779 (Fig. 1) in plasternests using the same methods as was done with breeding experiments of *Maculinea alcon* (see: Elfferich 1963a,b) but the caterpillars died within a few months. In a breeding experiment in nests of *Myrmica ruginodis* in a wooden box with soil, two butterflies were reared, but no observations of the behaviour of the caterpillars could be made. In the mean time the species became extinct in The Netherlands. In 1968 Hans Malicky kindly supplied *Sanguisorba*-flowers with caterpillars of *Maculinea nausithous* from Austria. Then, in a strong colony of *Myrmica ruginodis* in a plaster nest the first positive results were obtained. Two butterflies were bred, but still the feeding habits of the caterpillars were not quite clear. After the reintroduction of the species in The Netherlands in 1990 (Wynhoff 1992; Wynhoff & van der Made 1995) new attempts to breed the species were made. In 1996 and 1997 caterpillars were successfully bred and full observations of their feeding habits were made.

Figure 1 A fresh adult female of the dusky large blue *Maculinea nausithous*. [photo: N.W. Elfferich]

MATERIAL AND METHODS

Ant colonies were placed in wooden and plastic boxes and in plaster nests as described by Elfferich (1963a,b), but in the present situation a larger plastic waste box was used. The ant food basically consisted of pieces of mealworms, flies, larvae of the lesser wax moth and sugar. Larvae of *Tortix viridana* and *Cheimatobia brumata* were added to the menu in spring. Caterpillars of *Maculinea nausithous* were taken from the flowers of *Sanguisorba officinalis* from habitats in The Netherlands or Austria (through Hans Malicky). Vibrations and other behaviour were simultaneously recorded with a Watec video-camera and a Telefunken TD 20 microphone. For sound recording the insects were placed on a piece of sigarette-paper which was attached directly onto the microphone membrane (Elfferich 1998).

RESULTS

Adoption in the ants nest

After leaving the *Sanguisorba* flowers the caterpillar crawls around to meet a *Myrmica* ant. Shortly after the first contact, the caterpillar produces a small drop from the DNO (Dorsal Nectary Organ). Then the ant starts palpating the abdomen of the caterpillar and after a while imbibes the secretion. In a short time, the ant seizes the caterpillar at the thorax and transports it into the nest. The time from the first contact till the transportation ranges from 5 minutes (in *Myrmica sabuleti*) to 20 minutes (in *M. ruginodis*). Shortly before the transportation, the caterpillar inflates its thorax and releases its true legs, one by one, lastly the third pair. It seems important that only one ant has the first contact with the caterpillar. Ants that arrive later seem to have less interest in the caterpillar and soon leave. When caterpillars were put directly into the ants nest or were put close to the entrance, the ants reacted agressively and killed the caterpillar almost immediately. This might indicate some relationship between the pheromones/vibrations released by the caterpillar and the behaviour of the ants. The best way to introduce caterpillars in the plaster nests was to put them into the waste box. There the ant

