Host plant preferences in the comma butterfly (*Polygonia c-album*): Do parents and offspring agree?[1]

Sören NYLIN & Niklas JANZ, Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden,
e-mail: snylin@zoologi.su.se

Abstract: We studied the host plant preferences of parents and offspring in the comma butterfly, *Polygonia c-album* (L.), from the Stockholm area of central Sweden. Females of the spring and summer broods were given a choice of four host plants (*Urtica dioica, Ulmus glabra, Salix caprea* and *Betula pubescens*) in flight cages, whereas their newly hatched larval offspring were given a choice of leaf samples from the same plants in petri dishes. Disagreement regarding ranking of host plants among parents and offspring could indicate trade-offs between adult and offspring fitness correlates. In the case of *P. c-album,* we predicted that if such trade-offs are weak, the choices of parents and offspring should be in agreement, because a good correlation between female host plant preference and offspring laboratory performance has previously been found. Both female and larval plant preferences differed between broods. The preferences of females and offspring were in close agreement, also differing in the same way between broods. Larvae tended to avoid plants low in the preference hierarchy (*Salix* and *Betula*) to a higher degree than females. We found no evidence of a genetic correlation between the preferences of parents and offspring. We propose that comparisons between the preferences of parents and their offspring could be an important complement to the more common studies on preference-performance correlations, giving additional clues as to how insect-plant interactions evolve.
Keywords: Nymphalidae, host plant, oviposition, seasonality, inclusive fitness.

Résumé: Nous avons étudié quelles étaient les plantes-hôtes préférées du papillon *Polygonia c-album* (L.) dans la région de Stockholm (centre de la Suède). Non seulement les papillons géniteurs ont-ils été observés, mais également leur progéniture. Les femelles se reproduisant au printemps et en été ont été placées dans des cages avec quatre plantes-hôtes d'espèces différentes (*Urtica dioica, Ulmus glabra, Salix caprea* et *Betula pubescens*). Les larves fraîchement écloses issues de ces femelles ont été déposées dans des plats de pétri avec des morceaux de feuilles provenant des mêmes plantes. Une différence entre les préférences des larves et celles des papillons adultes quant aux plantes-hôtes pourrait indiquer des relations de réciprocité entre les adultes et les larves quant à la valeur adaptative de leur préférence. Dans le cas de *P. c-album*, si de tels échanges sont faibles, les choix des parents et des larves devraient correspondre, puisqu'une corrélation positive a été établie entre les plantes-hôtes préférées des femelles et la performance de la progéniture en laboratoire. Les préférences des femelles et des larves en provenance de lignées différentes (celles du printemps et celles de l'été) ne concordent pas. Les préférences des femelles et des larves d'une même lignée sont par contre similaires. Les larves évitent davantage que les femelles adultes les plantes qui ne sont pas très appréciées par les papillons (*Salix* et *Betula*). Il n'y a pas de preuve d'une corrélation génétique au niveau des préférences des parents et larves. Nous proposons que l'étude des comparaisons entre les préférences des parents et celles de leur progéniture envers des plantes-hôtes pourrait être complémentaire aux études corrélant les préférences alimentaires et la performance en reproduction, ce qui pourrait donner des indices supplémentaires sur la façon dont les interactions plantes-herbivores évoluent.
Mots-clés: Nymphalidae, plante-hôte, oviposition, saisonalité, valeur adaptative globale.

Introduction

The relationship between the female's oviposition plant preferences and the performance of offspring are important dimensions of the ecology and evolution of insect-plant interactions (Thompson, 1988). A strong, positive correlation between preference and performance, measured as larval survival, growth rate and other correlates of fitness, suggests that females base their choice of host plants on the "intrinsic" properties of plants (*sensu* Nylin, Janz & Wedell, in press) that can influence such performance differences (plant chemistry or morphology). Often, however, weak or even negative correlations are observed, and these can be due to adaptive or non-adaptive causes (Thompson, 1988). Among the adaptive explanations, most attention has been given to the possibility that "extrinsic" properties of plants may shape

the preference hierarchy, for instance plant abundance patterns or patterns of mortality due to predation or parasitism on offspring, differing between plants (Thompson, 1988; Bernays & Graham, 1988; Ohsaki & Sato, 1994). Reavy & Lawton (1991) also pointed out that there may be conflicts between different developmental stages regarding what constitutes a good site for oviposition. A host plant which is good for the larvae may, for instance, be dangerous for the pupae or eclosing adults.

