

Host-plant specialisation and habitat restriction in an endangered insect, *Lycaena dispar batavus* (Lepidoptera: Lycaenidae) I. Larval feeding and oviposition preferences

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Abstract. The Large Copper butterfly, *Lycaena dispar*, is extinct in Britain and rapidly declining in the rest of Europe, due predominantly to loss of its wetland habitats. In the Netherlands the sub-species *L. d. batavus* is at the edge of its range in Northern Europe and, as with most marginal butterflies, has more specialised food plant and habitat requirements than the core populations of *L. d. rutilus*. We investigate reasons for the relative specialisation of *L. d. batavus* on *Rumex hydrolapathum* in a fenland habitat when compared to the more widespread and common *L. d. rutilus*. Host-plant choice by ovipositing females and by larvae are measured as well as larval performance on alternative hosts. Laboratory experiments reveal that larvae are able to feed on other *Rumex* species without detriment to their overall survival and can utilise these alternative host plants at least as efficiently as their natural host plant. This suggests that plant chemistry is not responsible for their lack of utilisation in the wild. Under greenhouse conditions, females showed an equal willingness to oviposit on host and alternative *Rumex*, expressing no significant preference for any particular plant species. However, in field experiments using free-ranging females in a fenland habitat, eggs were laid only on *R. hydrolapathum*. Our interpretation is that there are no short distance cues discriminating between the three *Rumex* species but longer distance cues in the field situation may operate to maintain this host-plant specialisation. The selection pressure maintaining *L. d. batavus* as a specialist on *R. hydrolapathum* in a wetland may underlie its current rarity.

INTRODUCTION

The large copper butterfly, *Lycaena dispar*, is widespread throughout central and eastern Europe (Pullin et al., 1998) but has been declining on the north and western fringes of its range. This species went extinct in England in the late 19th Century and is currently reduced to a few populations in both the Netherlands and Finland as well as showing substantial declines in Germany and Poland. Declines in range have resulted from wetland drainage and have primarily concerned the univoltine populations. The still widespread bivoltine subspecies *L. d. rutilus* occurs over a range of wet and moist early successional habitats and feeds as a larva on a range of species in the genus *Rumex*.

Many butterfly populations have more specific habitat and food-plant requirements at the edges of their ranges, possibly due to the necessity of meeting certain thermal and nutritional criteria needed for optimum growth and development of the immature stages (Thomas, 1993; Thomas et al., 1999). *Papilio machaon britannicus* (Swallowtail) is a wetland subspecies, confined to the UK, which utilises only *Peucedanum palustre* as a larval host plant. On the continent this species uses a wider range of umbellifers (Dempster & Hall, 1980). The Dutch subspecies of the large copper, *L. dispar batavus*, is a univoltine, monophagous fen specialist (this was also true of the extinct English subspecies *L. d. dispar*) that is classified as endangered. This paper investigates the eco-

logical basis of the food plant and habitat specialisation in *L. d. batavus* by comparing its preferences and performance on its host plant *R. hydrolapathum* and two other *Rumex* species commonly used by *L. d. rutilus*, *R. crispus* and *R. obtusifolius*. A primary concern of this study was to investigate whether it is food plant specialisation that confines *L. d. batavus* to fenland and therefore has rendered populations more vulnerable to extinction than the more generalist *L. d. rutilus*. An alternative explanation is that *R. hydrolapathum* is used because *L. d. batavus* is confined to fenland for other reasons and only rarely encounters other *Rumex* species. Survival of larvae on the same plant species in the field is reported in another paper (Martin & Pullin, 2004).

METHODS

Study species

Except where stated otherwise, the individuals used in this study were derived from a captive colony of *L. d. batavus* maintained at Woodwalton Fen NNR, UK. The history of this colony is detailed in Duffey (1968) and Webb & Pullin (1997).

Comparison of larval growth and development on different *Rumex* hosts

1. Early instar larval survival: we initially tested whether 1st instar larvae were able to feed on other *Rumex* hosts. Eggs from one female were used in this experiment to minimise differences due to genetic variation and all were laid within 2 days, thus ensuring reasonable synchrony of development. Eggs were assigned to leaves of one of the three *Rumex* spe-

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cies (21 eggs per species), placed in petri-dishes and development monitored until the first larval moult. The data were analysed using the G-test (Fowler & Cohen, 1990).

