

Spatial and temporal patterns of caterpillar performance and the suitability of two host plant species

SASKYA VAN NOUHUYS,¹ MICHAEL C. SINGER² and MARKO

NIEMINEN¹ ¹Metapopulation Research Group, Department of Ecology and Systematics, University of Helsinki, Finland and ²Section of Integrative Biology, University of Texas, Austin, U.S.A.

Abstract. 1. The butterfly *Melitaea cinxia* uses two host plant species in the Åland Islands of south-west Finland. Survey data show that host plant use is spatially variable and that the two species are not used (fed on by *M. cinxia* larvae) in proportion to their abundances. The pattern of host plant use by *M. cinxia* has been attributed in part to plant distribution and spatial variation of butterfly oviposition preference.

2. The additional roles that may be played by spatial variation in host plant quality and larval physiology (performance ability) were investigated. Seven years of field survey data and a series of laboratory experiments were used to demonstrate that neither of these variables contributed measurably to the observed pattern of host plant use.

3. Specifically, while there was great variation among individuals in both the performance ability of caterpillars and host plant suitability, the two plant species appeared equally suitable, there was no within-species geographic variation in plant suitability, and there was no evidence for adaptation of caterpillars to the locally used host plants.

Key words. Herbivory, *Melitaea cinxia*, performance, *Plantago*, preference, *Veronica*

Introduction

Polyphagous herbivores, including many butterflies, frequently encounter an array of possible host plants of differing suitability. Under these conditions, the oviposition choice made by a female undoubtedly influences the fitness of its offspring. The consequences of its choice can influence survival of eggs, growth and survival of larvae, survival of pupae, and adult size and fecundity (Scriber & Slansky, 1981; Thompson, 1988; Weiss *et al.*, 1988; Janz *et al.*, 1994; Awmack & Leather, 2002). Researchers frequently measure the association (phenotypic or genetic) between the oviposition preference of females and the performance of their offspring. If there is a positive association between adult oviposition preference and the performance of offspring, compelling ecological and evolutionary stories can be told

(Rausher, 1982; Via, 1986; Singer *et al.*, 1988; Bossart & Scriber, 1995; Stein & Price, 1995; Gu *et al.*, 2001), but the scenarios are more complicated when no concordance is found (Courtney, 1981; Thompson, 1988; Fox, 1993; Janz *et al.*, 1994; Berdegué *et al.*, 1998; Gratton & Welter, 1998; Leyva *et al.*, 2000). In these cases, finding an ecologically or evolutionarily plausible explanation for the observed patterns of adult preference and juvenile performance can be daunting, but potentially revealing of what ecological processes are involved and might drive evolution in natural populations.

The butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae) uses two larval host plant species in the Åland Islands of south-west Finland. *Veronica spicata* is found primarily in the west and *Plantago lanceolata* is found throughout the Islands (Hanski, 1999). Adult butterflies choose among host plants when ovipositing. The young larvae are relatively immobile and must therefore rely on the host plant choice of the female. Kuussaari *et al.* (2000) showed that adult oviposition preference is spatially variable, and that the spatial genetic structure of oviposition preference is

Correspondence: Saskya van Nouhuys, Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, NY 14853, U.S.A. E-mail: saskya@cornell.edu

consistent with the observed regional pattern of host plant use in Åland. Individuals from local populations in regions dominated by *P. lanceolata* mostly preferred to lay eggs on *P. lanceolata*, but where the density of *V. spicata* was generally high, individuals preferred *V. spicata*. This causes disproportionately high use of *V. spicata* where it is regionally abundant. Hanski and Singer (2001) showed that along with population size and habitat patch connectivity, the regional pattern of host plant preference contributes to explaining the large-scale metapopulation dynamics of *M. cinxia* in Åland.

While it is clear that adult butterflies have preferences (Kuussaari *et al.*, 2000) that have large-scale consequences (Hanski & Singer, 2001), it is not clear why butterflies might prefer one plant species over another. In the work reported here, the performance of *M. cinxia* larvae was measured in order to detect spatial variation of both host plant suitability and larval physiology, and to look for an association between the pattern of adult butterfly preference and the performance of larvae. An analysis of the spatial and temporal pattern of survival of larvae on each host plant using 7 years of survey data from the entire Åland Islands is presented. These observational data present the realised survival of larvae on the two host plants in the Åland Islands but reveal nothing about possible underlying spatial variation in plant suitability or larval physiology. In order to test the hypothesis that differences among host plants cause differences in larval performance, five laboratory experiments and one field experiment were performed, comparing the weight and survival of larvae feeding on *P. lanceolata* and *V. spicata*, and between *P. lanceolata* plants from habitat patches in which *P. lanceolata* is used and in which it is not used. Using laboratory reciprocal transplant experiments, the hypothesis that *M. cinxia* larvae are adapted to the predominantly used host plant in their region of origin was tested. The results are discussed with respect to the observed pattern of host plant use and butterfly oviposition preference.

Material and methods

Natural history

The butterfly *Melitaea cinxia* lives in a fragmented rural landscape in the Åland Islands (50 × 70 km) of south-west Finland. Several hundred small local butterfly populations inhabit individual meadows. There are about 4000 of these meadows, clustered into habitat patch networks, separated by forest, water, or cultivated land. The butterflies have a classical metapopulation structure with a high rate of local population turnover (Hanski, 1999; Nieminen *et al.*, in press).

