

Vestiges of an ancestral host plant: preference and performance in the butterfly *Polygonia faunus* and its sister species *P. c-album*

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Abstract. 1. In the study of the evolution of insect–host plant interactions, important information is provided by host ranking correspondences among female preference, offspring preference, and offspring performance. Here, we contrast such patterns in two polyphagous sister species in the butterfly family Nymphalidae, the Nearctic *Polygonia faunus*, and the Palearctic *P. c-album*.

2. These two species have similar host ranges, but according to the literature *P. faunus* does not use the ancestral host plant clade – the ‘urticalean rosids’. Comparisons of the species can thus test the effects of a change in insect–plant associations over a long time scale. Cage experiments confirmed that *P. faunus* females avoid laying eggs on *Urtica dioica* (the preferred host of *P. c-album*), instead preferring *Salix*, *Betula*, and *Ribes*.

3. However, newly hatched larvae of both species readily accept and grow well on *U. dioica*, supporting the general theory that evolutionary changes in host range are initiated through shifts in female host preferences, whereas larvae are more conservative and also can retain the capacity to perform well on ancestral hosts over long time spans.

4. Similar rankings of host plants among female preference, offspring preference, and offspring performance were observed in *P. c-album* but not in *P. faunus*. This is probably a result of vestiges of larval adaptations to the lost ancestral host taxon in the latter species.

5. Female and larval preferences seem to be largely free to evolve independently, and consequently larval preferences warrant more attention.

Key words. Adaptation, constraints, host choice, insect behaviour, oviposition.

Introduction

Insect–host plant interactions have long been a central focus in research on the evolution of species associations and co-evolution, niche theory, and diversification (e.g. Ehrlich & Raven, 1964; Price *et al.*, 1980; Dres & Mallet, 2002; Nylin & Janz, 2009; Nyman, 2010; Janz, 2011; Forister *et al.*, 2012). Study of patterns of insect–plant associations led Thompson (1988) and Thompson and Pellmyr (1991) to note that correspondences among host plant ranking for female preference and

offspring performance can provide important information about how host plant utilisation evolves. A third correspondence variable of interest is added by studying the offspring preferences for different plants (e.g. Nylin *et al.*, 1996; Soler *et al.*, 2012; Gómez Jiménez *et al.*, 2014).

When similar host rankings are found in female preference hierarchies and offspring performance, the insect–plant association can be understood as a result of selection on females to prefer to oviposit on host plants that permit favourable growth and development of their offspring (Darwin, 1909; Thompson & Pellmyr, 1991; Gripenberg *et al.*, 2010; Videla *et al.*, 2012). However, discrepancies often occur between preference and performance rankings, and there can be many causes, including the one studied here: evolutionary lags after changes in host

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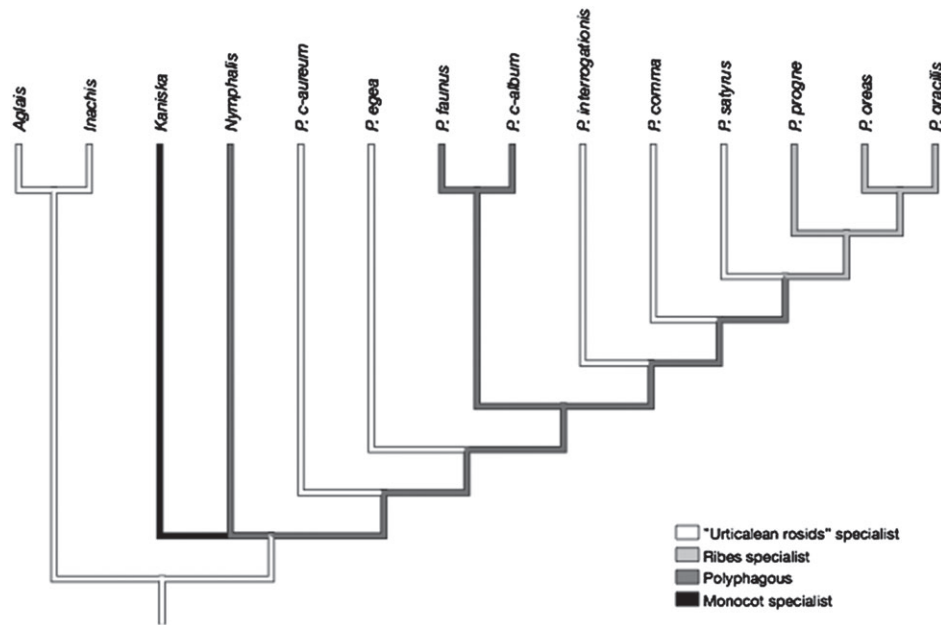