In the present paper, we address the possibility that between-generation conflicts or trade-offs between optimal female behaviour and offspring performance on various hosts can influence preference-performance correlations. The fitness of an ovipositing female is given by the product of her realized fecundity and the mean fitness of her offspring. Wiklund & Persson (1983) pointed out that the time spent

searching for suitable oviposition sites should be seen as a form of parental care, which counteracts the advantages of simply maximizing fecundity. The possible trade-off lies in the fact that her fitness is based on the number of eggs she lays, as well as the successful development of these offspring (Nylin, Janz & Wedell, 1996).

Therefore, females may search for more abundant hosts, even if offspring performance is poorer on these plants, if the resulting increase in oviposition rate more than compensates for the loss in offspring fitness (Rausher, 1980; 1985; Bernays & Chapman, 1994). This would lead to a negative preference-performance correlation. Similarly, even in cases when the preference hierarchy correlates well with offspring performance regarding the relative rank given to each host, females may accept plants low in the hierarchy (and associated with poor performance) to increase the oviposition rate. This would lead to a positive but weak preference-performance correlation. Finally, if a plant which results in good offspring performance is predictably associated with high mortality risks for the ovipositing female (*e.g.* because it grows in exposed habitats where predation risks are high), it is possible that such a plant should be avoided in order to increase the number of days that can be spent ovipositing before death (Weisser, Houston & Volkl, 1994). This could also weaken or remove the expected positive correlation between preference and performance.

Here, we report a study of individual females and their offspring, to test if parents and offspring show similar ranking of host plants and degree of specialization on the "best" hosts. The study organism is the polyphagous comma butterfly, *Polygonia c-album* (L.) (Nymphalidae), which occurs in two seasonal morphs that have earlier been shown to differ in the degree of specialization on host plants (Nylin, 1988). This difference in host plant choice between morphs is probably a result of time constraints on the second generation (favouring use of only plants permitting high growth rates) or of seasonal changes in the quality of plants, or both (Nylin, 1988).

In this species there is a basic agreement between female preference and offspring performance (Nylin, 1988; Nylin & Janz, 1993; Janz, Nylin & Wedell, 1994) and for this reason we predicted that females and offspring should also agree in their ranking of host plants, if strong trade-offs between adult and offspring fitness correlates are lacking in this system. Another prediction was that larvae should avoid plants low in the preference hierarchy, since, when given a choice, an individual larva should avoid a poor host plant (assuming a good correlation between female preference and larval performance). Several females and families of offspring were used, which means that we could also address the question of whether genetic correlations exist between the preferences of individual females and their offspring.

Materials and methods

P. c-album hibernates in the adult stage in a state of reproductive diapause. The overwintering morph differs in wing colour from the directly developing summer morph

(Nylin, 1988; 1989). The stock that we used originated from females caught near Stockholm, Sweden. In this area the population is normally univoltine and hence only the spring/autumn morph occurs, but the summer morph can be produced by exposing larvae to increasing daylengths (Nylin, 1989). In 1993, five females of the spring brood were caught in early May and they were used to found a laboratory stock of directly developing individuals, reared individually on *Urtica dioica* cultivated in water.

Later in the summer six females of this stock were given the choice of four host plants presented simultaneously in flight cages measuring 0.9 m × 0.9 m × 0.9 m (Nylin & Janz, 1993; Thompson, 1993; Janz, Nylin & Wedell, 1994): stinging nettle (*U. dioica*), elm (*Ulmus glabra*), sallow (*Salix caprea*) and birch (*Betula pubescens*). In earlier studies the two former plants were preferred on average by females and resulted in the best offspring performance, in terms of survival and growth rate, whereas birch was the least preferred and worst of these four as host for offspring (Nylin, 1988; Nylin & Janz, 1993; Janz, Nylin & Wedell, 1994).