2. Larval growth on the three *Rumex* species from hatching to pupation: to determine whether growth and survival rates during the whole larval period differed between *Rumex* species, surviving 2nd instar larvae, from the previous experiment, were transferred onto actively growing plants of the same *Rumex* species. When feeding ceased in the 4th instar they were placed in separate plastic beakers to allow them to pupate. Pupal and adult mass were measured by weighing the surviving pupae 24 hours after pupation (to allow the pupae to harden) and the surviving adults as soon after eclosion as possible (after their wings had dried and meconial fluid had been excreted). Developmental times, from hatching to pupation and adult emergence, were also recorded. The results were analysed using One-way ANOVA.

Feeding efficiencies of larvae on different species of *Rumex*

To test the nutritional suitability of each *Rumex* species, eggs of *L. d. batavus* were randomly assigned to each of the three plant species and reared on that species until they were newly moulted 4th instar larvae. This both tested for acceptability and avoided any possible larval conditioning that might occur if only one foodplant species was used (Jermy et al., 1968). Feeding efficiency was investigated using just 4th instar larvae, as they consume larger quantities of food in more easily measurable amounts than earlier instars. Newly moulted larvae were used because they provide a readily identifiable starting point as gut contents have been evacuated. Fourteen larvae from each treatment were weighed and transferred to individual plastic petri-dishes, 9 × 1.5 cm, lined with moistened filter paper to prevent desiccation of plant material and kept in an insectary with a photoperiod of 18.5L : 5.5D at 25°C. A known weight of food, from greenhouse grown plants was given to each individual at approximately the same time each day and any uneaten leaves from the previous day were removed, and dried in an oven to a constant weight. At the same time all frass produced was removed, dried and weighed. All food samples were taken from mature leaves to avoid any differences in feeding efficiency being due to the nutritional quality of different aged leaves. To find the dry weight of food consumed, extra samples were cut at the same time and their wet and dry weights recorded. Larvae were weighed at the same time each day until they had finished feeding and reached the prepupal stage, and again when the pupa had formed and hardened. The resulting adults were weighed as soon after emergence and excretion of meconial fluid as possible. Two larvae from each treatment were removed at the start and two at the end of the experiment and dried to a constant weight to provide a growth curve for each treatment for conversion of wet to dry weight. As larvae lose some weight prior to cessation of feeding and pupation, growth indices were calculated from the peak weight achieved, in all cases the day before pupation.

Growth and feeding efficiency indices were used following Waldbauer (1968):

Relative Consumption Rate (RCR)

$$RCR = \frac{\text{dry weight of food ingested over unit time}}{\text{mean dry weight of larva over unit time}} \times 100$$

Efficiency of Conversion of Ingested Food (ECI)

$$ECI = \frac{\text{dry weight gained}}{\text{dry weight of food ingested}} \times 100$$

Approximate Digestibility (AD)

$$AD = \frac{(\text{dry weight of food ingested}) - (\text{dry weight of frass})}{\text{dry weight of food ingested}} \times 100$$

Efficiency of Conversion of Digested Food (ECD)

$$ECD = \frac{\text{dry weight gained}}{(\text{dry weight of food ingested}) - (\text{dry weight of frass})} \times 100$$

The following were also calculated for each larva; the dry weight of food consumed (FC), dry weight of frass produced (FP) and the dry weight gained (DWG) over the whole experiment.

Host plant choice by larvae

In order to determine whether larvae showed any preference for a particular *Rumex* species, 33 second instar larvae were placed into separate petri-dishes lined with moist tissue paper containing 1 × 26 mm diameter discs of each of *R. crispus*, *R. obtusifolius* and *R. hydrolapathum* arranged in randomised triangular pattern. Second instar larvae were used as they are more mobile than 1st instar and may therefore be able to exhibit a preference more clearly. Larvae were reared through the first instar on their natural host plant *R. hydrolapathum*. Larvae were placed in the centre of the dish to avoid bias and checked after 2, 4, 6 and 24 hours and their position and species of any food eaten was recorded at each interval. Any larva not on a plant was excluded from that time period unless feeding damage, not present at the previous time period, was evident. The frequency data were analysed using a G-test and analysis on the number of discs utilised was carried out on 24 hour data, also using a G-test.

Oviposition preference on alternative *Rumex* species

To test for an oviposition preference, mated females were presented with a choice of the three *Rumex* species. For this experiment all larvae were reared to adults under summer light and temperature regimes (19L : 5D at 20°C) on their natural host-plant so that if any larval conditioning were to occur we would expect *R. hydrolapathum* to be chosen in preference to non-hosts. This was to encompass the Hopkin's Host Selection Principle, which suggests that an ovipositing female will preferentially choose the host-plant on which she was reared (Wiklund, 1974; Stanton, 1979). Female butterflies were mated prior to the experiment by releasing one male and one female into a cage 1m³, with an artificial food source of 5% honey, and left for 24 hours.