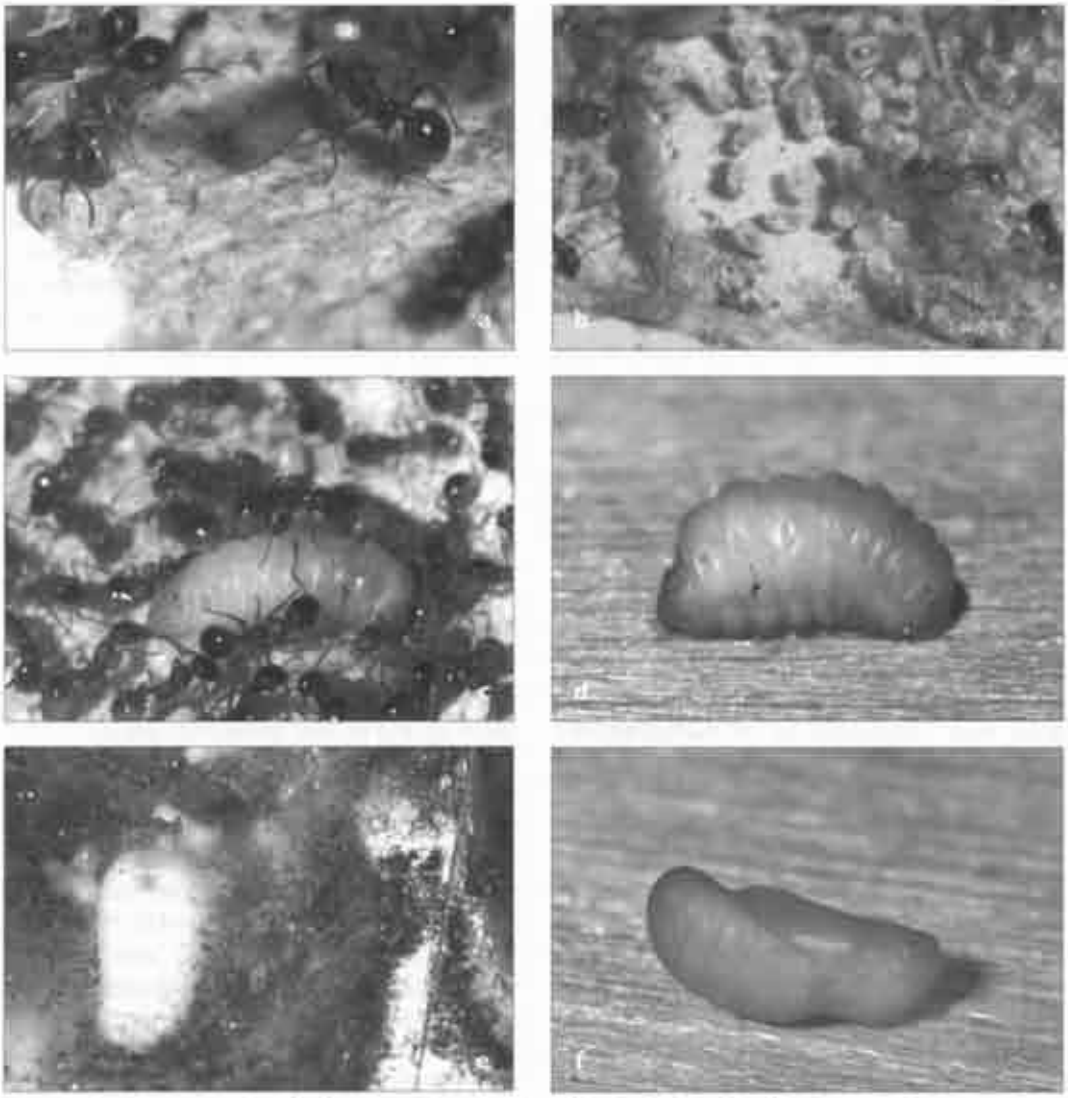

Figure 2. Various stages in the development of the caterpillar of the dusky large blue *Maculinea nausithous*: **a** caterpillar shortly after the adoption in the plaster nest with *Myrmica rugnodis*; **b** caterpillar during hibernation together with the ant-larvae; **c** almost fully grown caterpillar in the ants nest; **d** fully grown caterpillar removed from the nest; **e** fully grown caterpillar constructing a web in order to get foothold on the underside of the coverglass of the plaster nest; **f** the pupa. [photo's: N.W. Elfferich]

concentration was low and most caterpillars were adopted without difficulties.

In the ants nest

After entering the nest, the ant soon drops the caterpillar and leaves it alone (Fig. 2a). Still, ants are regulary close to the caterpillar and palpate the abdomen. The caterpillar crawls for hours to get into the broodchambers of the ants. The ants orderly collect all their larvae of the same age and bring them together in different 'rooms'. Sometimes, it takes a day for the caterpillar to reach a room with very small larvae or eggs. After devouring larvae and eggs

of the ants, the caterpillar will grow to about 6 mm before hibernation. In winter the caterpillars lay down in large heaps of antgrubs (Fig. 2b). In spring feeding starts again. The caterpillar determines the size of the larva by moving its head around it. Then the larva is pushed under the caterpillar against the bottom and is devoured. This will last about one hour. In the mean time, always some ants are palpating the DNO. Sometimes it was observed that an ant takes away a larva just before the caterpillar started attacking it. After devouring an ant larva, the caterpillar crawls around the nest for a long period. It was never observed that the ants fed the caterpillar mouth-to-mouth, as is done by caterpillars of *Maculinea alcon*. The *nausithous* caterpillar withdraws its head at once when meeting an ant. From observations based on the amount of caterpillars found in ants nests, Bink (1992) and Weidemann (1988) concluded that the *nausithous* caterpillars are fed by the ants as is done with caterpillars of *M. alcon* and *M. rebeli*, but the observations in plaster nests (as described here) show the opposite.

