

HOST DISCRIMINATION AND PRE-OVIPOSITION BEHAVIOUR OF *YPONOMEUTA PADELLUS*

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Summary

In a three choice test *Yponomeuta padellus* not only laid egg batches on its own host plant *Prunus spinosa* but also on the non-host plants *Euonymus europaeus* (host of *Y. cagnagellus*) and *Malus domestica* (host of *Y. malinellus*). In contrast, *Y. cagnagellus* laid its egg batches exclusively on its own host, while *Y. malinellus* laid most egg batches on its own host. *Yponomeuta padellus* did not show any preference for one of the host plant species *P. spinosa*, *Prunus cerasifera* or *Crataegus monogyna*.

INTRODUCTION

The genus *Yponomeuta* (Lepidoptera: Yponomeutidae) has been studied as a model system for the evolution of insect-plant associations and speciation in phytophagous insects (Menken *et al.*, 1992; Menken, 1996). The main objectives to study this model are obtaining insights into the phylogenetic relationships among taxa, the evolution of their host relations and the speciation processes that have led to the present-day host associations (Menken *et al.*, 1992).

Most small ermine moths feed on species belonging to the genus *Euonymus* (Celastraceae). Other plant families on which some *Yponomeuta* species feed are Crassulaceae, Rosaceae and Salicaceae. The species existing nowadays probably evolved from an ancestral association with Celastraceae through allopatric speciation, mostly on *Euonymus*, and through sympatric host shifts to other host plant genera. All small ermine moth species are monophagous, except *Y. padellus*, which is oligophagous on a number of Rosaceae, including *Prunus spinosa*, *Crataegus monogyna* and *Prunus cerasifera*.

Under laboratory conditions, *Y. padellus* is observed to oviposit 20-80% of its egg batches on the non-host *Euonymus europaeus*, when given a choice between the non-host and its own host plant (Hora & Roessingh, pers. com.). Under the same conditions, its sister species *Yponomeuta cagnagellus* deposits all of its batches on its host *E. europaeus* (Bremner *et al.*, 1997; Hora & Roessingh, 1999a,b). The aim of this study was to test the host fidelity of the oligophagous *Y. padellus* in comparison to the closely related specialists *Y. cagnagellus* and *Y. malinellus*. Two hypotheses about the above mentioned high level of oviposition mistakes were tested: (1) In previous experiments small cages were used and conditions did not allow moths to fly. Host discrimination may be enhanced in larger cages. (2) Another cause of *Y. padellus* "oviposition mistakes" may be confusion by the presence of host volatiles in the cages. Therefore, influence of plant volatiles was tested by observing the pre-oviposition behaviour of *Y. padellus* on host and non-host, in the presence or absence of host volatiles.

EXPERIMENTS

Host discrimination

For the host discrimination test, fourth and fifth instar larvae of *Y. padellus* and *Y. cagnagellus* were collected in May and June of 1997. Fifth instar larvae of *Y. malinellus* were collected in June 1997. The larvae and pupae were kept in glass jars at room temperature till emergence. The larvae were fed with their own host plant species. After emergence moths were kept individually in glass tubes (length 8 cm, Ø 2.1 cm) in a climate room (t=18°C, RH=60%, L:D=18:6) and fed with 10% honey solution in 1% water agar three times a week.

Two to three weeks after emergence, ten females and five to ten males were put into plastic cages (length 45 cm, Ø 20 cm) in a greenhouse. Twigs of different plant species were introduced in the cages through holes (Ø 4 cm). The twigs of the potted plants had a diameter of 0.1-0.5 cm and were one to two years old. Spaces between the cage and the twig were filled with cottonwool. All three moth species were offered a choice of their host plant and the two hosts of the other moth species. Since *Y. padellus* is oligophagous, we used 2 hosts (*P. spinosa* (*Y. padellus* in Fig. 2) and *C. monogyna* (*Y. padellus** in Fig. 2)) alternatively. Additionally, *Y. padellus* was given a choice between 2 or 3 of its hosts (figure 3).