Fig. 1. Host plant utilisation in *Polytonia* and relatives. Most species are specialists on 'urticalean rosids' (white branches) and this is also clearly the ancestral state in the tribe Nymphalini as a whole. The two species of *Polytonia* studied here and most species of *Nymphalis* are polyphagous on a diverse but very similar range of host taxa (dark grey branches). This suggests that simple parsimony reconstruction is misleading. Instead the figure shows an event where the host range was broadened in the ancestor of *Polytonia* + *Nymphalis* + *Kaniska* (about 20–30 Mya; Wahlberg *et al.*, 2009) and the capacity to feed on this range of hosts was retained for a long period of time (dark grey 'backbone' in the phylogeny). Specialisation in other *Polytonia* (either re-specialisation on various 'urticalean rosids', shown in white, or on *Ribes*, shown in light grey), in *Kaniska* (shift to monocotyledons, black) and in some *Nymphalis* (not shown) probably happened secondarily. Modified from Weingartner *et al.*, 2006

distribution and/or use (Thompson, 1988; Denno *et al.*, 1990; Janz *et al.*, 1994; Nylin *et al.*, 1996; Scheirs & De Bruyn, 2002; Gripenberg *et al.*, 2010). Correspondence between the preferences of females and their offspring are similarly unproblematic, whereas discrepancies could indicate selection on females to maximise fecundity, spread risks or avoid natural enemies (Nylin & Janz, 1996; Soler *et al.*, 2012), but could again also indicate an evolutionary lag if insect–host associations have changed.

Here, we take advantage of recent phylogenetic information to study the effects of an evolutionary lag at a much longer time-scale than in previous studies. We contrast two polyphagous sister species in the butterfly family Nymphalidae, the Nearctic *Polytonia faunus* (Edwards) and the Palearctic *P. c-album* (L.). The two species have similar host ranges (see Materials and methods), with the notable exception that the former species, according to the literature (e.g. Scott, 1986; Layberry *et al.*, 1998; James & Nunnallee, 2011), does not use the ancestral host plant clade for the subfamily Nymphalidae: the 'urticalean rosids' (including Urticaceae, Ulmaceae, Cannabaceae and Moraceae; circumscription according to Sytsma *et al.*, 2002). In contrast, hosts in this clade are strongly preferred by its sister species *P. c-album* (Nylin, 1988; Janz *et al.*, 1994; Nylin & Janz, 1996; Nylin *et al.*, 2009). This loss of an ancestral host taxon in *P. faunus* provides a rare opportunity to test the hypothesis that preference–performance patterns are affected by evolutionary lags. It is of particular interest because the family Nymphalidae has a very long history of an

association with the urticalean rosids, evidently since the origin of the plant clade about 70 Mya (Nylin & Wahlberg, 2008; Nylin *et al.*, 2014).

Moreover, in another Nearctic clade of *Polytonia*, there has been a shift to the novel host *Ribes* as the strongly preferred or – in some species – the only host (Fig. 1; Weingartner *et al.*, 2006). As *Ribes* is also included in the normal host range of both *P. faunus* and *P. c-album* (but is not reliably recorded as a host of any butterfly in the family Nymphalidae outside of *Polytonia*) preference and performance observed in these polyphagous butterflies might provide clues to how shifts to novel hosts can happen (Celorio-Mancera *et al.*, 2013). *Polytonia c-album* has been extensively studied by us, but here we investigate these aspects for the first time in its American sister species *P. faunus*. As plant communities differ between the two continents only *Urtica dioica* L. could be included in both studies, but we used closely related congeneric species in *Salix*, *Betula*, and *Ribes* in the respective experiments. It should also be noted that *Polytonia* larvae do not normally choose among host plants in the field; ovipositing females make this choice. However, they still can rank hosts in choice experiments, presumably as a side-effect of the search for suitable sites within host individuals (Nylin & Janz, 1996; Gamberale-Stille *et al.*, 2014).

We tested the following predictions from the hypothesis that evolutionary lags affect preference–performance patterns, in combination with other current hypotheses about insect–plant associations:

- 1 Larvae of the two species will show more similar preferences than females. This prediction follows the general theory that evolutionary shifts in host use are initiated by changes in female host preference, whereas larvae are more conservative (Wiklund, 1975; Janz & Nylin, 1997).
- 2 Larvae of the two species will show similar performance when reared on the same or closely related hosts, and similar variation in performance among different hosts, in spite of the 5 Myr divide between the two species (Wahlberg *et al.*, 2009). This prediction was based on the general observation that larvae often perform relatively well on related or ancestral hosts not used by females for oviposition (Wiklund, 1975; Janz *et al.*, 2001; Lehnert & Scriber, 2012), suggesting relatively broad and conservative digestive and metabolic capacities in larvae.
- 3 *Polygona faunus* will show discrepancies between rankings of female preference, offspring preference, and offspring performance, owing to differences in evolutionary dynamics between females and offspring after the loss of an important ancestral host. In *P. c-album* these variables will accord with each other, as in previous studies.

Materials and methods

Study species

Polygona faunus has a wide distribution in Canada and the U.S.A., from coast to coast in the north and mountainous areas further to the south. Several subspecies have been recognised, but genetic diversity among geographical populations does not correspond well to subspecies divisions (Kodandaramaiah *et al.*, 2012). The population studied here, from Alberta, Canada, can be assigned to the subspecies *P. f. arcticus* (Kodandaramaiah *et al.*, 2012).