Melitaea cinxia uses several host plant species in the genera *Plantago* and *Veronica* (Plantaginaceae) throughout Europe (Olmstead *et al.*, 2001; Wahlberg, 2001). In Åland, *Plantago lanceolata* and *Veronica spicata* are virtually the only host plants used. *Plantago lanceolata* is common and

found throughout the Åland Islands. *Veronica spicata* is completely absent from eastern Åland and increases in abundance toward the north-west (Kuussaari *et al.*, 2000). In the brief northern mid-summer (June), adult butterflies lay eggs on the undersides of leaves in clusters of 100–200. Eggs hatch into larvae that live gregariously in silken nests until just prior to pupation the following spring. Larval growth is interrupted by an obligate diapause that lasts from September until snowmelt in spring. Newly hatched larvae spin a communal silk nest and feed on the individual plant chosen by their mother. If the larvae defoliate a plant, they move as a group to an adjacent or nearby plant, which is usually of the same species. If the defoliation occurs prior to diapause, the larvae may starve because they cannot feed individually and their ability to search for another plant is poor. After diapause, in spring, larval groups typically consume several host plant individuals but rarely starve because they are more mobile (Kuussaari *et al.*, in press).

Melitaea cinxia larvae sequester iridoid glycosides from *P. lanceolata* and *V. spicata* (Suomi *et al.*, 2001). These compounds are most probably used by herbivorous insects, including melitaeine butterflies, as a chemical defence against predators (Bowers, 1980; Bowers *et al.*, 1992; Stamp, 1992; Camara, 1997). As expected of a chemically defended species, the caterpillars are conspicuous. Nevertheless, there is substantial mortality during the egg, pre-diapause, and diapause stages due to starvation, predation, and parasitism so that the number of larvae remaining in each group after diapause (in spring) is usually 15–25 individuals (Kuussaari *et al.*, in press; van Nouhuys & Hanski, in press).

Field survey of *M. cinxia* larvae in naturally occurring populations

The survival of *M. cinxia* larvae was compared between plant species (*P. lanceolata* or *V. spicata*), habitat types (only *P. lanceolata* or mixed), and over time (7 years) using survey data. Each autumn from 1993 to 1999, all of the known habitat patches for *M. cinxia* in the Åland Islands were surveyed, and larval groups on each host plant species were marked. In spring, the occupied habitat patches were revisited and the number of larvae in each group was recorded. The number of populations present in the spring varied among years from 190 to 332.

From these survey data, the within-group survival of larvae from hatching through diapause was estimated, and the survival of entire larval groups during diapause was measured. Mortality of post-diapause larvae is low and attributable primarily to a parasitoid (van Nouhuys & Hanski, 2002) that does not appear to favour either host plant species (S. van Nouhuys and J. Ehrnsten, unpublished). The survival of individual larvae from emergence until diapause was estimated by the number of larvae per group in spring, under the assumption that butterflies laid egg clusters of equal size on the two host plant species. Before making this assumption, the numbers of first-instar larvae were counted in a sample of larval groups in natural

populations (the number of eggs could not be counted accurately). The number of first-instar larvae per group did not differ significantly between host plant species or among larval groups in different parts of the study area. In the sample of 52 groups, the number of individuals ranged from six to 144. The average number of first-instar larvae on *P. lanceolata* was 46 (N = 33, SD = 31) and on *V. spicata* was 45 (N = 19, SD = 35) out of the 100–200 eggs laid per cluster. There is normally significant loss of eggs and young larvae, primarily due to predation (Kuussaari *et al.*, in press).

The average numbers of larvae per group on each of the two host plant species in each population in the spring were calculated and used as data points. Each of these data points was weighted by the relative size of the population (number of groups in a population/total number of groups in 1 year) because the population sizes varied from a single group up to 100 groups, and means from large populations are better estimates of the true mean than are means from small populations. The correlation of observations from the same sites in different years was accounted for using the repeated statement in SAS PROC MIXED to make statistical models with structured covariance (Littell *et al.*, 1996).

In order to test specific hypotheses about the survival of larvae, four statistical models are presented (Table 1). The entire data set was used for the *host species* and the *habitat type* models. There are two habitat types, those containing only *P. lanceolata* and those containing *P. lanceolata* and *V. spicata*. Data from butterfly populations using both host plants were analysed in the *mixed sites* model; data from larval groups on *P. lanceolata* in the *P. lanceolata*-use and

mixed-use populations were used for the *P. lanceolata* model (Table 1). Larval survival on *P. lanceolata* and *V. spicata* and in *P. lanceolata*-use and mixed-use populations were compared within each year separately by constructing specified contrasts in each model (Littell *et al.*, 1996).

The second measure of larval performance on each plant was the overwintering survival of entire groups, which ranged among years from 50 to 86% (autumn 1993 to spring 1999). The differences in overwintering survival of entire larval groups among years and between host species were analysed using multiple logistic regression (1 = larval group survived the winter, 0 = larval group did not survive the winter). The data were divided into subsets as described above in order to test particular hypotheses.

