1 **Specialist and generalist oviposition strategies in butterflies: maternal care or precocious young?**

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Why this study and paper deserves to be honored as a Highlighted Student Paper:

Through a unique comparison between several butterfly species we cast new light on the costs of polyphagy, but also show that the “generalist” and “specialist” categories hide substantial variation. The co-adaptation of female choice and larval abilities on the species level cannot only be explained by the specialist-generalist axis, but can be properly understood in a historical framework.

29 **Abstract**

30 Herbivorous insects specialized on a narrow set of plants are believed to be better adapted to their
31 specific hosts. For example, species with a broader diet breadth seem to pay a cost through
32 decreased oviposition accuracy. Despite many studies investigating female oviposition behavior we
33 lack knowledge on how larvae cope behaviorally with their mothers' egg-laying strategies. We
34 examined a unique system of five nymphalid butterfly species with different host plant ranges that
35 all feed on the same host plant, which allowed us to compare on the species level how oviposition
36 preference is related to neonate larval responses in several disadvantageous situations. We found a
37 general co-adaptation between female and larval abilities, where species with more discriminating
38 females had larvae that were less able to deal with a suboptimal initial feeding site. Conversely,
39 relatively indiscriminate females had more precocious larvae with higher abilities to cope with
40 suboptimal sites. Despite similarities between the tested species with similar host ranges, there were
41 also striking differences. Generalist and specialist species can be found side by side in many clades,
42 with each clade having a specific evolutionary history. Such clade-specific, phylogenetically
43 determined preconditions apparently have affected how precisely a broad or narrow diet breadth
44 can be realized.

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Introduction

Most insect species are resource specialists (Futuyma and Moreno 1988) and today there are many indications of a general trend that drives herbivorous insects towards host plant specialization. Such drivers of specialization can for example be trade-offs in performance due to variation in host plant chemistry (Joshi and Thompson 1997; Agrawal 2000), predator avoidance (Bernays and Graham 1988; Murphy 2004; Wiklund and Friberg 2008), neural limitations in information processing (Bernays and Wcislo 1994; Janz and Nylin 1997) or differential local selection pressures (Thompson 2005).

However, despite this ubiquitous trend towards specialization, there are still a considerable number of species that utilize a broader range of host plants. The frequency and extent of relative generalists vary between groups, but it is interesting to note that even otherwise specialized groups of insects appear to be occasionally interspersed by periods of polyphagy (Nylin et al. 2014). The apparent ephemeral nature of these events suggests that the benefits of specialization are occasionally reversed or relaxed. The relative benefits of generalization may be temporary, but they could play an important role in evolution, both for the formation of new species interactions and for host-driven diversification (Janz & Nylin 2008; Janz 2011). Hence, even though the trend towards specialization is important to explain, we also need to understand how polyphagy can evolve and be maintained in spite of its apparent costs.

As a general explanation for the predominance of specialists over generalists, the *information processing hypothesis* is particularly promising because of its wide applicability across a broad range of situations and contexts (Bernays and Wcislo 1994; Dukas 1998; Bernays 2001). Studies testing this hypothesis during the last two decades show strong evidence for a cost of generalization in the behavior leading to host plant selection (Janz and Nylin 1997; Janz 2003; Egan and Funk 2006). Empirical comparative studies have demonstrated that relative generalists pay a cost in e.g. speed or accuracy of decisions. For example, feeding generalist larvae are found to be less efficient and need

more time to start feeding on a host than their specialized counterparts (Bernays et al. 2004). Similarly, for an ovipositing adult, having a broader host range is correlated with both inferior host plant detection and longer host evaluation time (Janz and Nylin 1997; Janz 2003; Egan and Funk 2006).

With a broader diet, these constraints are believed to limit the possibility to differentiate between individuals of the same species, since much of their neural resources have to be directed towards the differentiation between and recognition of varying host plant species (Bernays 2001). Recent morphological and physiological studies have also corroborated that there are indeed differences in number of antennal sensilla and in the physiological responses towards host plant odors between generalist and specialist butterflies (Carlsson et al. 2011; Carlsson et al. 2013).