Host plants reliably reported for *P. faunus* belong to *Betula* (Fagales: Betulaceae), *Alnus* (Fagales: Betulaceae), *Salix* (Malpighiales: Salicaceae), *Ribes* (Saxifragales: Grossulariaceae), and *Rhododendron* (Ericales: Ericaceae) (Scott, 1986; Layberry *et al.*, 1998; James & Nunnallee, 2011). In addition, larvae survive to pupation on *Urtica* (Rosales: Urticaceae) in the laboratory (Scott, 1986; Janz *et al.*, 2001), but this genus has not been reliably reported as a host in the field.

Polygona c-album is distributed across the Palearctic, from U.K. and Morocco in the west to Japan and China in the east, but has surprisingly little genetic diversity across its range (Kodandaramaiah *et al.*, 2011).

Host plants of *P. c-album* belong to *Urtica* (Rosales: Urticaceae), *Humulus* (Rosales: Cannabaceae), *Ulmus* (Rosales: Ulmaceae), *Betula*, *Corylus* (Fagales: Betulaceae), *Salix*, and *Ribes* (Nylin, 1988). In addition, larvae survive to pupation on *Vaccinium* (Ericales: Ericaceae) in the laboratory (Janz *et al.*, 2001). Individual females spread their eggs over several host species, singly or in very small clutches (Bergström *et al.*, 2006; in the present study the same behaviour was observed in *P. faunus*. Females are known to have a wide host range including several trees and bushes in the spring generation of northern populations (such as in Sweden), but preferentially oviposit on

the herbaceous hosts *Urtica* and *Humulus* in summer generations (Nylin & Janz, 1996) and in more southern populations (Nylin *et al.*, 2009).

Female preference

Six gravid females of *P. faunus* were caught in the wild in and near Edmonton and near Jasper, Alberta, Canada, in May 2012. They were placed in cages for oviposition, provided with sugar water from a sponge, and with light and heat from light bulbs placed over each cage. Four potential host plants were simultaneously presented in each corner of the cage (with position and individual plants changed daily): *Urtica dioica* (stinging nettle); *Betula papyrifera* Marsh. (paper birch); *Ribes oxycanthoides* L. (Canadian gooseberry or the northern gooseberry), and *Salix discolor* Muhl. (American willow or pussy willow). Eggs were counted daily. Four females (in the following designated as A–D) laid enough eggs (at least 10 on a single day) to be included in the female preference study.

Twenty-seven gravid females of *P. c-album* were caught near Stockholm, Sweden, in late April 2014. They were treated as above, although local hosts in the same genera were used: in addition to *U. dioica* females were presented to *B. pubescens* Ehrh. (downy birch), *Ribes uva-crispa* L. (gooseberry), and *Salix caprea* L. (goat willow, pussy willow or sallow). Twenty females laid enough eggs to be included in the preference study.

The rationale for choosing these local host species was as follows: *U. dioica* is present on both continents. *Betula pubescens* is the only birch species used as a host by *P. c-album*, whereas *B. papyrifera* is one of the birch species listed as a host for *P. faunus* by Scott (1986). Relationships among birch species are very complex based on molecular studies, owing to extensive hybridisation and introgression (Järvinen *et al.*, 2004), but morphological studies have traditionally placed *B. pubescens* and *B. papyrifera* close together in the series Pubescentes (Roskam, 1985). *Salix* is similarly problematic (Chen *et al.*, 2010). We chose the main host in this genus for *P. c-album* and a locally available species of *Salix* in Canada that is morphologically similar and apparently belongs to the same subclade (subgenera *Chamaetia* + *Vetrix* in Chen *et al.*, 2010). From *Ribes* we chose two ‘gooseberry’ species (subgenus *Grossularia*; Schultheis & Donoghue, 2004).

We scored preferences as the fraction of eggs laid on each plant by a female in a given day and used the average fraction per day of each female in the analysis of species-level averages for each plant.

Larval preference

Offspring of females in the preference experiments described above were used here. Small (about 8 mm diameter) circular cut-out sections of fresh leaves of the same four host plants as in the female preference experiment were placed on a moist filter paper in a Petri dish and a newly hatched larva was placed in the middle, equidistant from all leaf sections. A larva was scored as having chosen one particular plant when it had eaten at least 10% of its leaf section. This leaf section was then removed, and

subsequent choices were scored, providing a ranking 1–4 of the four hosts by each larva (1 for the most preferred, 4 for the lowest preferred).

From *P. faunus*, a total of 74 larvae could be scored for all four plants, but they were unequally divided among families (A–F; $N=44, 14, 8, 4, 3, 1$, respectively; family F was discarded for analyses). From *P. c-album* 269 larvae could be scored, with 5–20 individuals from each of 18 families.

Rankings (1–4) of each plant were summed for all individuals of the same family, using values for each family as data points in the species contrasts. Family rankings were normalised by summing all ranks, then expressing the summed rank for a plant as a percentage of the total.

