



Sex-linked inheritance of host-plant specialization in a polyphagous butterfly

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I investigated the genetic background of intraspecific variation in oviposition specificity in the generalist butterfly *Polygonia c-album*. Using reciprocal crosses between two populations that differ in their degree of specialization, I show that specificity is strongly sex-linked. This indicates that genes determining this difference are located primarily on the paternally inherited X-chromosome. The results suggest that intraspecific differences in specificity are caused by the same genetic mechanisms that have been shown to determine interspecific differences in host-plant ranking in other butterflies. Accordingly, the common assumption that specialization and ranking are determined by fundamentally different mechanisms was not supported.

Keywords: host-plant range; oviposition preference; specificity; *Polygonia c-album*; evolutionary genetics; X-chromosome

1. INTRODUCTION

Speciation onto plant species through shifts in host-plant ranking and specialization is thought to account for a substantial part of the diversification of plant-feeding insects (Ehrlich & Raven 1964; Mitter *et al.* 1988; Janz & Nylin 1998), yet the underlying genetic basis of these traits is still poorly known. A few studies have investigated the inheritance patterns of oviposition preference and larval performance, either by interspecific hybridization of pairs of closely related insect species that differ in host-plant utilization (Thompson 1988*b*; Thompson *et al.* 1990; Scriber *et al.* 1991; Sheck & Gould 1995; Keese 1996), or by investigating within-population heritability (Tabashnik *et al.* 1981; Singer *et al.* 1988; Carriere & Roitberg 1995). Results are mixed but we should at least be able to draw two cautious general conclusions from these studies. First, in the few cases it has been studied, oviposition traits appear to be inherited differently from larval performance traits (Thompson 1988*b*; Thompson *et al.* 1990; Keese 1996), which, if it proves to be generally true, would have important implications for theories on coevolution and sympatric speciation in phytophagous insects (e.g. Bush & Diehl 1982; Feder *et al.* 1988; Thompson 1988*a*). Second, it appears that inheritance of oviposition traits are taxon specific, probably because the groups of phytophagous insects that have been studied (chrysomelid beetles, noctuid moths and butterflies) differ greatly in many traits associated with host use, such as adult feeding and larval mobility. Interestingly, both butterfly studies reported a strong sex-linked component of host-plant preference, with a strong tendency of the hybrids towards the preferred plant of the paternal species (Thompson 1988*b*; Scriber *et al.* 1991). As butterflies have chromosomal sex determination where females are heterogametic, host-plant preference must be strongly influenced by at least one locus on the X-chromosome.

The present study differs from the above studies in two fundamental ways: while they investigated interspecific or intrapopulation differences in host-plant ranking (Sheck & Gould (1995) actually measured both ranking and specificity, but the results were confounded because the species differed in both these traits), the present study attempts specifically to investigate the inheritance patterns of host-plant specialization between conspecific populations on a geographical scale, which remain virtually unknown in spite of their immense ecological importance (Jaenike 1989; Thompson 1994).

As the relationship between ranking and specificity is not at all clear, some definitions are required. In defining these terms I have tried as far as possible to follow (and merge) general convention in the field (e.g. Thompson 1988*b*; Singer *et al.* 1992). First, it is important to state that all terms refer to oviposition behaviour under controlled conditions, whereas actual host use may differ depending on abundance and availability of potential host-plants in nature. In this study, 'host-plant ranking' is defined as the order of host-plants ranked by the number of eggs oviposited on them (in simultaneous choice trials), or the order of plants as they become acceptable (in sequential choice trials). 'Host-plant range' is the number of plants actually oviposited on in any type of choice trial. 'Specificity' is used as a relative measure of how many eggs a female lays on a preferred plant in relation to other plants in the trial (in simultaneous choice trials), or of the length of the discrimination time on a preferred plant in relation to other plants (in sequential choice trials). 'Oviposition preference' is a vaguely defined term, often just referring to the fact that a female shows a higher willingness to oviposit on certain plants than on others. Most often it is used in a very pragmatic sense, simply meaning 'the aspect of host-plant choice that we are measuring'.