After two months - the moth's life span - the number of egg batches laid on the plant species offered were counted. The results were analysed with the χ^2 -test ($\alpha=0.05$).

Figure 1 shows the average number of egg batches per cage in the preference test between *P. spinosa*, *E. europaeus* and *M. domestica* for the three *Yponomeuta* species as well as the preference test for *Y. padellus* offered *C. monogyna*, *E. europaeus* and *M. domestica*. *Yponomeuta cagnagellus* laid their egg batches (11.38±0.40) exclusively on their own host plant *E. europaeus*. *Yponomeuta malinellus* only laid a small proportion of

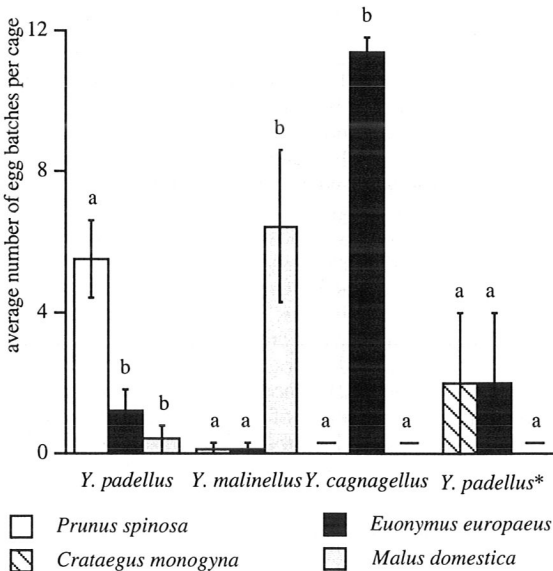


Figure 1-The average number of egg batches laid per cage. *Yponomeuta padellus* (n=10), *Y. malinellus* (n=7) and *Y. cagnagellus* (n=8) were offered *P. spinosa*, *M. domestica* and *E. europaeus*. *Yponomeuta padellus** (n=2) was offered the same species except *P. spinosa* which was replaced by *C. monogyna*.

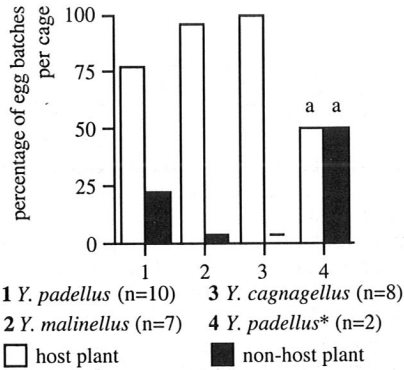


Figure 2-The percentages of egg batches laid on the host and non-host plants in the host discrimination test. Significances were tested with the χ^2 -test ($p < 0.05$). Only the numbers of egg batches laid by *Y. padellus** did not differ significantly.

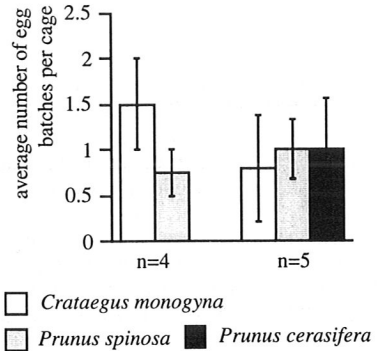


Figure 3-The average number of egg batches per cage laid by *Y. padellus*. The numbers did not differ significantly ($\chi^2 p < 0.05$).

egg batches on the non-hosts (6.43 ± 2.16 on its host, 0.14 ± 0.14 on *E. europaeus* as well as on *P. spinosa*). In contrast, *Y. padellus* made many mistakes in both tests. We found on average 5.50 ± 1.11 egg batches on the host *P. spinosa*, while 1.20 ± 0.59 (*E. europaeus*) and 0.40 ± 0.40 (*M. domestica*) egg batches were deposited on the non-host species.