The *Rumex* plants were grown in 20 cm pots under greenhouse conditions. For the experiment, one each of the plant species, matched for size, was placed in a muslin cage (150 cm × 91 cm × 91 cm), in a regular triangular pattern. The plants were randomly allocated to each of the three positions by drawing lots. The arrangement was re-randomised for each individual female. Females were released into experimental cages and left for 24 hours with a food source as above in natural daylight and temperatures regulated by air vents. After the allotted time period the female and the plants were removed and the number of eggs present on each species was noted.

Do females discriminate between *Rumex* species?

This experiment was undertaken to see if adult females discriminate between plant species before an oviposition event, or whether they accept the first plant on which they land. The experimental design was essentially the same as above, but in this case a mated female was released into the cage, left for 15 minutes and her movements recorded. Each female was released three times into the cage and plants were placed, on each occasion, in a different pattern, outlined above, which was changed

TABLE 1. Larval development from egg to pupation and eclosion of *Lycaena dispar batavus* feeding on each of three alternative *Rumex* species, n = 63, d.f. = 2.

	<i>R. hydrolapathum</i>		<i>R. obtusifolius</i>		<i>R. crispus</i>		F	Significance
	Mean	(SE)	Mean	(SE)	Mean	(SE)		
Larval duration (days)	26.05	1.635	27.03	1.662	26.35	0.609	2.56	P > 0.05
Pupal weight (g)	0.2183	0.02	0.2204	0.035	0.2136	0.019	0.37	P > 0.05
Adult weight (g)	0.0884	0.016	0.0794	0.023	0.0796	0.01	1.82	P > 0.05

on each release to avoid any possible pattern recognition by the female. A total of 8 butterflies was used.

Oviposition preferences in the field

Both captive and wild populations were used in this experiment to test for oviposition preference in their natural habitat. It was not possible to directly compare the captive and wild populations, as translocation of individuals to and from the Netherlands was not permitted at the time. Two locations were therefore used; 1. Woodwalton Fen National Nature Reserve, Cambridgeshire, UK, and 2. The Weerribben National Park in Overijssel, the Netherlands, and two field experiments undertaken in July 1999 during the natural flight period of the butterfly.

At Woodwalton Fen, a fenland habitat was chosen within compartment 39. Historically, *R. hydrolapathum* has been planted in this compartment to supplement naturally growing plants during the period when a *L. d. batavus* population was maintained on the fen (Duffey, 1968). However, in recent years this planting ceased with the extinction of the *L. d. batavus* population and the number of plants has fallen (A. Bowley, pers. comm.). Although *R. crispus* and to a lesser extent *R. obtusifolius* grow naturally within the fen boundary on disturbed, mown and grazed areas and on agricultural land outside the fen, they rarely occur within natural fen vegetation. Therefore, greenhouse-grown potted plants of each species were placed (pots buried to ground level) alongside the previously planted *R. hydrolapathum*. Within each 2m² plot, 5 species of each plant were used (25 in total). Captive bred butterflies, 8 females and 5 males were released onto the fen and 14 days later the plants were searched and any eggs found were recorded.

In the Weerribben National Park, three areas within the reserve were identified as suitable experimental sites due to eggs, larvae or adults being recorded there during the previous season. Compartment 78 is a fen meadow, which is cut annually during the summer. One plot was chosen along the waterside with five naturally growing *R. hydrolapathum* *in situ* and five each of *R. crispus* and *R. obtusifolius* were transplanted. Compartment 67 is a fen meadow that had already been cut (2nd week July). The experimental site was located along the central overgrown ditch and five *R. hydrolapathum* plants growing naturally amongst other vegetation were selected and five each of *R. crispus* and *R. obtusifolius* were transplanted. Compartment 86 contains a tall fen community characterised by *Phragmites australis*, *Juncus* spp and some *Betula* and *Alnus* scrub. *R. hydrolapathum* grows abundantly within the reed bed often in quite large clumps. Three experimental plots were sited in this area as eggs had already been recorded for 1999. Five *R. hydrolapathum* were selected and five each of *R. crispus* and *R. obtusifolius* were transplanted in each plot. As *R. crispus* and *R. obtusifolius* do occur within the reserve along roadsides and in grazed compartments it was possible to pot and translocate them to the chosen fenland sites for replanting.