It has become customary to view host-plant ranking (hierarchy) and specificity as two distinctly different phenomena (Wiklund 1981; Singer 1982; Papaj & Rausher 1983; Courtney *et al.* 1989; Singer *et al.* 1992; Carriere & Roitberg 1995; Carriere 1998). With this view, host-plant ranking is largely determined by responses to factors external to the ovipositing insect, such as plant-specific chemicals and enemies. Rank order should be relatively 'hard wired' because preference is not supposed to be able to readily evolve separately for each host-plant. Specificity, on the other hand, is thought to be determined by a 'threshold' value that is largely dependent on the insect's internal motivational state (e.g. egg load), and should therefore be much more evolutionarily labile. Thus, most changes in oviposition preference should involve changes in specificity, whereas reversals in rank order should be more rare (Courtney *et al.* 1989). According to the threshold model, host-plant specificity is a quantitative and plastic trait that is most easily interpreted in terms of quantitative genetics (Falconer 1981; Singer 1982; Courtney *et al.* 1989), and should therefore show polygenic inheritance, as opposed to the X-linked inheritance of host-plant ranking demonstrated for *Papilio* butterflies by Thompson (1988b) and Scriber *et al.* (1991).

When commenting on Thompson's (1988b) interspecific crosses of *Papilio* butterflies, Jaenike (1989) noticed the lack of similar studies using reciprocal crosses among differentiated populations of the same species. Such studies 'would provide a clue as to whether the genetic determinants of variation in host specificity within species are of the same sort that bring about differences among species' and this 'would constitute an important step towards understanding the macro-evolutionary patterns of butterfly–host-plant associations first brought to general attention by Ehrlich and Raven'. This paper investigates the inheritance of host-plant specialization, using reciprocal crosses of two populations of the nymphalid butterfly *Polygonia c-album* that differ in specificity, but not in host-plant ranking (Nylin 1988; Janz & Nylin 1997). *P. c-album* offers an unusual opportunity to understand the genetic basis of intraspecific differences in host-plant specialization on a geographical scale.

2. MATERIALS AND METHODS

Polygonia c-album (the comma butterfly) is distributed all over the Palaearctic region, from the British Isles in the west to Japan in the east. In Europe it occurs from Scandinavia in the north to north-west Africa in the south. These butterflies overwinter as adults, but whereas northern populations have one generation per season, southern populations can have two by producing a brighter coloured, directly developing summer form. The shifts in the number of generations per season are associated with changes in oviposition specificity: populations at southern latitudes, where two generations can be produced annually, show higher specialization on plants that support shorter larval development time than do populations at latitudes where the length of the summer does not allow for an additional generation (Nylin 1988; Janz & Nylin 1997). Larvae are polyphagous and can feed on plants from at least seven families in four orders (Urticales, Salicales, Fagales and Rosales), but generally prefer plants from the Urticales: *Urtica dioica*, *Ulmus glabra* and *Humulus lupulus* (Nylin 1988; Janz *et al.* 1994).

Study specimens had overwintered under laboratory conditions. The Swedish stock originated from females that were wild, caught in the Stockholm area in May 1996; the English stock originated from southern England, and was obtained from a commercial butterfly provider in May 1996.

Virgin males and females were crossed in all possible combinations and the mated females and their daughters were tested for host preference in identical simultaneous choice trials with the higher-ranked host *Urtica dioica* (stinging nettle) and the lower-ranked host *Salix caprea* (sallow). Females were placed individually in cages (0.5 m × 0.5 m × 0.5 m) directly after mating and were supplied with food (on a sponge with a diluted mixture of honey and sucrose). Each cage was illuminated by a 75 W light bulb directly above the cage (with a light regime of 7 h). All females were presented with the two host-plants that were cut to the same size. Each trial typically lasted for two days. The plants were standing at an equal distance from the central food and light source and their positions were shifted at least once every day to control for position effects. The plants were also rotated among the cages and were exchanged for fresh ones when showing signs of senescence. Eggs were counted and removed from the plants at the end of each day. F₁ larvae were reared individually on *U. dioica* under identical conditions.

One measure for each female was obtained by using the proportion of eggs laid on *U. dioica* over *S. caprea* as the dependent variable, so that each female represented one independent data point. Data were analysed with *t*-tests and a one-way ANOVA on arcsin-transformed data.