The larval stage of Lepidoptera is usually considered to be rather immobile, which is why much attention has been directed towards adult oviposition preferences as the determinant of the larval host plant. The careful female search for a suitable host plant has in some instances been interpreted as a form of parental care (Wiklund and Persson 1983; Janz and Nylin 1997). Although females do not actively tend or protect their offspring, they may reject seemingly suitable host plants to invest additional time to find even better oviposition sites to increase the fitness of individual offspring (Doak et al. 2006; Friberg et al. 2008). The more indiscriminate oviposition strategy seen in relative generalists (Janz and Nylin 1997) could have the potential benefit of increased realized fecundity, but at the rather serious expense of mean offspring fitness. However, this negative impact could potentially be lowered if larval capabilities were co-adapted; i.e. if larvae of indiscriminate females were more precocious and able to search for and locate suitable resources on their own (Wiklund 1984; Zalucki et al. 2002; Gamberale-Stille et al. 2014). Conversely, in species with a more “caring” oviposition strategy, the newly hatched larvae would not need to be able to cover longer distances or to evaluate potential food resources themselves. As a consequence, these species would not have to allocate as much resources to each egg.

This general idea of co-adaptation between adult oviposition strategy and larval ability has been very little explored and can best be tested experimentally in a comparative framework. We took advantage of a unique system of several closely related butterfly species with different degrees of host plant specialization that all include the same species in their host plant repertoire. Thus, we could assess female oviposition preference of species with different life-histories and relate to neonate larval behavior and performance in a variety of situations, without any inter-species effects that may arise from comparing different host plants.

We expected species that are host plant specialists to have discriminating females and larvae with poor host location and evaluation abilities, and species that are relative host plant generalists to have less discriminating females but larvae that are more capable of coping with unfavorable oviposition sites. In summary, we hypothesized that (a) larval precociousness and female care during oviposition are generally negatively associated and (b) that these differences should correspond to the specialist-generalist axis. Specifically, we first established female oviposition preference between host plant individuals of varying quality, and then related larval behavior and performance to female preference in a row of experiments: we investigated (i) larval capability to survive in unfavorable conditions and (ii) larval ability to leave an unfavorable site and to find another feeding site.

Material and Methods

Study organisms and rearing

We compared five species of nymphalid butterflies, two generalists (*Polygonia c-album* and *Vanessa cardui*) and three specialists (*Aglais urticae*, *A. io* and *Vanessa atalanta*) that all feed and oviposit on the host plant *Urtica dioica* (Urticaceae). *P. c-album* feeds on a number of species from several plant orders, although preferably *U. dioica* (Nylin 1988). *V. cardui* is a generalist reported to feed on over hundred species from many plant orders although they seem to prefer plants in Asteraceae and

Malvaceae (Ebert 1993; Stefanescu 1997). *U. dioica* is among their host plants and can at times be a preferred host (Seppänen 1970; Ebert 1993). *A. urticae* and *A. io* are specialists on *U. dioica* (Eliasson et al. 2005). In Europe *V. atalanta* is a specialist on *Urtica* and *Parietaria* (both Urticaceae) (Stefanescu 2001). The stinging nettle, *U. dioica* (Urticaceae) is a common, perennial herb that prefers nitrogen-rich soils. Plant quality declines with advancing season, but re-growth foliage can provide high quality leaves throughout the season (Pullin 1987).

The species *P. c-album*, *V. cardui*, *A. urticae* and *A. io* were caught in the Stockholm area in May 2013 and 2014. In April 2014, *V. atalanta* were collected in Catalonia, Spain. Additional stock of *V. cardui* was obtained in 2013 from a commercial butterfly provider (Worldwide Butterflies, www.wwb.co.uk). All applicable institutional and/or national guidelines for the care and use of animals were followed. Experiments were conducted from May-July in both 2013 and 2014. Offspring of all species were reared on *U. dioica* at long day-length to pupation as described by Janz et al. (2009). Eclosed individuals were placed in mating cages (approx. 70x70x50cm) with a light regime of 18/6 L/D.