Larval performance

The larvae of *P. faunus* used in the preference experiment described above were reared to adulthood so that performance on different host plants could be scored in the same individuals. After 2–4 days, when larvae had ranked the host plants, they were randomly assigned to being reared on one of the four plant species. We also tested performance separately on another urticalean rosid – the tree *Ulmus americana* – representing another of the preferred host genera of *P. c-album*. *Ulmus* is not native to Edmonton but occurs in many other parts of the geographical distribution of *P. faunus*. For this test we used 10 individuals from family A that were not used in the preference experiment. From *P. c-album* 259 individuals belonging to 18 families were split among the four host plants used in the preference studies and reared to adulthood.

Rearings were performed in laboratory rooms with a controlled temperature at 19–21 °C, and a short daylength photoperiod of LD 13:11 h (*P. faunus*, Canada) or LD 12:12 h (*P. c-album*, Sweden), to ensure development to adult reproductive diapause in both species, mimicking the normally univoltine life cycles of the two populations. Larvae were reared individually in Petri dishes where fresh leaves of the plants were given on moist filter paper (Canada) or similarly in small jars (Sweden). Leaves were changed daily. When larvae of *P. faunus* reached the fifth (ultimate) instar they were moved to jars covered by nets for pupation, with twigs of the plants given water from moist wads of cotton. Survival and larval development time to pupation were recorded, and pupae were sexed and weighed on the second day after pupation. The weights and development times were used to calculate average growth rates (log pupal weight/days).

Statistical analysis

Female preference data were statistically tested in a Factorial ANOVA with the average preferences of individual females for each plant as data points in the response variable ($N=4$ for *P. faunus*, $N=20$ for *P. c-album*). The tested model was Preference = Host + Species + Host × Species. Both factors were treated as fixed factors. Note that we were primarily interested in the Host × Species interaction, and thus used a parametric ANOVA with a model analysing both species together, although this is problematic because of partial dependence

between the different plant preferences of the same female (to an unknown extent, as in a four-way choice plant preferences can correlate positively as well as negatively). For this reason, we also investigated species' differences in preferences for each plant separately, with non-parametric Mann–Whitney *U*-tests.

Larval preference data were statistically tested with the same model, using the average ranking of a family of larvae (normalised as described above) as data points in the response variable ($N=5$ for *P. faunus*, $N=18$ for *P. c-album*). As for female preference, non-parametric Mann–Whitney *U*-tests were also employed for each plant separately.

Larval performance data were tested in two steps: within species and among species. Within species we used a Main effects ANOVA with the growth rates of individual larvae as data points ($N=67$ for *P. faunus*, $N=175$ for *P. c-album*). The models were Rate = Family + Host + Sex. Family was treated as a random factor, Host and Sex as fixed factors. For *P. c-album*, where we had enough data for more extensive testing, we also explored the effects of pooling sexes to investigate if families were differentially affected by the hosts (testing Rate = Host + Family + Host × Family).

Families were subsequently pooled for the among-species contrast. We used a full Factorial ANOVA: Rate = Host + Sex + Species + Host × Sex + Host × Species + Sex × Species + Host × Sex × Species.

All statistics were performed using the package Statistica 11 (Stat Soft Inc., 2012).

Results

Female preference

All four *P. faunus* females included in the analysis (A–D) ranked *Salix* highest, *Ribes* and *Betula* received intermediate numbers of eggs by two females whereas the other two laid all their eggs on *Salix* (cf. Fig. 2, data from individual females not shown). None of the females (including E–F) laid any eggs on *U. dioica*. This ranking of hosts is consistent with that suggested by earlier faunistic accounts (see Discussion).

This is in sharp contrast to *P. c-album*, where females (as in earlier studies) tended to prefer *U. dioica* over *Salix*, *Ribes* and (in particular) *Betula* (Fig. 2), although the differences are not very pronounced in early spring experiments such as this. The difference between the two sister species in their host preference was highly significant [ANOVA; Host: $F(3, 88)=14.44$, $P<0.001$; Species: $F(1, 88)=0.56$, $P=0.456$; Host × Species interaction: $F(3, 88)=17.6$, $P<0.001$]. Non-parametric Mann–Whitney *U*-tests also showed higher preferences for *Urtica* in *P. c-album* ($Z=3.06$, $P<0.001$) and also for *Ribes* ($Z=2.98$, $P<0.001$), with no species difference in preference for *Betula* ($Z=0$, $P=0.97$) and a tendency for higher preference for *Salix* in *P. faunus* ($Z=1.78$, $P=0.068$).