3. RESULTS

The results from the choice trial involving hibernated butterflies confirmed previous findings that the English population is more specialized on nettles than the Swedish population (Nylin 1988; Janz & Nylin 1997). English females laid 92.6 ± 2.8% (mean ± s.e.) of their eggs on nettle, whereas Swedish females laid an equal amount of eggs on nettle (46.6 ± 7.3%) as they did on willow (*t*-test, *n* = 7 and 4, *t* = 5.94, *p* = 0.002; figure 1).

Second generation females (F₁) with both parents coming from either the English population (hereafter called EE, with the maternal source population given first) or the Swedish population (SS), differed significantly in their egg-laying patterns from their parental generations. In both populations the second generation showed a higher degree of specialization on nettles than the first (figures 1 and 2). For the Swedish population the proportion of eggs laid on *Urtica* increased from 46.6 ± 7.3% in the first generation, to 77.1 ± 4.6% in the second generation (*t*-test, *n* = 4 and 11, *t* = -3.05, *p* = 0.009), whereas for the English population it increased from 92.6 ± 2.8% to 97.2 ± 2.5% (*t*-test, *n* = 7 and 13, *t* = -2.28, *p* = 0.035) (figures 1 and 2). However, the difference in specificity between populations remained in both generations (figures 1 and 2).

The hybrid crosses had a very similar degree of specialization as their paternal source populations, and were in fact indistinguishable from the paternal sources in *post hoc* tests (figure 2). Sex-linkage was quite striking and there were no detectable polygenic influences at all, although it is entirely possible that such influences would be detected with larger sample sizes. There is a larger variance in the ES crossing than in the other groups,

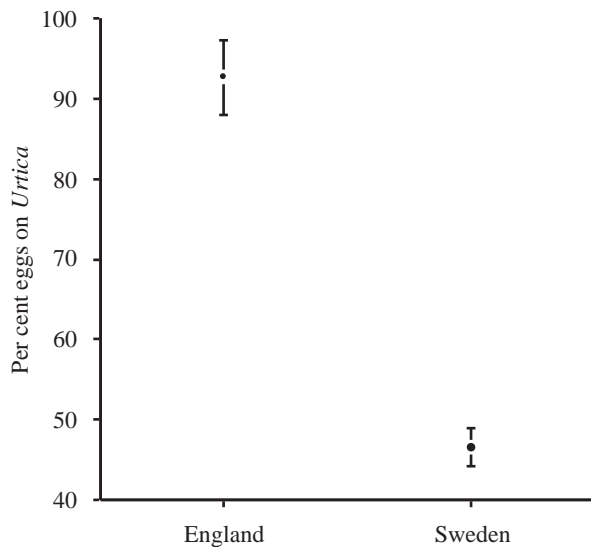


Figure 1. Degree of specialization on *Urtica dioica* by the parental generation, measured as the strength of preference for *Urtica* over the lower-ranked *Salix caprea*. Means \pm s.e. Sample sizes: England, 7; Sweden, 4.

especially when compared with the other hybrid group. Some females of this group behaved more like the English stock, but this large variation may reflect a higher natural variation in oviposition specificity in the Swedish population (Janz *et al.* 1994).

4. DISCUSSION

The higher degree of specialization on nettle in the summer generations than in the spring generations of both populations may be an effect of the different developmental pathways associated with direct development and diapausing. Larvae of the summer generation are generally under more severe time stress, as they all have to complete development in the relatively shorter time available before weather conditions become too harsh, and thus the females need to oviposit on the plants that can support the shortest development times (Nylin 1988). In addition, the differences between the hosts increase over the season as the trees degenerate faster in quality than nettle, the only herb in the repertoire (N. Wedell, N. Janz and S. Nylin, unpublished data). In any case, the difference in specificity between populations remained in both generations.

The results from the F_1 oviposition trials revealed a very close correspondence of the degree of specialization of the hybrids and their paternal source populations. This suggests that the difference in host-plant specificity between these populations is largely controlled by at least one gene on the X-chromosome. In contrast to what was predicted from the threshold model (Courtney *et al.* 1989), it appears that the difference in specificity between the English and Swedish populations of the comma butterfly is caused by the same genetic mechanisms that were shown to determine host-plant ranking in the *Papilio* species pairs studied by Thompson (1991).