Field experiment comparing larval performance on P. lanceolata and V. spicata in different habitats

Adult female butterflies that were ready to lay eggs (had not oviposited on the day on which they were caught, and were neither newly emerged nor old) were caught in four *P. lanceolata*-use and four mixed-use populations in 1998, and induced to oviposit on suitable plants that did not already bear natural egg clusters. Butterflies were not moved from their patches of origin in order to avoid influencing gene flow among this set of insect populations, in which population genetic studies were under way. In each of the *P. lanceolata*-use sites, *V. spicata* was absent so eggs were laid only on *P. lanceolata*. In each of the mixed-use

Table 1. Analysis of variance of survival (mean number of larvae per post-diapause group) of naturally occurring *M. cinxia* larvae from 1994 to 1999†.

Source	Numerator degrees of freedom	Denominator degrees of freedom	F value‡	P
Host species model (n = 1923 populations)				
Difference in larval survival among years	5	1799	12.14	0.0001
Difference in larval survival between <i>P. lanceolata</i> and <i>V. spicata</i>	1	1799	8.69	0.0032
Difference among years in the effects of <i>P. lanceolata</i> and <i>V. spicata</i> (interaction)	5	1799	3.53	0.0035
Habitat type model (n = 1923 populations)				
Difference in larval survival among years	5	1799	12.55	0.0001
Difference in larval survival between habitat types	1	1799	0.43	0.5098
Difference among years in the effects of habitat type (interaction)	5	1799	3.03	0.0099
Difference in larval survival between <i>P. lanceolata</i> and <i>V. spicata</i> (not due to habitat)	1	1799	2.42	0.1198
Difference among years in the effects of <i>P. lanceolata</i> and <i>V. spicata</i> (interaction)	5	1799	0.82	0.5361
Mixed sites model (n = 578 populations)				
Difference in larval survival among years	5	455	8.28	0.0008
Difference in larval survival between <i>P. lanceolata</i> and <i>V. spicata</i> with both present	1	455	3.86	0.0502
Difference among years in the effects of <i>P. lanceolata</i> and <i>V. spicata</i> (interaction)	5	455	1.44	0.2070
<i>P. lanceolata</i> model (n = 1545 populations)				
Difference in larval survival on <i>P. lanceolata</i> among years	5	1533	7.61	0.0001
Difference in larval survival on <i>P. lanceolata</i> between habitat types	1	1533	0.01	0.9506
Difference among years in the effects of habitat type (interaction)	5	1533	2.13	0.0599

†Each overall model likelihood ratio test χ^2 value is significant at $P < 0.001$. Year, species, host, and use-type are fixed effects, sites are repeated within years.

‡Calculated using type III sums of squares.

sites, eggs were laid on both *P. lanceolata* and *V. spicata*. After each butterfly had laid ≈ 100 eggs, it was removed from the plant in order to standardise larval group size. Twenty-four egg clusters were laid on *P. lanceolata* in *P. lanceolata*-use sites, 22 on *P. lanceolata* in mixed-use sites, and 23 on *V. spicata* in mixed-use sites. The number of egg clusters placed in a population varied from three to 13 because the number of adult butterflies that could be used depended on the size of the butterfly population. Each egg cluster then each larval group was checked weekly from oviposition to diapause. The growth stages and approximate number of individuals were recorded along with any notes about the group. When the larval groups had moulted to diapause form, they were collected, weighed, and counted accurately. In the following spring, the larvae were reared in order to record the level of parasitism by internal parasitoid wasps.

The effects of host species, butterfly population, and habitat type (*P. lanceolata* or mixed) on the weight and fraction of larvae surviving per group were analysed using ANOVA (SAS PROC MIXED; Littell *et al.*, 1996). Butterfly population was a random effect nested in habitat type.

Laboratory comparison of host plant suitability and larval performance

Five laboratory experiments were conducted in order to contrast (1) the suitability for larval growth and survival of *P. lanceolata* and *V. spicata*, (2) the suitability of *P. lanceolata* from habitats in which it is not used with *P. lanceolata* from habitats in which it is used by the butterfly, and (3) the performance of larvae from populations with differing adult preferences on each host plant type (*V. spicata* and *P. lanceolata* from where they were used, *P. lanceolata* from where it was not used).

Suitability of P. lanceolata and V. spicata from habitats where butterflies have different preferences. This experiment, performed in 1998, compared the suitability of the two host species for larval development between two habitat patches. Patches were chosen that resembled each other closely in relative abundance of the two host species and differed strongly in relative use of the two hosts by the butterflies (Kuussaari *et al.*, 2000). In one patch, both hosts were used equally, and in the other, only *V. spicata* was used, although the two species were about equally abundant.

The test plants from each patch were selected by locating a group of plants, assigning a number to each plant, then choosing a random plant. The next plant was selected from a different group. The groups from which the plants were taken were not chosen at random but spaced deliberately across the habitat patch so that any spatial variation in plant quality would be included in the sample. Each plant was transplanted into a 13-cm diameter pot, along with its accompanying soil. In this manner, 18 individual plants of each species were collected from each patch for a total of 72

potted plants. These plants were separated into three blocks, each made up of three treatment replicates.