Larval preference

Larval preference in *P. faunus* clearly differed from that of their mothers. Overall, *Ribes* was the highest ranked

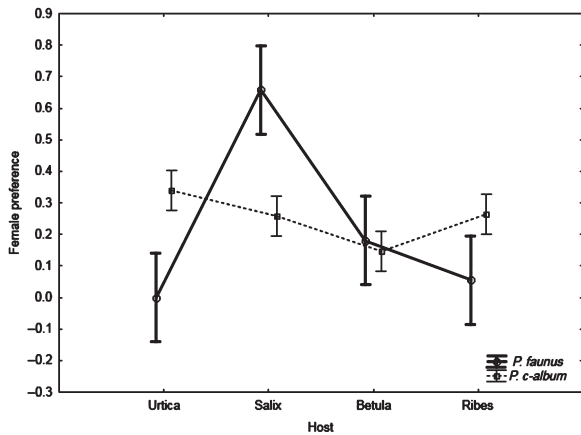


Fig. 2. Female host plant preferences (average fraction of eggs/day \pm CI) in *Polygona faunus* (circles, solid line) and *P. c-album* (squares, dashed line) when local representatives of four host plant genera were simultaneously presented. Connecting lines are for visual aid only.

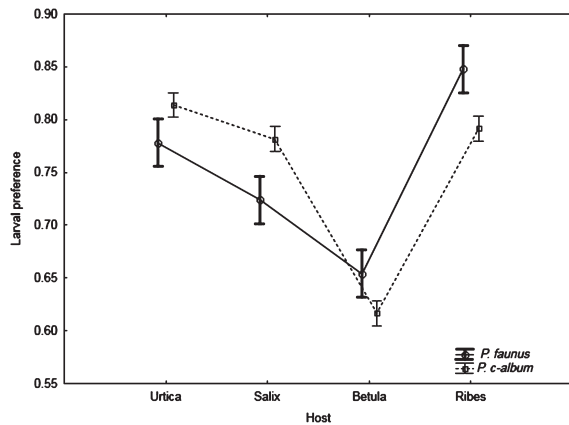


Fig. 3. Larval host plant preferences in *Polygona faunus* (circles, solid line) and *P. c-album* (squares, dashed line) when leaf sections cut from local representatives of four host plant genera were presented. Connecting lines are for visual aid only. Values are averages of normalised sequential rankings by larvae from different families, shown as 1-(percentage of summed rankings) \pm CI. Since the plant with the lowest summed ranks was in fact the most preferred, for clarity of illustration the percentage was deducted from 1 in order to make values for preferred plants higher than those for less preferred plants in the figure.

plant, followed by *Urtica*, *Salix*, and *Betula* in succession, and this ranking was almost completely consistent among families (cf. Fig. 3, results from individual families not shown).

This is quite similar to the situation in *P. c-album* (Fig. 3), although in this species larvae preferred *Urtica* over *Ribes*, *Salix* and (in particular) over *Betula* (Fig. 3). Thus, the overall preferences shown by females and their offspring were in accordance in *P. c-album* (Figs 2 and 3; $U > S = R > B$ in both cases) but clearly conflicted in *P. faunus* ($S > B > R > U$ vs. $R > U > S > B$).

Even though broadly similar, the species differed significantly in their behavioural response to host plants [ANOVA; Host: $F(3, 84) = 165.5$, $P < 0.001$; Species: $F(1, 84) = 0.0$, $P = 1$; Host \times Species interaction: $F(3, 84) = 18.9$; $P < 0.001$]. Likewise, non-parametric Mann–Whitney U-tests showed a significantly higher preference for *Urtica* in *P. c-album* ($Z = 2.61$, $P < 0.01$) as well as for *Salix* ($Z = 3.17$, $P < 0.001$), but no species difference in preference for *Betula* ($Z = 1.16$, $P = 0.26$) and a higher preference for *Ribes* in *P. faunus* ($Z = 2.87$, $P < 0.01$).

Larval performance

Survival. In *P. faunus* survival to pupation was 100% on all four plants in the main investigation ($N = 17$ – 18) except *Urtica* where it was 85% ($N = 20$). Another seven pupae later failed to emerge: three from larvae reared on *Ribes*, two from *Urtica*, and one each from *Salix* and *Betula*. None of the larvae survived to pupation in the side investigation on *Ulmus* ($N = 10$). However, three larvae survived to the third, fourth, and fifth instar, respectively, indicating that successful pupation occasionally could occur on this plant if females were to oviposit on it.

In *P. c-album* survival to pupation was good on all hosts (*Urtica* 89%, $N = 63$; *Salix* 73%, $N = 64$; *Ribes* 90%, $N = 67$), except on *Betula* (14%, $N = 65$).

Growth rate. There was a significant effect of Family in the rearing of *P. faunus* [Main effects ANOVA, $F(4, 58) = 7.35$, $P < 0.001$] as well as a Host effect [$F(3, 58) = 5.15$, $P < 0.01$] but no significant effect of Sex [$F(1, 58) = 1.56$, $P = 0.22$], although females tended to grow faster (and have shorter development times, not shown). Results were similar if the smaller families B–E were excluded, so families were subsequently pooled in order to make the species contrast possible and still make use of all of the data (families of *P. faunus* were too unbalanced in size for a nested analysis as well as for models including interactions with Family). In *P. faunus* growth rates were overall highest on *Ribes* and *Salix*, and lower on *Betula* and *Urtica*, although there was much overlap between hosts (cf. Fig. 4).