Females were kept individually in the cages. Plants were cut to similar size and placed in bottles of water in each corner of the cages. Eggs were collected and counted every second day, at which time the plants were exchanged and their relative positions in the cage were changed. Eggs are laid singly or (on preferred hosts) in small clutches (Janz, N. & S. Nylin, unpubl.), so that the relative number of eggs on each plant is a good measure of preference. Eggs from each female were saved for larval choice tests.

Over a period of a few days twenty-four newly hatched (naive) larvae of each of the six families, or a total of 144 larvae, were placed in the centre of petri dishes (one larva to each dish) on moist filter paper and were given the same choice of host plant as their parent females. We used circular pieces of leaves, drilled out using a test tube of about 8 mm in diameter. The filter papers were moistened daily, and we observed traces of eating first after one day and later at intervals of two or three days, for a total period of about two weeks, as the larvae moved from disc to disc, feeding on them sequentially. Discs which had not been touched by the larvae were replaced with fresh ones at these times.

We took note of how much had been eaten of each plant piece (a rough percentage of area) and which plant was being eaten at the time of observation. The plant of which 10% had first been eaten was scored as the first choice of this particular larva. Larvae typically moved several times over the whole area of the petri dishes before starting to feed, evidently also inspecting the leaf discs. However, most often a clear first choice was made in one day, and the following observations could generally be used to indicate second and third choices. When 10% of more than one plant had been eaten between observations the disk with the largest area eaten was scored as the next choice. All four choices were determined in less than half of the trials, because of ties in the area eaten or because the larva died before having eaten from all four plants.

In 1994, we repeated these procedures using three females of the spring brood caught wild at the same Stockholm site in the beginning of the flight season, and

24 larvae of each of the three resulting families. In this experiment we also took note of the plant on which the egg was originally laid in the four-choice trials, to test the possibility that the individual egg could be conditioned by the plant or be genetically determined to give rise to larvae with a particular preference (evidence of a strong genetic link between the preferences of individual females and their offspring).

Results

FEMALE CHOICE AND FIRST CHOICE OF OFFSPRING

A summary of the results, showing the percentage of eggs laid by all females of each brood together, and the first choice of all larvae in each brood together, is given in Figure 1. One prediction from considerations of between-generation trade-offs was borne out: although a relatively large number of eggs were laid by females on the two less preferred hosts, *S. caprea* and *B. pubescens*, very few larvae took *S. caprea* as their first choice and only one took *B. pubescens*. This is also reflected in Figure 3, by mostly negative slopes of the lines connecting the preferences for *Salix* and *Betula* in parents and offspring.
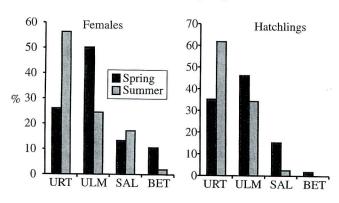

FIGURE 1. Host plant preferences of female *Polygonia c-album* (left; egg distributions in four-choice cage trials) of the spring and summer broods, and the preferences of their offspring (right; first choice of hatchlings). URT = *Urtica dioica*, ULM = *Ulmus glabra*, SAL = *Salix caprea*, BET = *Betula pubescens*. See legend of Figure 3 for sample sizes.

Even more striking, though, is the overall similarity between the choices of females and offspring. This similarity extends even to the differences between the first and second brood in ranking of host plants and in degree of preference for hosts in Urticales (Figure 1). In the summer brood, females strongly preferred *U. dioica*, followed by *U. glabra*, *S. caprea* and *B. pubescens*. In the spring brood a larger proportion of the eggs were laid on the three non-herbaceous host plants, *U. glabra* being the preferred plant rather than *U. dioica*. Overall eggs were spread more evenly over the four hosts than in the summer brood, but with a similar ranking order. These results agree with those found previously (Nylin, 1988; Nylin & Janz, 1993; Janz, Nylin & Wedell, 1994) but we found that the choices of larvae in all of these respects echoed those of their parents (Figure 1).