Oviposition experiment, egg-weights and leaf quality

Mating pairs of *P. c-album*, *V. atalanta* and *V. cardui* were separated and females were placed individually in oviposition cages (approx. 50x50x50cm). Mating pairs are not easily observed in *A. io* and *A. urticae*, so individuals were allowed to mate freely over several days. After that time period they were given a host plant choice following the protocol described below, and mated females were identified through continuous observation. The single egg-layers *P. c-album*, *V. cardui* and *V. atalanta* were allowed to oviposit for periods of 3 hours until they had laid at least 10 eggs on 3 different days - if they did not lay this minimum of eggs, they were not included in the analyses. The protocol of the oviposition experiment was similar to the one used by Janz & Nylin (1997). A simultaneous choice between two host plant shoots of differing quality was given: (1) a 'high' quality leaf pair of *U. dioica* with fresh, green leaves and (2) a 'poor' quality leaf pair with partly yellow or brown leaves, without

being wilted. Parameters of leaf quality such as leaf toughness, water content and nitrogen levels were taken. On 15 July 2013 data on leaf toughness of high and poor quality *Urtica dioica* were taken by measuring the weight required to punch the leaf with a device constructed after description in Feeny (1970). Water content was obtained from leaves weighed before and after drying at 50°C in an oven. Nitrogen content was obtained from high/poor quality *U. dioica* leaves collected on several sites on Campus of Stockholm University, Stockholm, Sweden, on 24/25 June 2014.

Eggs were counted and collected daily and egg-clutches were separated from the leaves. At least 30 eggs from 5 or more females of each species were weighed within 24 hours of oviposition.

Experiments: test of neonate larval performance

After oviposition the eggs were transferred into small plastic jars and stored at room temperature. When larvae hatched, care was taken to test them as soon as possible but no more than 12 hours after hatching.

Survival experiment: To investigate whether there is a general relationship between female oviposition preference and larval ability to feed on leaves of high or low quality, newly hatched larvae were placed in Petri dishes on either a 'high' or 'low' quality leaf. Survival and larval instar were checked every 24 hours for 8 days. At the end of the period the surviving larvae were weighed. All larvae were tested singly; however, to test for an effect of gregariousness in the clutch laying species *A. urticae* and *A. io*, larvae of these species were also tested with 10 larvae together on the same treatment as described above.

Mobility: To obtain a measure reflecting the ability of neonate larvae to cover a distance, we recorded the distance and direction that neonate larvae covered within 30 minutes. With a soft brush, larvae were placed singly in the middle of a vertical, 1m long wooden rod and the position was recorded every 5 minutes. The minimum distance covered was calculated.

Nettle stalk experiment: This experiment combined the survival and mobility test in a more realistic setup. With this setup it was possible to investigate to what extent neonate larvae had the ability to (i) move from an inferior position, which may have resulted from an inaccurate female oviposition on a host plant stalk, to (ii) locate a leaf and (iii) whether they were able to establish on that leaf and start feeding. For that purpose, we placed one cut shoot of nettle each in a flask with water and removed all edible parts (leaves, reproductive parts, shoots) between an upper and lower leaf pair so that about 12-20 cm of bare stalk remained between the leaf pairs. Newly hatched larvae were placed on the stem at equal distance from the upper and lower leaf pair. Larval position on the shoot and survival was checked after 1, 2, 3, 6, 24 and 48 hours. Plants that showed signs of wilting during the experiment were excluded.

Statistical analyses

Statistical analyses were conducted in R (R Core Team 2014). Leaf water content and the inverted data of leaf toughness were analyzed with Students t-test. To compare levels of leaf nitrogen between the 'high' and 'poor' quality host plants we used a linear mixed model with site as random factor. Since *Aglaia urticae* and *A. io* deposit their eggs in clutches their oviposition choice was analyzed with a binomial test. The preference of the other species was tested with a paired Wilcoxon signed-rank test. Egg weights were compared between species with a one-way ANOVA, followed by Tukey's post hoc test. The survival on the leaves of different quality was tested with a General linear model (logistic regression). To test for differences in larval weight after 8 days on the different leaf qualities we used Wilcoxon signed-rank tests. When analyzing survival and weight after 8 days on a specific leaf quality, the two *Aglaia* species were tested in several experiments both in the 'single' and 'gregarious' treatment, as well as compared between treatments; in these cases we conducted Bonferroni correction, where multiple testing of the same data required it. The distances covered on the wooden rod were square-root transformed to obtain normality of data and then tested with a one-way ANOVA, followed by Tukey's post hoc test to detect where differences in mobility occurred.

The survival on the nettle stalk and the percentage of larvae that reached a leaf after 48h were also tested with a General linear model (logistic regression).