Similarly, sexes did not differ significantly in *P. c-album* when data from this species was analysed separately, although males tended to grow faster and have shorter development times (not shown), but families did differ [ANOVA with Host, Sex and Family as main effects; Host: $F(3, 56) = 35.64$, $P < 0.001$; Sex: $F(1, 56) = 1.41$, $P = 0.24$; Family: $F(5, 56) = 4.16$, $P < 0.01$]. However, families responded similarly to the different host plants when sexes were pooled [ANOVA; Host: $F(3, 42) = 35.1$, $P < 0.001$; Family: $F(5, 42) = 4.32$, $P < 0.01$; Host \times Family: $F(15, 42) = 1.22$, $P = 0.30$]. Thus, families of *P. c-album* were subsequently pooled to make analysis of the species' contrast possible.

In the species' contrast, larvae of *P. faunus* had higher growth rates overall [Fig. 4; Species main effect in ANOVA with Host, Sex, Species, and all interactions, $F(1, 197) = 337.4$, $P < 0.001$], however, it should be noted that the two species were not reared together, so precise rearing temperatures will have differed, as

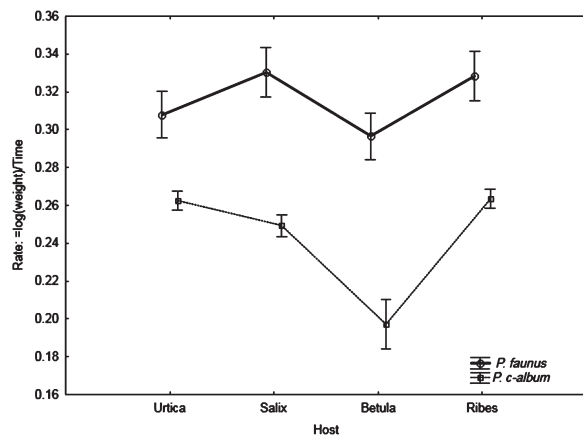


Fig. 4. Growth rates (log (pupal weight)/development time; average \pm CI) in *Polytonia faunus* (circles, solid line) and *P. c-album* (squares, dashed line) when larvae were reared on local representatives of four host plant genera. Connecting lines are for visual aid only.

did the exact photoperiod. Effects of Sex were not significant in this analysis, and neither were any interactions involving Sex. The larval Host affected growth rates significantly as a main effect [$F(3, 197) = 17.5, P < 0.001$] and the species differed as to the nature of these effects [Host \times Species interaction, $F(1, 197) = 9.3, P < 0.001$], in particular in that *Betula* resulted in much lower growth rates (relative to preferred hosts) in *P. c-album* than in *P. faunus* (Fig. 4). However, also in *P. faunus* *Betula* as larval host resulted in the lowest growth rates, with the striking result that *P. faunus* larvae in fact tended to perform slightly better on the non-host *Urtica* than on *Betula*, the host second-most preferred by females (Fig. 2).

Discussion

We have shown here that the loss of the ancestral host clade in *P. faunus* has resulted in discrepancies among female preference, offspring preference, and offspring performance, corroborating the general hypothesis regarding the importance of evolutionary lags in shaping the current patterns of insect–plant associations. From theory (Janz & Nylin, 1997), we hypothesised that the loss of the ancestral host in *P. faunus* happened first in female preference. Selection normally drives larvae to accept a wider range of hosts than their mothers (Wiklund, 1975), and larvae often seem to perform relatively well on related or ancestral hosts not used by females for oviposition (Wiklund, 1975; Janz *et al.*, 2001; Lehnert & Scriber, 2012). Hence we predicted: (i) more similar larval than female preferences between the two species; (ii) similar larval performance in the two species across hosts (or alternatively evidence of loss of adaptation to *Urtica* in *P. faunus* if trade-offs are strong enough); and (iii) correspondence between preference and performance in *P. c-album*, but not in *P. faunus*, where female preferences have changed.

The first prediction was clearly borne out (Figs 2 and 3). In *P. faunus*, females apparently no longer accept *Urtica* (or *Ulmus*) for oviposition, but larvae still readily accept *Urtica* as a host,

and they will in a pinch also accept *Ulmus*. Our results from the female preference experiment are based on a low number of females in the case of *P. faunus*, so the precise figures should be taken with caution, but it is important to note that in both species we found the same ranking of hosts as indicated by earlier literature (e.g. Scott, 1986; Layberry *et al.*, 1998; James & Nunnallee, 2011). Furthermore, from our four-way choice experiment alone we cannot entirely rule out the possibility that the absence of eggs laid by *P. faunus* on *Urtica* reflects a strong preference for *Salix* (and hence few eggs left to lay on the other plants) rather than females avoiding *Urtica* (cf. Mercader & Scriber, 2007), but again the field records indicate that it is not an experimental artifact.