In the spring brood, where this was tested, there was no relationship between the original plant on which each individual egg was laid and the preferences of larvae.

Larvae took as first choice the plant from which they originated in only 14 out of 72 cases, despite the fact that the similar average preferences of adults and larvae could lead to a spurious correlation also at the level of individuals. This result is a consequence of the fact that females spread their eggs over several plants, including some suboptimal ones, and the fact that there was no correlation between the preferences of individual females and their offspring (see below).

FURTHER CHOICES OF OFFSPRING

Data for hatchlings in Figure 1 is based on the 137 and 72 cases, in each brood respectively, where we could determine the first choice of larvae using the criteria mentioned in the Methods section. In a number of those cases it was also possible to determine further choices of larvae, and it was found that they too reflected the female preference hierarchy. Thus, among offspring of the summer brood, 86 out of 137 larvae (62.8%) took *U. dioica* as their first choice and 48 larvae (35.0%) took *U. glabra* (Figure 1). Out of 106 cases where it was possible to score a second choice, 39 (36.8%) were in favour of *U. dioica* and 53 (50%) in favour of *U. glabra*. In contrast, *S. caprea* were taken as first choice in only 2.2% of the cases and as second choice in 13.2% of the trials. *B. pubescens* was never taken as first or second choice. *S. caprea* was instead the commonest third choice (54 out of 66 cases, or 81.8%), and *B. pubescens* the commonest fourth choice (66 out of 69 cases, or 95.7%). These patterns can also be seen in Figure 2, which summarizes the results of the 66 cases where all four choices could be scored.
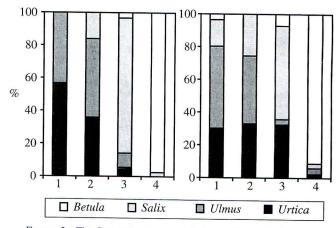

FIGURE 2. The first and subsequent choices of larvae in the 66 cases (offspring of summer brood) and 33 cases (spring brood), respectively, where all four choices could be scored.

Results for offspring of the spring brood are similar. *U. glabra* was the preferred first choice (Figure 1), taken in 34 out of 72 cases (47.2%), followed by *U. dioica* (26 cases, or 36.1%). Among the 67 cases where we could score a second choice, *U. glabra* was again preferred (33 cases, or 49.3%), followed by *U. dioica* (22 cases, or 32.8%). Reflecting the lower degree of specialization on hosts in Urticales in this brood, *S. caprea* was taken as first choice in 11 cases (15.3%) and as second choice in 12 cases (17.9%). *B. pubescens* was taken first by one larva (1.4%), but never as second choice. As in the summer brood, *S. caprea*

was the commonest third choice (19 cases out of 33, or 57.6%) and *B. pubescens* the commonest fourth choice (33 cases out of 35, or 94.3%). In 33 cases all four choices could be scored, and these results are shown in Figure 2.

VARIATION AMONG FEMALES AND FAMILIES OF OFFSPRING

Variation in preferences among females and offspring can be seen in Figure 3. The variation in preference among females was not statistically significant (female-plant interaction in ANCOVA on number of eggs laid at each count, using count # as covariate, $0.1 > p < 0.05$ for summer brood, spring brood $p > 0.01$). Neither was the variation in preference among families of larvae statistically significant in any of the two broods (Kruskal-Wallis ANOVA by ranks), although the results bordered on significance in the case of preference for *U. glabra* in the spring brood ($0.1 > p < 0.05$). Thus, it is possible that variation among females and families, suggesting genetic variation, could be found in larger samples.
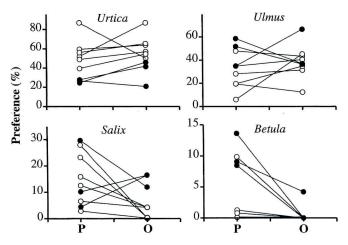

FIGURE 3. Preferences of female *Polygonia c-album* of the summer (open symbols) and spring (closed symbols) broods, and their offspring, for four host plants. The preferences of parents (P) and offspring (O) are connected by lines. Female preferences measured as percentage of eggs laid on the plants. N (total number of eggs) ranged from 117 to 324 for each female. Preferences of naive larvae measured as the percentage of larvae in each family which took each plant as first choice. N (total number of larvae) ranged from 22 to 24 in each family.