Results

'High' quality leaves had a water content of $75.4\% \pm 1.1$ (mean $\pm$ SD, $n=10$) and 'poor' quality leaves $69.6\% \pm 1.9$ ($n=10$; $t=8.47$, $df=14.5$, $p<0.001$). Further, 'high' quality leaves required $133.3g (\pm 11.1, n=10)$ of weight to punch a hole through the leaf, whereas 'poor' quality leaves required $186.9g (\pm 29.2, n=10)$ of weight; a significant difference in toughness between the two categories ($t=5.95$, $df=16.5$, $p<0.001$). Lastly, nitrogen level of 'high' quality leaves was $3.62\% \pm 0.72$ (mean $\pm$ SD) and 2.25 ± 0.38 of 'poor' quality leaves, a significant difference (both categories $n=15$; $\chi^2=74.4$, $df=1$, $p<0.001$).

Before investigating larval behavior, we assessed female oviposition preference in the five species of butterflies with a choice between poor and high quality host plant (Figure 1). Both *A. urticae* ($n=22$) and *A. io* ($n=11$) showed significantly stronger preference for the high quality host plant (binomial test, both $p<0.001$), whereas the remaining three species did not show a significant preference for either alternative (paired Wilcoxon signed-rank test; *V. atalanta*: $n=6$, $V=1$, $p=0.063$; *V. cardui*: $n=16$, $V=87$, $p=0.348$; *P. c-album*: $n=19$, $V=46$, $p=0.156$). Egg weight was significantly different between but not within genera (ANOVA, $F_{(4,32)}=579.1$, $p<0.001$; followed by a post-hoc Tukey test); data for the five species is shown in figure 2.

Next we observed survival of neonate larvae manually distributed on either poor or high quality host plant (Figure 3). After eight days we found that only *A. urticae* larvae had survived significantly better on high than on poor quality host plant (logistic regression, $\chi^2=27.87$, $df=1$, $p<0.001$). We did not detect any difference in survival after 8 days between the two leaf categories for the other species (logistic regression, $p>0.05$). We further tested differences in survival on the two plant qualities

219 when larvae were allowed to feed in groups (gregarious), which was considered to be a more realistic
220 environment for species laying eggs in clutches (*A. urticae* and *A. io*). For *A. urticae*, similarly to
221 solitary larvae, gregarious larvae survived better on high than on low quality leaves ($\chi^2= 55.19$, $df= 1$,
222 $p<0.001$). For *A. io*, however, there was no difference in survival on the two leaf categories within the
223 gregarious treatment ($\chi^2= 0.45$, $df= 1$, $p=0.76$), but it did matter for larval survival whether larvae
224 were tested solitarily or gregariously: independently of leaf quality did the larvae in the gregarious
225 treatment survive better ($\chi^2= 87.44$, $df= 1$, $p<0.001$).

226 The weight after eight days was significantly higher for larvae feeding on high quality host plants
227 compared to those feeding on poor hosts (Wilcoxon signed-rank test, *A. urticae*: $W=18$, $p<0.001$; *A.*
228 *io*: $W=11$, $p<0.01$; *V. atalanta*: $W=378.5$, $p<0.001$, *P. c-album*: $W=25.5$, $p<0.001$) except for *V. cardui*
229 ($W=135$, $p=0.20$), in which larval weight was similar on both plant qualities (Figure 4). Likewise, the
230 weight of gregariously feeding larvae was significantly higher on high than on poor quality leaves (*A.*
231 *urticae*: $W=23.5$, $p<0.001$; *A. io*: $W=145$, $p<0.001$). In addition, when comparing weights between
232 single and gregarious larvae within leaf quality category, weight was significantly higher of
233 gregariously feeding larvae on good leaves in both *A. urticae* ($W=1371$, $p<0.001$) and *A. io* ($W=748$,
234 $p<0.001$). On poor leaves, gregariously feeding larvae had a higher weight after 8 days than singly
235 feeding larvae in *A.io* ($W=544$, $p<0.001$), but weight was not different in *A. urticae* ($W=92$, $p=0.101$).