After a month the caterpillars start growing faster (Fig. 2c). About every two hours an antgrub is eaten. This lasts half an hour. In the nest, the amount of suitable larvae diminishes rapidly. In the mean time, a part of the antbrood has become prepupal and the first pupae are being formed. It is remarkable that the big white caterpillars (Fig. 2d) only eat the smaller ant larvae. The larvae suitable as food for the caterpillars show a dark contents of the intestine. A shortage of these kind of larvae results in a smaller butterfly or death of the caterpillar. From these observations is concluded that the *nausithous* caterpillars can survive only in strong ant colonies with a large amount of brood. An ant queen with active workers is necessary to keep the colony in good condition. In the present breeding experiments eight caterpillars were placed in the waste nest, five survived the winter and resulted in five butterflies. The last butterfly that was bred, was too small because of the shortness of ant larvae during the end of its development.

The pupation

The fully grown caterpillar starts wandering about the nest and tries to reach higher places, consequently in the 'room' close to the exit. In the plaster nest the caterpillar walks and constructs a web in order to get some foothold on the underside of the coverglas (Fig. 2e). After half a day it starts constructing a very thin web and stays motionless for about 36 hours. During this time some ants are on or around the caterpillar. The pupation is very slow and almost motionless. Only the movement of the small black head shows the metamorphosis. At last the pupa hangs with the head down in an angle of various degrees. The ants start to remove the larval skin from the abdomen, and after a few hours the pupa drops to the floor. The total development of the butterfly takes about 17 days at 21°C. During that period ants were always on and around the pupae.

The emergence of the butterfly

The butterflies always emerged between 00.00 and 06.00 hour and walked out of the nest through a 7 mm wide glass tube that connected the nest with the outside world. During the observations, this part of the ants nest was illuminated. Probably, this helped the butterfly in finding the way out. During the night there was only a little ant activity and there was no real aggressive behaviour. The emerged butterfly was placed on a piece of paper before wing expansion. Afterwards the ants showed no attention for this paper, so we may conclude that no pheromone to reduce aggression of the ants is produced by the butterfly.

Vibrations

During the breeding experiments recordings of the vibrations were made. The caterpillar produces a grunting vibration during crawling. About the same signal was recorded from the fully grown caterpillar, so we can state that during its stay in the ants' nest a vibration is studied constantly being produced. In the pre-

pupal stage, only temporary vibrations were recorded, but during the pupation the insect gives strong, continuous vibrations. The pupa produces about the same signals, but only during caterpillar disturbance. It is remarkable that in the period of 'drying' of the butterfly in the pupa, about a day before the emergence, the pupa starts a strong vibration. This vibration is produced about 30 times per minute and lasts for about half a second. It is produced for more than 24 hours. After that, the butterfly produces fewer signals until the emergence takes place. Just before and during emergence, the butterfly produces a vibration signal similar to the signal given after disturbance. During the 'drying' period of the wings the normal humming vibration is produced by 'closing' the wings.

CONCLUSIONS AND DISCUSSION

Caterpillars of *Maculinea nausithous* will only develop in a strong ant colony, with an active and healthy queen. The breeding experiments in plaster nests were only successful in a good 'working' colony. In the present study the colony was nine years old. As there seems to be a synchronisation between the development stage of the caterpillar and the availability of its food, is it very important that the ants' nests are kept under natural (outside) temperature conditions. Only then the caterpillar is able to find the ant larvae in the right stage of development. Both a large amount of antbrood and a large number of ants is necessary for successful breeding. The palpating and licking of the ants keep the caterpillars free of mould.

During the stay in the ants nest the caterpillar, the pupae and the adult produce vibration signals. The function of these signals is not clear, but the ability of the Lycaenidae and the myrmecophilous Riodinidae to produce vibrations (DeVries 1991; Elfferich 1998), suggests that they are related to communication with ants (Elfferich 1998). DeVries *et al.* (1993) suggested a resemblance between *Myrmica* stridulation and *Maculinea* vibrations, and Thomas *et al.* (1989) have studied the host-specificity of *Maculinea* butterflies.

It is known that *Myrmica ruginodis*, *M. rubra*, *M. schenkii*, *M. sabuleti* and *M. scabrinodis* are good hosts for *Maculinea* carterpillars (Elfferich 1963a,b). In this study, successful breeding results with *Maculinea nausithous* caterpillars were obtained in colonies of *Myrmica ruginodis*. This ant species appeared to be one of the best to keep in captivity. Possibly, the habitat from which the ants originate is also of importance to the breeding results: when *Maculinea* species occur in the natural habitat of *Myrmica ruginodis*, the ants will easily accept a caterpillar and the insect will develop well.

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