RESULTS

Comparison of larval growth and development on different *Rumex* hosts

All larvae in the early instar experiment, with the exception of two from the *R. obtusifolius* treatment and one from the *R. crispus* treatment, commenced feeding and successfully moulted to the 2nd instar. There was no significant difference in early instar larval survival between the three plant species ($G = 4.54$, d.f. = 2, $P > 0.05$).

The growth and development of larvae from hatching to adults on the three *Rumex* species, showed no significant difference between treatments in the length of the larval stage (ANOVA: $F_{2,57} = 2.56$, $P = > 0.05$) (Table 1). Pupal and adult weights were very similar with no significant difference between the treatments (ANOVA: $F_{2,56} = 0.37$, $P = > 0.05$ and ANOVA: $F_{2,57} = 1.82$, $P = > 0.05$ respectively).

Feeding efficiencies of larvae on different species of *Rumex*

All larvae reached a similar dry weight at pupation regardless of which treatment they received (Table 2a). Using ANOVA followed by Tukey's test for pairwise comparisons of means, showed there was no significant difference between the groups in the dry weight (DWG) gained over the experiment (ANOVA: $F_{2,27} = 1.34$, $P > 0.05$). However, significant differences were found within the nutritional indices tested (Tables 2a & 2b) suggesting that the host-plants varied in their nutritional value.

The *R. crispus* group had significantly higher ECI values than either *R. obtusifolius* or *R. hydrolapathum* (ANOVA: $F_{2,27} = 26.13$, $P < 0.001$) indicating that they were more efficient at the conversion of ingested food to biomass (Table 2b). The *R. obtusifolius* group had significantly higher AD values than either of the other two groups (ANOVA: $F_{2,27} = 8.08$, $P < 0.01$) indicating that a higher proportion of *R. obtusifolius* was digested, often indicative of a more nutritious food. No significant difference existed between *R. crispus* and *R. hydrolapathum* groups (Table 2b). *R. crispus*-fed larvae had a significantly higher ECD than either *R. obtusifolius* or *R. hydrolapathum* groups (ANOVA: $F_{2,27} = 5.81$, $P < 0.01$). Despite the fact that *R. obtusifolius*-fed larvae had the highest AD, they actually had the lowest ECD values, which suggests that this larval group were apparently not as efficient in turning digested food into biomass (Table 2b).

The relative consumption rate (RCR) differed significantly between all three groups (ANOVA: $F_{2,27} = 20.9$, P

TABLE 2. Feeding and growth measurements of *Lycaena dispar batavus* larvae feeding on each of three alternative *Rumex* species over the 4th instar. Means with the same letter were not significantly different from each other (n = 30).

a	<i>R. hydrolapathum</i>		<i>R. obtusifolius</i>		<i>R. crispus</i>		F _(2,27)	Significance
	Mean (mg)	(SE)	Mean (mg)	(SE)	Mean (mg)	(SE)		
FC	0.250 a	0.011	0.224 a	0.009	0.163 b	0.008	23.0	P < 0.001
DWG	0.039 a	0.002	0.038 a	0.002	0.042 a	0.001	1.340	P > 0.05
FP	0.187 b	0.008	0.134 a	0.004	0.115 a	0.004	46.52	P < 0.001
b	<i>R. hydrolapathum</i>		<i>R. obtusifolius</i>		<i>R. crispus</i>		F _(2,27)	Significance
	Mean (mg)	(SE)	Mean (mg)	(SE)	Mean (mg)	(SE)		
ECI	15.58 a	0.97	17.64 a	0.96	25.91 b	1.26	26.13	P < 0.001
AD	24.84 a	2.25	37.80 b	1.74	28.61 a	2.90	8.08	P = 0.002
ECD	67.53 a	9.23	47.48 a	4.39	88.35 b	10.74	5.81	P = 0.008
RCR	2.70 a	0.12	2.29 b	0.10	1.76 c	0.09	20.9	P < 0.001

< 0.001) with *R. crispus* larvae having the lowest daily consumption rate as a proportion of body weight, and the *R. hydrolapathum* larvae having the highest rate (Table 2b). The *R. crispus* group consumed, in terms of quantity, significantly less food (FC) over the duration of the experiment than either of the other two groups (ANOVA: F_{F2,27} = 23.0, P < 0.001), which were not significantly different from each other.