For each block, newly hatched larvae from a single large egg cluster produced by a butterfly from a laboratory colony were divided into sub-groups of 10 and put on leaf pieces 2–3 cm long. Once feeding was initiated, the leaf pieces were placed back onto the whole plants, and the larvae moved to fresh leaves. The plants were kept in a common environment outside in individual mesh bags in a randomised order for the duration of the experiment. When most of the larvae had moulted to diapause form, they were counted and weighed.

The association of larval survival with host species and host origin was analysed using logistic regression (SAS PROC CATMOD; Stokes *et al.*, 1995). The association of larval weight with host species and host origin was analysed using ANOVA (SAS PROC GLM; SAS Institute Inc., 1988). In each case, block (egg cluster) was included as a random factor, as well as the two-way interactions.

Within- and between-species variation of host plant suitability, and the performance of larvae from different origins. Ten potted *P. lanceolata* and 10 potted *V. spicata* were selected from a set of plants collected in spring 1999 from a variety of locations, not including the patches that served as sources for the insects in the study. These plants were chosen to include natural extremes of colour, leaf hairiness, and growth form. Mated female butterflies were collected from four populations that had a history of using only *P. lanceolata* (*P. lanceolata*-use), six that had used only *V. spicata* (*V. spicata*-use), and two that used both hosts (mixed-use). The butterflies oviposited in the laboratory and, on hatching, the larvae from each egg batch were separated into sub-groups of 10. Three groups of 10 first-instar larvae, each group from a different origin, were reared on leaf pieces taken from each plant individual. Larvae from each egg cluster were reared on both plant species, and each individual plant was host to larval groups from three different egg clusters for a total of 60 larval groups. An unequal number of larval sub-groups from each type of population was used. The larvae were counted and weighed after 30–33 days when they had moulted to diapause form.

Analysis of variance (SAS PROC MIXED; Littell *et al.*, 1996) was used to evaluate the association of larval weight and number of larvae surviving per sub-group with plant species, plant individual nested in plant species, and larval origin type. Egg cluster nested in origin type was included as a random effect. Plant individual was coded as a fixed effect because individuals were chosen to represent a range of physical characteristics.

The performance on P. lanceolata and V. spicata of larvae from populations with different adult preferences. The performance of larvae from a population with strong preference for *P. lanceolata* (from Kumlinge) and a population with strong preference for *V. spicata* (from Sälis) (Kuussaari *et al.*, 2000) was compared feeding on *P. lanceolata* and *V. spicata*. The progeny of butterflies from these two populations maintained in the laboratory for three generations

were reared from egg to third instar on potted *V. spicata* and *P. lanceolata* plants under uniform laboratory conditions in a reciprocal transplant experiment in 1997. The plants, which were grown in pots in a greenhouse, were a haphazard collection from several populations, not including populations from which the butterflies were collected. Each plant hosted a single egg cluster from one butterfly. Egg clusters from nine to 11 females from each population were put on plants of both species for a total of 43 larval groups. At the end of the third-instar, the number of larvae surviving in each group was counted and the larvae were weighed. Analysis of variance (SAS PROC MIXED; Littell *et al.*, 1996) was used to test whether larval weight and number of larvae surviving differed between plant species and between larval origins, and to test for local adaptation by the larvae.

The suitability of P. lanceolata collected from habitat patches in which it was used (P. lanceolata-use) and habitat patches in which it was not used (V. spicata-use). This experiment tested the effect of habitat origin on the suitability of *P. lanceolata*, using larvae from a single population. Five *P. lanceolata* plants were collected from each of four *P. lanceolata*-use sites and each of four *V. spicata*-use sites in 1997. The plants were selected as described above then kept in pots for 2 weeks prior to use. The larvae used were the progeny of three butterflies collected as adults from a *V. spicata*-use population from which no plants were collected. Each female butterfly was allowed to lay a single egg cluster in the laboratory. When the cluster hatched, larvae were fed leaves from a single plant for 3 days then separated into sub-groups of 10. These sub-groups were assigned randomly to plants, and fed leaf pieces every other day until the fourth instar, when they were counted and weighed. Analysis of variance (SAS PROC GLM; SAS Institute Inc., 1988) was used to analyse the influence of plant origin on larval weight (all of the larvae survived so there was no analysis of survival). The egg batch from which the larvae came was included in the statistical model as a random blocking factor.

The suitability of P. lanceolata from different habitats and the local adaptation of larvae. The performance of larvae from *V. spicata*-use, *P. lanceolata*-use, and mixed-use populations was compared feeding on *P. lanceolata* from each habitat type. Ten *P. lanceolata* plants were collected from random locations in each of 12 populations in 1999. The set comprised five *P. lanceolata*-use populations, four where only *V. spicata* was used but *P. lanceolata* was available (*V. spicata*-use), and three where both hosts received substantial butterfly oviposition (mixed-use). The plants were transplanted into 13-cm diameter pots using 50% fine gravel and 50% peat, and kept in a common outdoor environment for 2 weeks prior to the experiment. A mated female butterfly was taken from each population but only nine could be persuaded to lay eggs in the laboratory. Butterflies from three *V. spicata*-use populations were used instead of three butterflies from *P. lanceolata*-use populations that did not lay eggs. On hatching, each cluster was split into 10 sub-groups of 10 larvae. Five of these sub-groups were reared on

leaf pieces from five different plants from the population of origin of the larvae. The other five sub-groups were reared on five plants from a single population of a different type. In this way, larvae from each origin were tested on *home* and *away* plants, and plants from each origin were tested with *home* and *away* larvae, but not all larval origins were tested on all plant origins. The larvae were given fresh leaf pieces every other day. After 33 days, the number surviving, weight, and instar were recorded.