Although larval preferences were broadly similar between the two species (Fig. 3), there were some indications of adaptation to the new situation in *P. faunus*, for instance in that *Ribes* was the very most preferred host rather than *Urtica*. A similar conclusion can be made regarding the second prediction. Larvae of *P. c-album* performed similarly to results from previous studies on the different hosts: best on *Urtica* and worst on *Betula*, with *Salix* and *Ribes* being intermediate (Nylin, 1988; Janz *et al.*, 1994; Nylin & Janz, 1996; Nylin *et al.*, 2009). Larvae of *P. faunus* were also found to survive and develop well on the ancestral host *Urtica*, and even *Ulmus* seems to be a possible host, albeit a poor one. At the same time, larvae of *P. c-album* performed relatively better on *Urtica*, whereas *P. faunus* larvae instead performed better on the alternative hosts, in particular on *Betula* (Fig. 4). Thus, *P. faunus* larvae seem to be slowly adapting to the loss of the ancestral host, but in the meanwhile female preference, larval preference, and larval performance show discrepancies in this species, whereas they all correspond in *P. c-album*, corroborating our third prediction.

The loss of ‘urticalean rosids’ as hosts for *P. faunus* stands in dramatic contrast not only to the many related species specialising on this host clade (Fig. 1), but also to the sister species, *P. c-album*. In the Swedish population of the latter species, urticalean rosoid hosts (specifically the genera *Urtica*, *Ulmus* and *Humulus*) are preferred over host plants in *Salix*, *Ribes*, *Betula*, and *Corylus* that are also used by ovipositing females and on which development to adulthood can be completed (this study; Nylin, 1988; Janz *et al.*, 1994; Nylin & Janz, 1996; Nylin *et al.*, 2009).

Whatever the reason for the loss of ‘urticalean rosids’ as hosts for *P. faunus* (discussed below), it is an interesting demonstration of an often assumed principle in the evolution of insect–plant associations: it is easier to lose a host plant from the repertoire than to gain a novel association. The reason why this is assumed is because an insect–plant association is a complex trait (Forister *et al.*, 2007; and see Orr, 2000, regarding the general theory of complexity in evolution). To make use of a plant as a host, it needs to be accepted as a host by ovipositing females, as well as accepted by neonate larvae so that they attempt feeding rather than searching for alternative food. It also has to be suitable as food for larvae not only for metabolism and (if necessary) detoxification, but also for ecological contexts such as phenology and natural enemies. To add a host to the repertoire, all these conditions need to be simultaneously fulfilled to some degree. We suggest that an increase in host

breadth can only be achieved through either an extension to similar plants, as in oligophagous species feeding on many members of the same family, or by passing through a polyphagous stage where females oviposit relatively indiscriminately, so that sub-optimal hosts are also used (cf. Nyman, 2010). Over time, offspring can become better adapted to the new hosts, and finally even complete shifts to specialisation on such novel hosts can become possible (Nylin & Janz, 2009; Agosta *et al.*, 2010; Nylin *et al.*, 2014). The flipside is that the chain can in principle be broken at any of these stages, resulting in a loss of a host in a single step while (at least for some evolutionary time) preserving vestiges of the earlier host plant adaptation (Lehnert & Scriber, 2012).

We can only speculate on the reason why urticalean rosids have been lost as hosts by *P. faunus* and not by *P. c-album*. It is possible that a contributing factor may have been community structure as there is only one *Polygonia* species in Northern Europe, whereas several species co-occur with *P. faunus* – among them the *Urtica* specialist *P. satyrus*. Although direct competition for resources seems unlikely because of the abundance and size of the host plants and because eggs are laid singly, factors such as shared generalist parasites could conceivably select for avoidance of *Urtica*. It is also likely that *Urtica* was rarer in North America in the past as nitrogen-rich soil favours the plant and hence by human settlements (Jornsgard *et al.*, 1996). However, we believe that the difference in life history between *P. faunus* and *P. c-album* is the most important cause. The Swedish population of *P. c-album* prefers the herbaceous hosts *Urtica* and *Humulus* in the laboratory much more strongly later in the summer season, if direct development is induced by artificially increasing photoperiods in this normally univoltine population (Nylin, 1988; Nylin & Janz, 1996). We have suggested that this seasonal pattern occurs because plants belonging to the other host genera (that are all trees or bushes) decline more strongly in quality in the summer than the herbs. Strong selection against using trees in the summer is also most likely an important part of the reason why the bi- and multivoltine populations of *P. c-album* to the south of Sweden specialise more or less completely on the herbaceous urticalean rosids (Nylin *et al.*, 2009). Another part of the explanation may be stronger selection favouring the use of herbaceous host plants supporting fast growth (in order to achieve additional generations in the same season), compared with univoltine areas to the north (Nylin, 1988).

Following the same line of reasoning, it may be that the loss of the ancestral host clade in *P. faunus* occurred because the species has had only a single generation per year for all or most of the time as the ancestor of *P. faunus* + *P. c-album* colonised the Nearctic along a northern route (about 5 Mya; Wahlberg *et al.*, 2009). A univoltine life cycle could have contributed to the loss of *Urtica* as a host (cf. Scriber, 1988, regarding polyphagy and univoltinism in northern taxa of the *Papilio glaucus*–*canadensis* species complex), because of relaxed selection in favour of using herbaceous hosts, probably in combination with some advantage of using the trees and bushes (enemy-free space and/or abundance of oviposition targets being likely candidates). The other main herbaceous host of *P. c-album*, *Humulus*, is not native to North America, and the loss of the related tree *Ulmus* as

a host could simply be a consequence of oviposition stimulants shared across urticalean rosids.