In all cages in which the females of the three *Yponomeuta* species had a choice between *P. spinosa*, *E. europaeus* and *M. domestica*, significantly more egg batches were laid on the host plant than on the non-host plants (figure 2). As mentioned before, *Y. cagnagellus* only laid egg batches on its host plant. *Yponomeuta malinellus* laid 95.7% on its host plant species, whereas *Y. padellus* laid 77.5% of its egg batches on its host. When *C. monogyna* was offered as a host, an even larger proportion of egg batches was deposited on the non-hosts. This latter result, however, may not be representative since the number of egg batches was very low.

Figure 3 shows the average number of egg batches that were laid on different host plant species by *Y. padellus*. In both cases no preference seemed to be shown.

Pre-oviposition behaviour

Fourth and fifth instar larvae of *Y. padellus* were collected in the field in May and June of 1997 and 1998. The larvae were fed with leaves of *C. monogyna* and kept at room temperature in glass jars till pupation. After emergence, the moths were kept separately in glass tubes (length 8 cm, \varnothing 2.1 cm) in an observation room ($t=22^\circ\text{C}$, L:D=18:6). The moths were fed with 10% honey solution in 1% water agar three times a week. Five days after emergence from pupae, four males and four females were put together in a glass tube to give the opportunity to mate. *Yponomeuta padellus* starts to be sexually active five days after pupation and is most active at the end of the scotophase (Hendrikse, 1990). At the end of the scotophase, moths which were in copula were transferred to another glass tube. The use of mated females maximised the chance to observe oviposition. Five to thirteen days after mating the females were observed. Females that were not used within five days, were kept in a climate box ($t=8^\circ\text{C}$, RH=60%, L:D=18:6 with the same

photoperiodic regime as in the observation room). All females were fed with honey-agar three times a week.

Observations were done according to the protocol described in Hora & Roessingh (1999a). The behaviour of the females was observed for 40 minutes. Each female was observed twice to compare the behaviour of the same individual on the twigs of the potted plants of host plant *P. spinosa* and on the non-host plant *E. europaeus*. During both observations pressurised air (103-246 ln/h) was passed over the twig. The behaviour of 23 females was observed on both plant species with clean air, 23 other females were observed when *P. spinosa* volatiles of one twig were added to the air flow. The pre-oviposition behaviour, the location and the motion were scored with the computer program "The Observer 3.0" (Noldus Information Technology, Wageningen, Holland). The Wilcoxon's signed rank test (2-tailed $p < 0.05$) was used to test the significance between the percentages of the time spent on the host plant twig compared with the time spent on the non-host plant twig. The same test was used to compare the activity (defined as % of time that females show active behaviour (walking as well as pre-oviposition)) on the host twig and the non-host twig as well as comparing the activity on the arena wall with the host plant and the non-host plant in the tube. All tests were done for individual females. We did not find any significant differences between these parameters (table 1).

DISCUSSION

Experiments were done in order to test the host fidelity of the oligophagous *Y. padellus* relative to that of the specialists *Y. cagnagellus* and *Y. malinellus* under laboratory conditions. The moths were offered a selection of various host and non-host plants. In this study *Y. padellus* deposited a significantly larger proportion of egg batches on non-host plants than the other species. To test if this is a consequence of *Y. padellus* being more dependent on - and thus easier confused by - host volatiles, pre-oviposition behaviour was studied. The behaviour of females on the host *P. spinosa* was compared with the behaviour on the non-host *E. europaeus*, either under natural conditions or with extra *P. spinosa* odour added. The preliminary results of this study did not suggest any