The null hypothesis that *P. lanceolata* from *P. lanceolata*-use and mixed-use populations were equally suitable for larval growth was tested using ANOVA (SAS PROC MIXED; Littell *et al.*, 1996). The population origin of the larvae on each host plant was included in the model as a fixed effect and the population origin of the plant, nested in use-type, was included as a random effect. Analysis of variance was also used to test the null hypothesis that larvae survived equally well on *P. lanceolata* from their own population origin type (*home*) as on *P. lanceolata* from the other two population types (*away*). Plant population origin nested in population use-type was included in the model as a random covariate.

Results

Field survey

In all of the populations over 7 years, on average 20 larvae survived per group on *P. lanceolata* and 19 larvae per group on *V. spicata*. These numbers are remarkably similar but the difference is statistically significant (Table 1; *host species* model, effect of host species), because the sample size is very large ($n = 1923$ populations). It is unlikely that such a small difference between averages is biologically meaningful. There were, however, biologically meaningful differences in survival of larvae among years, which in a few cases differed between host plant species (Table 1; *host species* model). In particular, in 1994, 1998, and 1999, survival on *P. lanceolata* was significantly greater than survival on *V. spicata* (Fig. 1a). In an attempt to distinguish between plant species *per se* and other effects of the habitats in which one host plant or the other was used primarily, habitat types (only *P. lanceolata* present or both species present) were compared in addition to plant species. Larval survival varied from a low mean of 12 larvae per group in mixed-host habitats in 1994, to a high of 29 larvae per group in *P. lanceolata*-only sites in 1998. There was no overall difference in survival between habitats or between species when differences among habitat were accounted for (Table 1; *habitat type* model). While on average the habitats did not differ, there was variation between habitats among years (Table 1; year \times use-type interaction in *habitat type* model; Fig. 1b).

In habitat patches containing both plant species (mixed sites), survival of larvae on both plants varied among years and overall larval survival was almost significantly higher on *P. lanceolata* than on *V. spicata* (Table 1; *mixed-site*

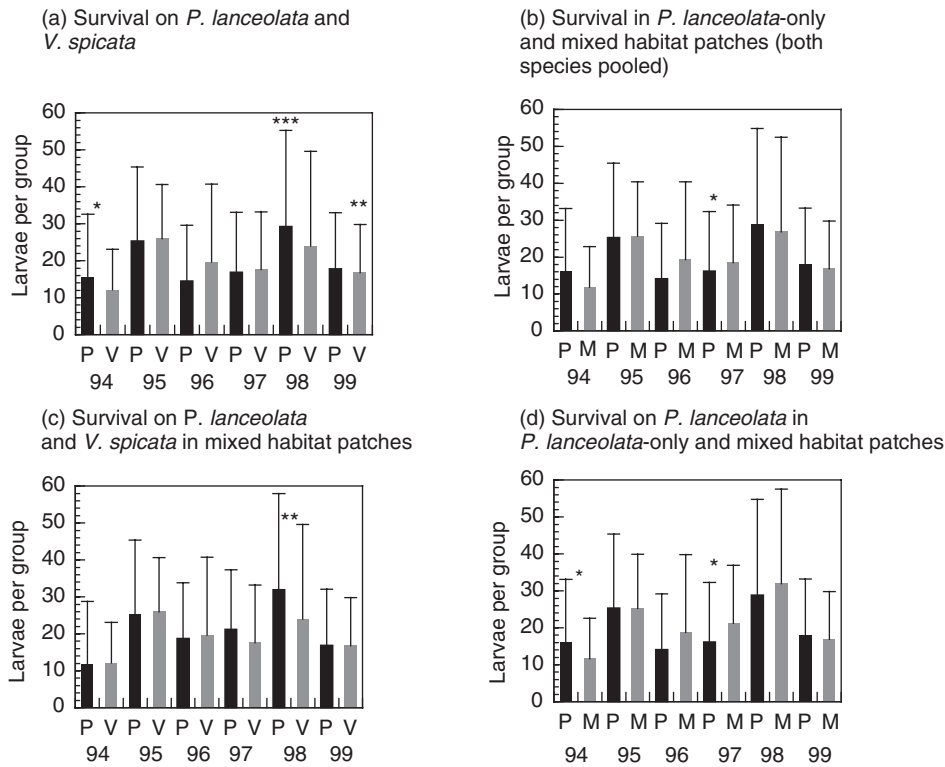


Fig. 1. The mean survival of naturally occurring *M. cinxia* larvae on *P. lanceolata* and *V. spicata* each year from spring 1994 to spring 1999. The mean number (+1 SD) of larvae per nest surviving in the spring is partitioned into (a) those surviving on each plant species ($n = 1923$), (b) those surviving in each host habitat patch type with both species pooled ($n = 1923$), (c) sub-set into those surviving on each plant in mixed-use habitat patches ($n = 578$), and (d) those surviving on *P. lanceolata* in *P. lanceolata*-only and mixed-use habitat patches ($n = 1545$). Within-year differences between groups were tested as planned contrasts within each of the four statistical models presented in Table 1. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