Given the lack of significant genetic variation in either parents or offspring, it is not surprising that we found no evidence of a genetic correlation between the preferences of individual females and their offspring. It should be stressed, however, that there was not even a tendency towards a correlation (note the many crossing parent-offspring lines in Figure 3). For instance, in both broods the correlation between the percentage of eggs that a female laid on *U. dioica* and the percentage of its offspring that took *U. dioica* as its first choice was in fact negative rather than positive (not statistically significant).

Discussion

The fact that larvae of *P. c-album* are at all capable of making a choice of host plant is of interest in itself, since the choice of host plant is under natural circumstances made by the mother. There are, however, a number of both ecological and physiological reasons why larvae in general should be able to make such choices, (Bernays & Chapman, 1994). In some cases, eggs are laid near the host plant rather than on it, and larvae have to find a suitable plant, or the original host plant is not enough to feed the larva or larvae. These circumstances do not normally apply to the comma butterfly, which most often places the eggs directly on host leaves, and has solitary larvae living on large plants. The choice of host plant made by the mother is not always perfect, however. Females of *P. c-album* seem to discriminate very poorly among plants of different quality within species, and larvae may therefore need the ability to find better plant individuals or plant parts. There is also a need to guard against possibilities such as host plant species growing intermingled with non-hosts, or larvae falling off plants, which could select for the ability to recognize and rank host plants. Apart from these ecological considerations, it is known from other Lepidoptera (Blaney & Simmonds, 1988) that the chemoreceptors of adults and larvae respond very similarly to stimulation with sugars, amino acids and deterrent compounds.

We found variation in preference among females, and among families of larvae, which in both cases bordered on being statistically significant. However, we found no hint of a genetic correlation between female preferences and those of their offspring. This may be just a question of sample size, but could also reflect the fact that the preference hierarchies of females and larvae are proximately formed in different ways. The former probably includes a much stronger component of visual search for host plants.

The result that the mean preferences of larvae almost exactly echoed those of the adult females, even down to the differences observed between broods, is striking. Together with the basic agreement between female preferences and offspring performance (in terms of larval laboratory survival and growth rate) found in earlier studies (Nylin, 1988; Nylin & Janz, 1993; Janz, Nylin & Wedell, 1994) this provides evidence that there are no strong trade-offs operating between generations in this system, except possibly in the case of degree of specialization on the "best" hosts (*i.e.*, *Urtica, Ulmus* and, for the spring brood, *Salix*; Nylin, 1988; Janz, Nylin & Wedell, 1994). It is not surprising that larvae should avoid poor host plants when given a choice, but this does not necessarily mean that there is a parent-offspring conflict in the classic sense (Hamilton, 1964; Trivers, 1974; Clutton-Brock & Godfray, 1991). The fitness of an offspring may be low on a poor plant, but its inclusive fitness benefits from having many sibs, as would be the case if a female increases her realized fecundity by accepting the poor plant. It is to be expected from theoretical considerations that classic parent-offspring conflicts concerning the choice of oviposition site should not generally be found in insect systems, where there are often many relatively closely related sibs (D. Raubenheimer, pers. comm.). Trade-offs between the realized fecundity of females and offspring performance is, however, a strong possibility (as it is in parasitoid systems; Weisser, Houston & Volkl, 1994). Such trade-offs could cause both lack of correlation between the preferences of females and larvae and lack of correlation between female preference and offspring performance, for

instance if the females prefer abundant plants which are relatively poor for larvae, or avoid plants which are dangerous to ovipositing females but good for larvae.