This interpretation of a long-lasting univoltine past in *P. faunus* is supported by three observations: (i) the current distribution pattern and life cycle, where the species occurs only in the north, or at high altitudes further south, with good evidence of a second generation lacking even in the south (but see Scott, 1986); (ii) the fact that *P. faunus* has lost the strong seasonal polyphenism in wing colour that occurs in *P. c-album* and indeed evidently is ancestral to the genus *Polygonia* (Nylin *et al.*, 2005a); (iii) the evident lack of protandry. It is a general pattern in butterflies that males have shorter development times than females and thus under direct development enter the population earlier, presumably as a result of sexual selection on males to maximise mating opportunities and natural selection on females to minimise the pre-reproductive stage (Wiklund & Fagerström, 1977; Wiklund & Solbreck, 1982). If there is a sex difference in development time in *P. faunus*, it seems to be in the opposite direction, perhaps because males are heavier (cf. Wiklund *et al.*, 1991). In contrast, protandry during juvenile development is typically observed in the potentially multivoltine *P. c-album*, even under conditions inducing adult diapause (Nylin, 1992).

It has long been known that female preference and larval performance are genetically separate traits that can evolve independently (Thompson *et al.*, 1990) and it is interesting in this context to note that adult and larval host preference do not seem to be strongly genetically connected either. This is evident in *P. faunus*, where mothers and larval offspring have very different preferences (Figs 2 and 3), but is to some extent true also in *P. c-album*. Although in the latter species we have found evidence of a genetic correlation between the host preferences of mothers and their adult female offspring (Nylin *et al.*, 2005b), the correlation is much weaker between the preferences of mothers and that of their neonate larval offspring (Nylin & Janz, 1996; Gamberale-Stille *et al.*, 2014). In addition, individual females can show radically different host preferences as neonate larvae, compared to later in their life when they have reached adulthood and oviposit (Gamberale-Stille *et al.*, 2014).

Taken together, these results suggest that even though a positive genetic correlation between female and larval preference is likely sometimes to be found, the two traits can to a large degree evolve independently. One reason for this is probably the differences between the environments that the two life stages are exposed to (particularly in holometabolous insects where the adults do not themselves feed on the plants).

With the exception of insects where females are very indiscriminate in their oviposition, instead forcing larvae to seek out hosts (e.g. Tammaru *et al.*, 1995), very few studies (at least in holometabolous insects) have so far addressed preference correlations between different life stages, which is important because positive genetic correlations could aid the evolution of host shifts, whereas negative correlations could constrain them (Forister *et al.*, 2007). One major event in the evolution of host plant associations in *Polygonia* is the colonisation of hosts in *Ribes*, and the specialisation on this genus by one Nearctic clade (Fig. 1: Weingartner *et al.*, 2006). This plant genus is not used by any other nymphalid butterflies, and the association with it is thus an evolutionary novelty (Celorio-Mancera *et al.*, 2013). We

have argued elsewhere that the polyphagous state in *P. c-album* and *P. faunus*, with a range of hosts that more specialised species in *Polygonia* (and the closely related genus *Nymphalis*) use a subset of, can be seen as something of a model of the generalist state that we believe was present in the ancestor of the *Polygonia* + *Nymphalis* clade. In other words an evolutionary potential for using this particular range of hosts seems to have persisted for millions of years (Fig. 1; Janz *et al.*, 2001; Weingartner *et al.*, 2006; Celorio-Mancera *et al.*, 2013).

It is interesting in this context to note that *P. faunus* larvae consistently ranked *Ribes* highest among the tested hosts, in spite of the fact that their mothers preferred *Salix* (in line with field records (Scott, 1986; James & Nunnallee, 2011)). Preference for *Ribes* was high also in *P. c-album* larvae, indicating that larval preference would not be an evolutionary hurdle for specialisation on *Ribes* in these butterflies. Larvae also perform well on this host taxon in both species, so from the likely state represented by the ancestor of *P. faunus* + *P. c-album* only female preference need to shift towards specialisation on *Ribes* for a host shift to be completed. This would presumably then be due to fitness advantages such as superior ability in specialists to judge the quality of individual oviposition sites (Janz & Nylin, 1997).

In conclusion, female preference, larval preference, and larval performance among the hosts presented here for two sister species of *Polygonia* highlight how evolutionary lags at long time scales can affect preference–performance patterns, through vestiges of ancestral host plant adaptations. They also lend support to several often assumed general principles in the evolution of insect–plant associations, as well as provide some clues about how host shifts may have proceeded, at least in the case of *Polygonia* and relatives. We propose that larval preference should be investigated and contrasted with female preference in a wider range of holometabolic phytophagous insects, so that a clearer view of the general patterns of correlations among life stages and larval performance can emerge.

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