model). This difference was most evident in 1998 (Fig. 1c). Analysis of larvae feeding on *P. lanceolata* growing in *P. lanceolata*-use (only *P. lanceolata* present) and mixed sites again showed significant variation of survival among years but no overall difference in survival between habitat types (Table 1; *P. lanceolata* model). While on average survival was equal in the two habitat types, there was variation between the two habitats within years, with significantly higher survival in *P. lanceolata*-use habitats in 1994 and mixed habitats in 1997 (Table 1; year \times habitat interaction in *P. lanceolata* model, and Fig. 1d).

Overwintering survival of larval groups varied significantly among years from 50 to 86%. On average, there was no significant difference in survival between groups on the two host plant species. During one winter (1996/97), survival of larval groups on *V. spicata* was 10% greater than on *P. lanceolata*, which was statistically significant (logistic regression 1996/97 \times host plant species interaction, $P = 0.001$). In two winters, there was a non-significant trend for higher survival on *V. spicata*, and in the three remaining winters, there was a non-significant trend towards higher survival on *P. lanceolata*. There was no significant difference between the two host plant species in the mixed-

use sites and no difference in overwintering survival on *P. lanceolata* between mixed-use and *P. lanceolata*-use sites.

Field experiment comparing larval performance on *P. lanceolata* and *V. spicata* in different habitats

The fraction of larval groups surviving from oviposition to diapause ranged from complete survival in one population to complete mortality in two populations (Table 2). High predation of eggs and predation or disappearance of small larvae resulted in few of the larval groups in the *P. lanceolata*-use sites surviving, so on average the performance of larvae on *Plantago* was low. A significantly larger fraction of larvae per group survived in mixed habitats than in the *P. lanceolata* habitats ($F_{\text{habitat } 1,68} = 17.47$, $P < 0.001$). Within the mixed habitats, more entire groups survived on *V. spicata* than on *P. lanceolata* but the difference was not significant ($\chi^2 = 3.09$, $P = 0.08$; Table 2). The number of larvae in surviving groups was equal on the two host plants ($F_{\text{species } 1,44} = 0.04$, $P = \text{NS}$), and the weight of the surviving larvae did not differ significantly between host plant species

Table 2. Survival of *M. cinxia* larval groups on *P. lanceolata* and *V. spicata* in different host use regions.

Habitat type	Host species	Number of groups dying	Number of groups surviving	Number of groups parasitised
<i>P. lanceolata</i>	<i>P. lanceolata</i>	9	0	–
<i>P. lanceolata</i>	<i>P. lanceolata</i>	6	3	3
<i>P. lanceolata</i>	<i>P. lanceolata</i>	3	0	–
<i>P. lanceolata</i>	<i>P. lanceolata</i>	1	2	1
	<i>P. lanceolata</i>	3	3	2
Mixed	<i>V. spicata</i>	0	5	4
	<i>P. lanceolata</i>	4	3	3
Mixed	<i>V. spicata</i>	2	6	3
	<i>P. lanceolata</i>	4	3	3
Mixed	<i>V. spicata</i>	3	3	3
	<i>P. lanceolata</i>	0	3	3
Mixed	<i>V. spicata</i>	0	3	3

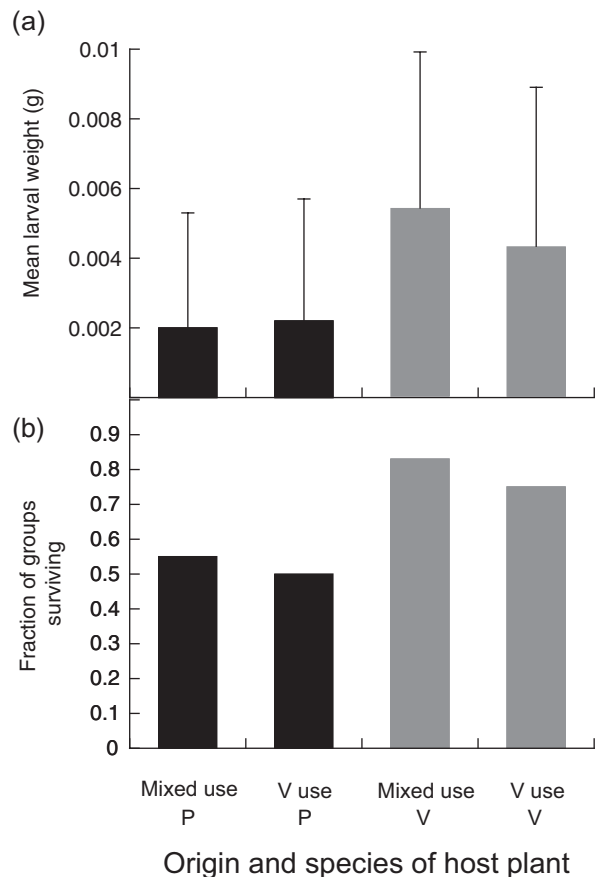
or habitat types. In the majority of the larval groups that survived, at least some larvae were parasitised by the primary parasitoids *Cotesia vestalis* (Haliday) [= *melitaearum* (Wilkinson)] or *Hyposoter horticola* (Table 2). Parasitism rate did not differ between host plant species or between habitat types.