We conclude, from the combined results of this study and our earlier studies on preference-performance correlations, that extrinsic plant properties such as plant abundance patterns and field mortality risks evidently are of less importance in shaping the preference hierarchy of *P. c-album* females than are the intrinsic plant properties which determine offspring performance as measured in the laboratory. Studies of preference-performance correlations suffer from the problem that there may be many alternative explanations for the absence of such correlations (Thompson, 1988). When this is found, it could be due to constraints of various types, could indicate that extrinsic plant properties are more important than intrinsic properties, or may reflect the fact that different performance variables (components of offspring fitness) are not always positively correlated (Thompson, 1988; Janz, Nylin & Wedell, 1994; Nylin, Janz & Wedell, 1996) and that one or several performance variables of particular importance to fitness have been omitted in the study.

We propose that comparisons of parent and offspring preferences for host plants could be an important complement to preference-performance correlations in the study of insect-plant interactions, which could help distinguishing among these alternatives. If parents and offspring agree on how to rank the host plants, but the preference is uncorrelated with laboratory performance, we should consider other performance variables to explain this apparently suboptimal behaviour, including extrinsic plant properties of importance to offspring fitness. If parents and offspring disagree, but offspring preference and performance is correlated, we should further investigate the role of extrinsic plant properties for the realized fecundity of females.

Acknowledgements

Literature cited

Bernays, E. A. & R. F. Chapman, 1994. Host-Plant Selection by Phytophagous Insects. Chapman & Hall, New York.

Bernays, E. A. & M. Graham, 1988. On the evolution of host specificity in phytophagous arthropods. Ecology, 69: 886-892.

Blaney, W. M. & M. S. J. Simmonds, 1988. Food selection in adults and larvae of three species of Lepidoptera: A behavioural and electrophysiological study. Entomologia Experimentalis et Applicata, 49: 111-121.

Clutton-Brock, T. & C. Godfray, 1991. Parental investment. Pages 234-262 *in* J. R. Krebs & N. B. Davies. Behavioural Ecology. Blackwell, London, England.

Hamilton, W. D., 1964. The genetical evolution of social behaviour. Journal of Theoretical Biology, 7: 1-52.

Janz, N., S. Nylin & N. Wedell, 1994. Host plant utilization in the comma butterfly: Sources of variation and evolutionary implications. Oecologia, 99: 132-140.

Nylin, S., 1988. Host plant specialization and seasonality in a polyphagous butterfly, *Polygonia c-album* (Nymphalidae). Oikos, 53: 381-386.

Nylin, S., 1989. Effects of changing photoperiods in the life cycle regulation of the comma butterfly, *Polygonia c-album* (Nymphalidae). Ecological Entomology, 14: 209-218.

Nylin, S. & N. Janz, 1993. Oviposition preference and larval performance in *Polygonia c-album* (Lepidoptera: Nymphalidae) - the choice between bad and worse. Ecological Entomology, 18: 394-398.

Nylin, S., N. Janz & N. Wedell, 1996. Oviposition plant preference and offspring performance in the comma butterfly: Correlations and conflicts. Entomologia Experimentalis et Applicata, in press.

Ohsaki, N. & Y. Sato, 1994. Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. Ecology, 75: 59-68.

Rausher, M. D., 1980. Host abundance, juvenile survival, and oviposition preference in *Battus philenor*. Evolution, 34: 342-355.

Rausher, M. D., 1985. Variability for host preference in insect populations: Mechanistic and evolutionary models. Journal of Insect Physiology, 31: 873-889.

Reavey, D. & J. H. Lawton, 1991. Larval contribution to fitness in leaf-eating insects. Pages 293-329 *in* W. J. Bailey & J. Ridsdill-Smith (ed.). Reproductive Behaviour of Insects: Individuals and Populations. Chapman & Hall, London.

Thompson, J. N., 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologia Experimentalis et Applicata, 47: 3-14.

Thompson, J. N., 1993. Preference hierarchies and the origin of geographic specialization in host use in swallowtail butterflies. Evolution, 47: 1585-1594.

Trivers, R. L., 1974. Parent-offspring conflict. American Zoologist, 14: 249-264.

Weisser, W. W., A. I. Houston & W. Volkl, 1994. Foraging strategies in solitary parasitoids: The trade-off between female and offspring mortality risks. Evolutionary Ecology, 8: 587-597.

Wiklund, C. & A. Persson, 1983. Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? Oikos, 40: 53-63.