Table 1-The duration (in percentages) of the twig contact, the activity on the twig and the activity on the arena with the host plant *Prunus spinosa* and the non-host plant *Euonymus europaeus*. The comparison on host and non-host was done without odour or with *Prunus* odour. Differences between the duration of the twig contact and the activity of individual females were tested for significance with the help of Wilcoxon's signed rank test (2-tailed $p < 0.05$). The observations on *Prunus* without odour was compared with *Euonymus* without odour. While the observations on *Prunus* with *Prunus* odour were compared with the observations on *Euonymus* with *Prunus* odour. Significances were tested for individual females. The differences were not significant.

comparing host and non-host	n	duration twig contact (%)	activity on twig (%)	activity on wall (%)
<u>without odour</u>				
<i>Prunus</i>	23	25.1	5.7	12.0
<i>Euonymus</i>	23	29.0	4.1	11.6
<u>with <i>Prunus</i> odour</u>				
<i>Prunus</i>	23	17.0	5.8	13.4
<i>Euonymus</i>	23	9.1	3.1	13.7

differences in behaviour on host or non-host, irrespectively of whether extra volatiles were added.

Contrary to results found by Kooi (1991), *Y. padellus* did not seem to prefer one of the host plants tested, although *P. spinosa* is a more suitable host for the larvae than *P. cerasifera* and *C. monogyna* (Kooi *et al.*, 1991). Performance is best on *P. spinosa* (Menken *et al.*, 1992), but the selective advantage as seen in the more successful development may be diminished by the relative scarcity of this host plant (Gerrits-Heybroek *et al.*, 1987). The host rarity may select for risk spreading over a number of plant species so polyphagy may be favoured due to limitations of the rate at which the most suitable plant for oviposition can be found (Larsson & Ekbom, 1995; Menken, 1996). Polyphagy may have been initiated by changes in sensitivity of chemoreceptors for plant compounds (loss of sensitivity for deterrents and/or gain of sensitivity for stimulants), enabling the species to accept new plants as host (Menken & Roessingh, 1998). As a less adaptive side-effect of this loss of sensitivity, *Y. padellus* may have become less exact in discrimination between host and non-host plant species.

Although *Y. padellus* deposited eggs on *E. europaeus* in our study, we would not expect this species to lay such a large proportion of eggs on *E. europaeus* in nature, since *Y. padellus* caterpillars do not survive on a diet of *E. europaeus* (Kooi, 1988; Hora, pers. com.). A large disadvantage of feeding on a non-host during the first instar may be a decrease of fitness, enhanced by the necessity of migration, during which the larvae are exposed to predators or may be unable to locate the correct host. First instars enter diapause upon emergence and hibernate under the protective shield of the empty egg shells on the original host; in spring feeding marks can be observed on the bark under the hibernaculum. That *Y. padellus* deposited eggs on *E. europaeus* in our bioassays, may still be explained by the relatively short distance between the different twigs, which may be bridged by the first instar larvae. Experiments under semi-field conditions will be needed to further evaluate this hypothesis.

Another explanation may be that *Y. padellus* is influenced by volatiles to a larger extent than *Y. cagnagellus* or *Y. malinellus*. This may cause the host discrimination through contact chemicals becoming less reliable in the presence of host volatiles in case of *Y. padellus*. *Yponomeuta cagnagellus* uses surface chemicals for host recognition (Hora & Roessingh, 1999b). Volatiles do not have a large effect on the pre-oviposition behaviour of this species (Hora & Roessingh, 1999a). Furthermore, twigs of *Crataegus* (host plant of *Y. padellus*) can be made more acceptable for *Y. cagnagellus* females by placing them with twigs of its host plant in the same jar of water (Bremner *et al.*, 1997; Roessingh *et al.*, in press). These results suggest a dependence on contact cues. In our experiments, the monophagous species *Y. cagnagellus* and *Y. malinellus* were more consistent in host plant recognition, so *Y. malinellus* may also use surface chemicals rather than plant volatiles. Unfortunately, on the basis of the preliminary observation data we cannot conclude whether *Y. padellus* is more influenced by volatiles than the aforementioned species. Therefore the reason behind the high level of oviposition mistakes still remains an open question.

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