Laboratory experiment one: suitability of P. lanceolata and V. spicata from habitats in which butterflies have different preferences

Larvae grew larger (ANOVA, $F_{\text{species } 1,60} = 16.44$, $P = 0.001$; Fig. 2a), and larval groups were more likely to survive (logistic regression, $\chi^2 = 5.04$, $P < 0.05$; Fig. 2b) when fed *V. spicata* than when fed *P. lanceolata*, regardless of the origin of the host plant (ANOVA, $F_{\text{origin} \times \text{species } 1,2} = 3.44$, $P = \text{NS}$; Fig. 2a). Larval weight and survival did not vary significantly among egg clusters (blocks).

Laboratory experiment two: within- and between-species variation of host plant suitability, and the performance of larvae from different origins

Larval sub-group (10 larvae per sub-group) survival to fourth instar varied from 0 to 100%. Suitability varied greatly among individual host plants (ANOVA, $F_{\text{ind } 18,23} = 2.84$, $P = 0.01$; Fig. 3), but did not differ significantly between host plant species (ANOVA, $F_{\text{species } 1,23} = 3.38$, $P = \text{NS}$), larval origins ($F_{\text{origin } 2,9} = 0.99$, $P = \text{NS}$), or interaction between plant species and larval origin ($F_{\text{species} \times \text{origin } 2,23} = 0.11$, $P = \text{NS}$). The final larval weight did not vary significantly among host plant species, plant individuals, or larval origins.

**Fig. 2.** (a) Mean larval weight (+1 SD) and (b) fraction of *M. cinxia* larval groups surviving on *V. spicata* and *P. lanceolata* from a mixed-use patch and a *V. spicata*-use patch in expt 2. $n = 18$ plants per species per patch.

Laboratory experiment three: the performance on P. lanceolata and V. spicata of larvae from populations with different adult preferences

Larvae reared to third instar on potted *V. spicata* plants weighed more on average than those reared on potted *P. lanceolata* ($F_{\text{species } 1,35} = 9.00$, $P = 0.005$), regardless of the origin of the larvae ($F_{\text{origins } 1,35} = 0.41$, $P = \text{NS}$). There was no difference in survival or larval weight between the progeny of butterflies from populations preferring *P. lanceolata* and those preferring *V. spicata*, and the interaction between larval origin and plant species was not statistically significant.

Laboratory experiment four: the suitability of P. lanceolata collected from habitat patches in which it was used (P. lanceolata-use) and habitat patches in which it was not used (V. spicata-use)

In this experiment, all of the larvae in all of the treatments survived to fourth instar. There was no significant

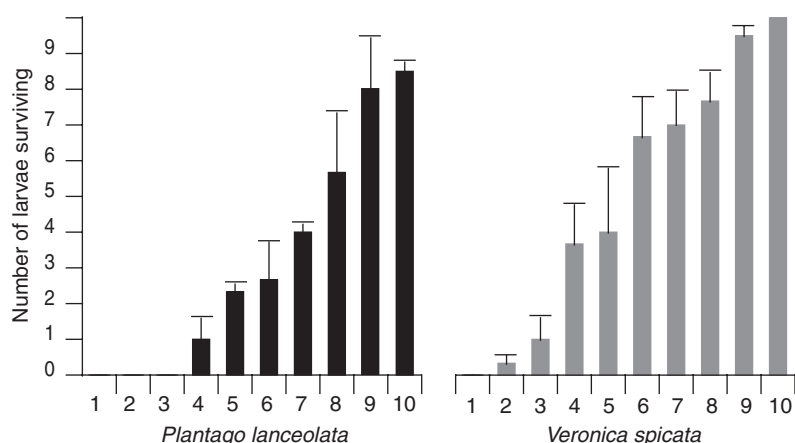


Fig. 3. The mean number (+1 SD) of *M. cinxia* larvae surviving to the end of expt 3 on 10 *P. lanceolata* plants and 10 *V. spicata* plants selected to represent a range of plant phenotypes. Each bar represents the mean of three groups of 10 larvae feeding on leaves from a single plant.

difference in final weight or rate of growth between host plant origins.

Laboratory experiment five: the suitability of *P. lanceolata* from different habitats and the local adaptation of larvae

Larvae survived equally well on *P. lanceolata* from *P. lanceolata*-use, *V. spicata*-use, and mixed-use origins ($F_{\text{plant origins } 2,9} = 0.21$, $P = \text{NS}$). Survival differed significantly among the three types of larval origin ($F_{\text{larval origins } 2,98} = 5.17$, $P = 0.01$), the expression of which depended on plant origin ($F_{\text{larval origins} \times \text{plant origin } 4,98} = 2.83$, $P < 0.05$). The difference between larval origins was due to the poor performance of larvae from the three mixed-use sites. The interaction between larval origin and host origin was due largely to the extreme differences in survival between larvae from *P. lanceolata*-use and *V. spicata*-use populations on the mixed and *P. lanceolata*-use plants. Locally adapted larvae would perform best on *P. lanceolata* from the type of population from which they originated. No evidence of this was found ($F_{\text{home vs away } 5,101} = 1.94$, $P = 0.09$; the trend was in the direction of poor performance on home *P. lanceolata*).