236 Finally, we investigated neonate larval mobility and performance when placed off a feeding site (a
237 vertical rod or a nettle stalk). There were significant differences in the distance covered on the rod
238 between the species (ANOVA, $F_{(4,115)}=105$, $p<0.001$; Figure 5). The most mobile species was *V.*
239 *atalanta* followed by *P. c-album* and *V. cardui*, the least mobile species were *A. urticae* and *A. io*. The
240 mobility of the *Aglais* species did not differ from each other ($p=0.918$), however, Tukey's post hoc
241 test revealed that all other comparisons were significantly different from each other ($p<0.05$). In the
242 second part of this experiment we placed neonate larvae on a naked nettle stalk with only the upper
243 and lower leaf pairs left intact and subsequently recorded the percentage of larvae that reached a

leaf within 48h and the percentage survival after 48h. We found significant differences between species in likelihood to reach a leaf (logistic regression, $\chi^2=51.4$, $df=4$, $p<0.001$) and to survive on the nettle stalk for 48h ($\chi^2=92.8$, $df=4$, $p<0.001$). For details on species specific differences see Table 1 and 2. The two *Vanessa* species did occasionally feed on the stalk (25% of *V. atalanta* and 2.5% of *V. cardui*), which may partly explain why more larvae of *V. atalanta* survived than actually reached the leaves.

We found significant differences in survival after 48h on the nettle stalk between the species (logistic regression, $\chi^2=99.1$, $df=4$, $p<0.001$); for species specific differences see Table 1 (Bonferroni correction was conducted where multiple testing of the data required it). In general, the two *Aglais* species performed worse than the other three species, in that fewer larvae survived and reached a leaf. *V. cardui* seemed to be somewhat intermediate

Discussion

As we predicted, there was a general negative association between female and larval abilities. Species with relatively indiscriminate females had more precocious larvae with higher ability to cope with suboptimal oviposition sites, either by an increased ability to feed on poor-quality leaves or to relocate to a better feeding site (or both). Conversely, species with more discriminating females had larvae that were less able to deal with a suboptimal initial feeding site (Figure 3-4 and table 1).

Although there was a general correspondence between our results and the previous findings of Janz & Nylin (1997), there were also some differences. Most of these can be summarized by the observation that the observed differences did not correspond very well to the "specialist" and "generalist" categorization. To some extent, clutch size appears to describe host specificity better than host range in our study system: females of the clutch-laying species *A. urticae* and *A. io* had strong preferences for high quality leaves, whereas the females of single egg-laying species accepted low quality leaves for oviposition to a relatively high extent (Figure 1). However, there were also

other, more individual differences between the tested species as demonstrated in the larval assays. Larval mobility varied greatly between the species, with *V. atalanta* being most mobile, followed by the two generalists *P. c-album* and *V. cardui* and *A. urticae* and *A. io* being substantially least mobile (Figure 5). The measured mobility levels of *V. atalanta* and *P. c-album* matched those observed by Reavey (1992).

In the larval feeding assays, rates of neonate survival on high and poor quality leaves were comparable within species (except for *A. urticae*). Larvae from feeding on low- quality leaves paid a significant cost in terms of growth during the eight days that this experiment lasted – except the generalist *V. cardui* (Figure 4); a fitness cost that is likely to be severe if extrapolated to the whole period of larval growth (c.f. Feeny 1970; Kelber 1999; De Bruyn et al. 2002). Indeed, leaves of the ‘low’ quality category were found to contain less water, were tougher and contained less nitrogen than high quality leaves. Thus, as expected, most species gained more weight on high-quality plants, where especially *V. atalanta* showed a remarkable weight gain after 8 days both compared to its relative *V. cardui* and considering that egg and neonate weight is at best half that of *P. c-album* (this study and Reavey 1992). The outcome of the mobility and survival experiments could be confirmed in the more natural setup of the nettle stalk experiment, where larval ability to move from an inferior position, to locate a new feeding site and to establish at this site were investigated. All these findings indicate that in parallel to the general coadaptation between female preference and larval abilities, species-specific variation in the measured traits exists across all species.

The “hypothesized specialist”, exhibiting parental care via high female oviposition accuracy, but paired with low larval mobility and rather poor larval performance in unfavorable situations, fitted well to the clutch-laying species. However, even in the closely related *A. urticae* and *A. io*, which share a similar ecological niche (Bryant et al. 2000) the species’ larval performance differed in the respect that larval *A. urticae* were most affected by host plant quality, whereas social context appeared to be an additional factor important for neonate *A. io* survival and weight gain. On the