Apparently, a quantitative-threshold model cannot sufficiently explain the mechanisms behind specialization

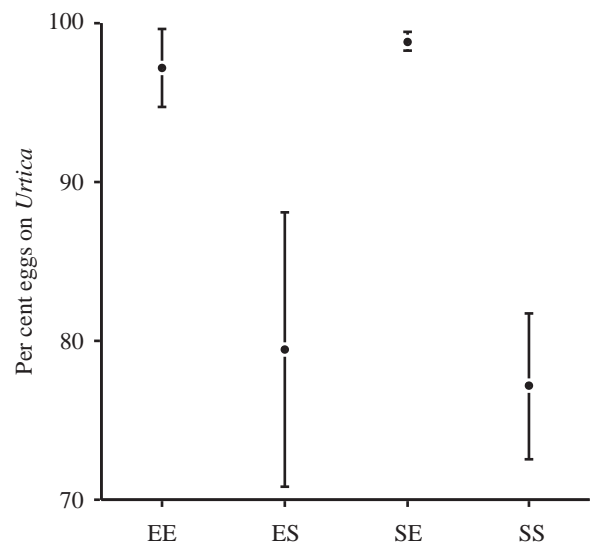


Figure 2. Degree of specialization on *Urtica dioica* by the reciprocal F_1 crosses, measured as the strength of preference for *Urtica* over the lower-ranked *Salix caprea*. E = English, S = Swedish, female origin given first. Means \pm s.e. One-way ANOVA, d.f. = 3,39, $p < 0.001$. Sheffe *post hoc* comparisons: EE–ES, $p = 0.003$; EE–SE, n.s.; EE–SS, $p < 0.001$; ES–SE, $p = 0.015$; ES–SS, n.s.; SE–SS, $p = 0.002$. Sample sizes: EE, 13; ES, 11; SE, 8; SS, 11.

in *P. c-album*. Even if no comparable investigations of inheritance of specificity have been made, quantitative models have been quite successful in explaining variation in oviposition behaviour in other species (Tabashnik *et al.* 1981; Ng 1988; Singer *et al.* 1988, 1989; Fox 1993). These studies, however, investigated oviposition preference within populations, whereas the present study focuses on geographical differences between populations. This does not preclude that there may exist fundamental differences in how the host-plant hierarchy is established in these species compared with *P. c-album*. One possible difference is the diversity of the host-plant range in terms of phytochemistry and other plant-specific characteristics. *Polygonia c-album* is polyphagous on a diverse range of plants, as are the *Papilio* species where host-plant rankings were demonstrated to be X-linked (Thompson 1988b; Scriber *et al.* 1991). When the number of plants included in the oviposition repertoire is large, and differences in, for example, chemistry between them are significant, it will become increasingly difficult for ovipositing females to evaluate the potential host-plants along a single axis. In addition, the same piece of information need not necessarily carry the same meaning for different plant species in the repertoire. An alternative view is that each plant in the repertoire has its own probability of acceptance, which is tightly linked to the identification of the host-plant species. Formulated in the language of the threshold model, the ovipositing female would possess a different threshold for each host-plant species.

With this view, preference will more easily be able to vary separately for each plant species in the repertoire. Even so, changes in preference rank have to be of a certain magnitude to actually shift preference in favour of a previously lower-ranked plant; smaller changes in the strength of preference will instead be manifested as

changes in specificity. As smaller changes will be more common than large, most changes in preference would still be modifications of specialization rather than host-plant ranking. If host-plant ranking and specialization are two expressions of the same phenomenon, differing only in quantity, the process leading to host shifts is easier to understand as a result of a continuous process in which preference of a low-ranked host increases over time, first leading to a lowered degree of specialization on the preferred plant, and eventually to a shift in rank order.

Even if there are additional quantitative components of specificity, especially within populations, the present study points at an overlooked genetic component of specialization that could contribute to much of the variation in host-plant specificity seen in nature. Furthermore, X-linkage of this and other important life-history traits in Lepidoptera may also function as a means to keep important combinations of traits intact in the face of recombination and gene flow (Charlesworth *et al.* 1987; Scriber 1994; Sperling 1994).

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