Discussion

Natural variation of larval survival

This large data set spans hundreds of local populations over 7 years. As would be expected in a heterogeneous landscape over time, there is quite a bit of variation in larval survival (Fig. 1). Larval survival differs among years, and during some years differs among habitat patches, indicating that natural selection on larvae is spatially and temporally variable. There is not, however, any evidence that caterpillars are better off on average on one host plant species or the other in either habitat type. If larval performance generated natural selection on adult preference, the observed

variation in larval performance would not tend to cause evolution of adult preference in any consistent direction.

Variation of plant suitability

Veronica spicata is found mainly in the western part of Åland. Where it is most abundant, it is preferred for oviposition by the adult butterflies (Kuussaari *et al.*, 2000). If larvae performed better on *V. spicata* than on *P. lanceolata*, this preference would be adaptive. In the field experiment, larvae were indeed more successful for that one year in habitat patches containing both host plants than in the *P. lanceolata* habitat patches, but within the mixed habitat patches, larvae were equally successful on *V. spicata* and *P. lanceolata*. The suitabilities of *V. spicata* and *P. lanceolata* were compared in three laboratory experiments. In expt 1, the suitability of *V. spicata* and *P. lanceolata* from two habitat patches that contained both plants but differed in relative use by the butterflies was compared. In this case, larval performance was higher on *V. spicata* regardless of the origin of the plants. In expt 3, larval performance on *V. spicata* was again higher than on *P. lanceolata*. When plants were selected specifically to encompass the range of phenotypes observed in the field (rather than randomly), the differences between host plant species were not evident (expt 2).

Preference for *V. spicata*, where it is available, could also be adaptive if *P. lanceolata* were unsuitable for larval development in habitats where *V. spicata* grows. The suitability of *P. lanceolata* from where it is used and where it is not used was tested in three laboratory experiments (1, 4, and 5). In each experiment, *P. lanceolata* from the two habitat types were equally suitable for larval growth and survival.

The experiments lead to the conclusion that *V. spicata* is more suitable for *M. cinxia* larvae than is *P. lanceolata*, and that *P. lanceolata* is equally suitable where it is unused as where it is used; however the 7 years of survey data over the entire study area reveal that the advantage of *V. spicata*

does not manifest itself under natural conditions. With this information, the three possible conclusions are that the advantage of *V. spicata* is so small that it is unmeasurable under natural conditions, that butterflies make up for the superiority of *V. spicata* on average by selecting especially suitable *P. lanceolata*, or that the larvae are locally adapted. The first explanation may be important because the laboratory differs from natural settings in ways that affect larval performance. The second explanation may also be important because there is great potential variability of suitability within plant species (expt 2), and butterflies are choosy, even among individual plants of the same species (Singer & Lee, 2000; Singer *et al.*, 2002). The third explanation will be discussed below.

Variation among larvae from different origins

If larvae were adapted to the locally used host plant, the variation in performance among larvae observed in natural populations would be minimised, so little information about local adaptation of larvae is gained from the survey data or field experiment. The results of the laboratory experiments suggest that the larvae are not locally adapted. In expt 3, the survival and weight of larvae from populations using only *P. lanceolata* were compared with larvae from populations using almost exclusively *V. spicata*, feeding on haphazardly collected suitable *P. lanceolata* and *V. spicata*. Larvae fed *V. spicata* weighed more than larvae fed *P. lanceolata*, regardless of their origin. In expt 2, in which plants were chosen for their phenotypic diversity, there was also no difference in larval weight or survival on the two host plants among larval origins. Experiment 5 addressed specifically whether larvae from populations that used *P. lanceolata* performed better on *P. lanceolata* than larvae from populations using *V. spicata* or those from mixed-use populations. Whether larvae performed best on *P. lanceolata* from the habitat patch from which they originated was also considered. Again, no evidence of local adaptation for *P. lanceolata* or for home *P. lanceolata* was found. Experiments 2, 3, and 5 also demonstrate that larval performance is not correlated with adult preference at the population level. In particular, adults appear to have preferences that have no direct consequence for their larvae. This last point deserves further study, however, because there is within-population variation in preference and the correlation between individual females and their offspring was not studied.

The pattern of host plant use by *M. cinxia* in the Åland Islands has consequences for their population dynamics (Hanski & Singer, 2001). Kuussaari *et al.* (2000) showed that this pattern of host plant use is consistent with the interaction between host plant distribution and spatial variation of adult butterfly preference. Here it is shown that variation in larval performance does not contribute to the observed pattern and does not explain adult oviposition preference.

The preferences of adult butterflies can be perplexing, and how preference relates to the performance of their offspring even more so (Thompson, 1988). Analysis of the survey data of larval survival alone would suggest that butterflies should use the two host plants in proportion to their abundance. The results of the experiments alone suggest that butterflies should prefer *V. spicata* and use it whenever possible. Because the butterflies do neither, something other than larval growth and survival must drive the evolution of host plant use.

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