other hand, the “hypothesized generalist” with indiscriminate female oviposition behavior and precocious larvae was represented by the generalist species *V. cardui* and *P. c-album*, but - unexpectedly - also to some extent by the specialist *V. atalanta*. In our experiments the larvae of these three species showed a relatively high probability to survive in disadvantageous situations and to find resources of better quality, which suggests a potential to counterbalance inaccurate or indiscriminate female oviposition behavior – as also suggested by Nylin & Janz (1996) and Gamberale-Stille et al. (2014). The specialist *V. atalanta* exhibits a marginally significant preference for high quality leaves, but, intriguingly, also accepts to a relatively high extent poor quality host leaves. With their precocious larvae they seem to behave like a “hypothesized generalist”, which is puzzling since their host range is narrow. One should note, however, that *V. atalanta* larvae typically weave characteristic tents from the leaves of its host plant (Thomas and Lewington 2010), which likely require mobile larvae both to find a suitable place for the tent and to actually build it. In addition, a comparably high growth rate on both the poor and high quality leaves suggest a good ability of *V. atalanta* larvae to exploit its plant resource rather effectively.

Even though both generalist species fit relatively well into the “generalist category”, with their indiscriminate oviposition behavior and precocious larvae, they were surprisingly different from each other in almost every aspect measured. Where *P. c-album* females laid an average of 25 comparably large eggs during the 3h interval, *V. cardui* averaged at about 120 eggs in the same time, but then with relatively high larval mortality rates on the host plant (see also Janz 2005) when compared to *P. c-album*. Moreover, they also differed in egg weight, larval mobility and survival in the nettle stalk setup, indicating that these species are realizing their broad host ranges quite differently.

Egg weight is likely to be an important cause for these differences, as it seems to be a relatively phylogenetically constrained trait in the tribe Nymphalini. The relatively high egg weight in the closely related genera *Polygonia* and *Nymphalis* (S Nylin & N Janz, unpublished data) may be an adaptation to the colonization of trees as host plants at the time when these genera diverged (Janz

et al. 2001; Weingartner et al. 2006), since Lepidoptera feeding on woody plants were found to have larger eggs than those feeding on herbaceous plants (Reavey 1992). Ancestors to the genera *Vanessa* and *Aglais* have not colonized trees (Janz et al. 2001), and thus have retained the ancestral small egg size. We suggest that phylogenetically constrained egg sizes provided different preconditions for how host plant generalization could be achieved in the two generalist species studied here. This implies that the “generalist” and “specialist” categories should not be treated as fixed life-history syndromes, and that the pathways to generalization (and perhaps specialization) could be varied and highly context-dependent.

Another example of such varied pathways to evolution of host range and co-adapted traits is the pattern regarding clutch size. Batch laying was well correlated to a “hypothesized specialist” in our study and can be understood as the outcome of a choosy specialist laying many eggs once a suitable oviposition site is found. Still, broader host ranges are known from clutch laying species such as some *Nymphalis* butterflies (Tolman and Lewington 1997), *Spodoptera* moths (Salama et al. 1971) or indeed species with wingless females (Hunter 1995). Hence, species at both sides of the specialist-generalist axis can have such specific traits in common, and the evolutionary outcome would depend on many different ecological factors (Stamp 1980).

In conclusion, we found female oviposition strategy and degree of larval precociousness to be co-adapted. Contrary to our expectations, these traits do not fully match the generalist-specialist gradient. Hence, traits such as discriminate/ indiscriminate oviposition strategy or degree of larval precociousness can be assumed to be found both among generalist and specialist species.. Specific traits that arose in the evolutionary history of a species can clearly alter the conditions for precisely how a wider or narrower diet niche can be accomplished.

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Table 1: Overview of larval performance on nettle stalk after 48h; The letters in the asterisk column describe significant differences in survival after Bonferroni correction (logistic regression; $p < 0.05$), where different letters indicate significant differences between each other; for more statistical details see Table 2

species	% that reached leaf after 48h	*	% that feed on stalk	% survival after 48h	*
<i>Aglaia urticae</i>	48.3	a	0.0	18.2	a
<i>Aglaia io</i>	13.3	b	0.0	3.3	a
<i>Vanessa atalanta</i>	80.6	c	25.0	88.9	b
<i>Vanessa cardui</i>	70.0	a, c	2.5	62.5	c
<i>Polygonia c-album</i>	85.4	c	0.0	81.3	b, c

Table 2: Species comparisons on larval performance on nettle stalk after 48h; each pairwise comparison was made with a logistic regression. p-values in italic are statistical significant even after Bonferroni correction. Sample size for ‘% reach leaf’ and ‘survival’, respectively: *A. urticae* (31,33), *A. io* (30,30), *Vanessa atalanta* (37,36), *V. cardui* (40,40), *Polygonia c-album* (48,48);

Comparison	% that reached leaf		% survival	
	χ^2	P	χ^2	P
<i>Aglaia urticae</i> – <i>A. io</i>	9.2	<i>0.0025</i>	3.9	0.049
<i>A. urticae</i> – <i>Vanessa atalanta</i>	6.7	<i>0.0095</i>	38.5	<i><0.001</i>
<i>A. urticae</i> – <i>V. cardui</i>	3.4	0.064	15.3	<i><0.001</i>
<i>A. urticae</i> – <i>Polygonia c-album</i>	12.5	<i><0.001</i>	33.7	<i><0.001</i>
<i>A. io</i> – <i>V. atalanta</i>	30.7	<i><0.001</i>	57.6	<i><0.001</i>
<i>A. io</i> – <i>V. cardui</i>	24.1	<i><0.001</i>	30.7	<i><0.001</i>
<i>A. io</i> – <i>P. c-album</i>	42.8	<i><0.001</i>	53.0	<i><0.001</i>
<i>V. atalanta</i> – <i>V. cardui</i>	0.7	0.40	7.4	<i>0.0064</i>
<i>V. atalanta</i> – <i>P. c-album</i>	0.7	0.40	0.9	0.33
<i>V. cardui</i> – <i>P. c-album</i>	3.1	0.080	3.8	0.049

Figure legends

Fig 1 Oviposition preference between a poor and a high quality host plant. On the left side the mean proportion of preference for the clutch laying species *Aglais urticae* (n=26) and *A. io*, (n=14) is shown (binomial test, *** $p < 0.001$). Observe that the data is binomial and that no *A. io* females laid eggs on poor quality leaves. For the remaining three species box plots show the median proportion of preference (horizontal line), the 25th and 75th percentile (lower and upper margin of the box) together with whiskers showing the minimum and maximum values (Wilcoxon signed-rank test, ns= not significantly different); *Vanessa atalanta* ($p = 0.063$, n=6), *V. cardui* ($p = 0.348$, n=19) and *Polygonia c-album* ($p = 0.156$, n=16).

Fig 2 Egg weights of the five species. The box plots show the median egg weight (horizontal line), the 25th and 75th percentile (lower and upper margin of the box) together with whiskers showing the minimum and maximum weights. Box plots capped by different letters differ significantly (ANOVA, $F_{(4,32)} = 579.1$, followed by post-hoc Tukey test, $p < 0.01$). *A. urticae*: n=30; *A. io*: n=44; *V. atalanta*: n=41; *V. cardui*: n=42 and *P. c-album*: n=50.

Fig 3 Larval survival after eight days of feeding on poor or high quality host plant. Bars show the proportion of larvae alive after eight days. To detect differences in survival on the two leaf categories we performed a logistic regression (*** $p < 0.001$, ns= not significant); respective n-values are shown in bars. For *Aglais urticae* and *A. io*, data are shown for both single and gregarious treatment. Significant p-values of the multiply tested *Aglais* species were still significant after Bonferroni correction. The line above singly vs gregariously tested *A. io* larvae refers to significantly higher survival of the gregariously tested larvae – independently from leaf quality.

480 Fig 4 Larval weight after eight days of feeding on poor or high quality host plant. The box plots show
481 median weight (horizontal line), the 25th and 75th percentile (lower and upper margin of the box)
482 together with whiskers showing the minimum and maximum weights (Wilcoxon's signed-rank test,
483 *** $p < 0.001$, * $p < 0.05$, ns=not significant, n-values shown below the boxes). Significant p-values of the
484 multiply tested *Aglaia* species were still significant after Bonferroni correction.

485

486 Fig 5 Larval mobility on wooden rod. The box plots show the median distance (cm) covered per hour
487 (horizontal line), the 25th and 75th percentile (lower and upper margin of the box) together with
488 whiskers showing the minimum and maximum speed. Box plots capped by different letters differ
489 significantly (ANOVA [$F_{(4,115)}=105$, $df=1$] followed by Tukey post hoc test, $p < 0.05$); n=20, 28, 33, 19
490 and 20, respectively)

491

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5