Host plant choice by larvae

Using G-test for a one-way classification of frequencies (Fowler & Cohen, 1990), at each of the four time periods, *R. hydrolapathum* had significantly less feeding damage than *R. obtusifolius* and *R. crispus*. There was no significant difference between *R. crispus* and *R. obtusifolius*, at each time period (Table 3). In assessing the number of discs eaten of each species, significantly fewer *R. hydrolapathum* discs (only 10%) were damaged compared to *R. crispus* (70%) and *R. obtusifolius* (63%), (G = 17.58, d.f. = 2, P < 0.01).

Oviposition preference on alternative *Rumex* species

Chi Square analysis showed that females exhibited no significant preference for any species of *Rumex* ($\chi^2 = 3.8$, d.f. = 2, P > 0.05) suggesting that all three species were equally acceptable as host plants (Table 4). Of the 31 females, 23 laid eggs on more than one species. Eleven females laid on two species and 12 on all three species. Of the eight females that laid on only one species, six chose *R. obtusifolius*.

Pooling all females, there was a significant difference in the median number of eggs laid on each plant species (H = 11.63, d.f. = 2, P < 0.01, Kruskal Wallis Test). *R. obtusifolius* received a significantly higher number of

eggs (694), with each female laying an average of 22.4 eggs per plant, than *R. crispus* (438); 14.1 eggs per plant and *R. hydrolapathum* (381); 12.3 eggs per plant.

Do females discriminate between *Rumex* species?

None of the eight females showed any discrimination between the different *Rumex* species prior to an oviposition event. Each female oviposited on the first plant she landed on, even if she had laid eggs on a different species previously, indicating that all three species of *Rumex* are acceptable as host plants in this situation. The numbers of eggs laid varied between plants, as in the above experiment, but was not significant (H = 3.99, d.f. = 2, P.0.05, Kruskal Wallis Test). *R. obtusifolius* received larger egg loads (between 5 & 35 eggs) than *R. crispus* (between 9 & 14 eggs) and *R. hydrolapathum* (between 1 & 6 eggs).

Oviposition preferences in the field

In natural field conditions at Woodwalton Fen and in the Weerribben, eggs were found only on *R. hydrolapathum* plants. A total of 30 eggs were found on 10 out of 25 experimental plants at Woodwalton Fen and a total of 5 eggs were found on 4 out of 25 plants in the Weerribben.

DISCUSSION

Performance of larvae on alternative species of *Rumex*

The fact that newly hatched larvae readily accepted all three plant species and commenced feeding indicates that the three *Rumex* species all contain the appropriate feeding stimulants. This is to be expected as Whittaker & Feeny (1971) state that related plant species typically contain similar chemical profiles. But there is evidence that some small larvae of other species can, in certain

TABLE 3. Feeding preference of early instar *L. d. batavus* larvae among three alternative *Rumex* species.

Time Period	Number of larvae feeding on each <i>Rumex</i> species at each time Period				Significance
	<i>R. hydrol</i>	<i>R. obtusi</i>	<i>R. crispus</i>	G	
2 hours (n=32)	2	17	13	14.394	P < 0.01
2 hours (n=32)	2	14	11	10.51	P < 0.01
6 hours (n=25)	1	16	8	15.98	P < 0.01
Overnight (n=20)	1	11	8	9.85	P < 0.01

TABLE 4. Oviposition preference of *Lycaena dispar batavus* among three alternative *Rumex* species in caged conditions over a 24 hour period.

Test	Chi Sq	df	P. Value
RC v RO v RH	3.8	2	> 0.05
RC v RO	2.9	1	> 0.05
RC v RH	0.06	1	> 0.05
RO v RH	2.14	1	> 0.05

instances, be unable to detoxify high concentrations of allelochemicals, that present no problem to older larvae (Chew, 1980).

As larval survival rates were similar between the plant species, it is unlikely that interspecific differences in allelochemical concentrations between the plants have any effect on the larvae. Secondary plant metabolites can affect a larva's performance on its host plant and the Polygonaceae family, to which *Rumex* belongs, has high levels of oxalates which can act as a feeding deterrent to many herbivores (Libert & Franceschi, 1987). Hatcher et al. (1995) found that *R. crispus* had 20–30% higher oxalate concentrations than *R. obtusifolius* (unfortunately, no comparative information is available for *R. hydrolapathum*). As final instar *L. d. batavus* larvae are able to feed on and utilise *R. crispus* more efficiently than *R. obtusifolius*, it would appear that oxalate concentration has little or no harmful effect on *L. d. batavus* larvae and that allelochemicals are not the reason for other *Rumex* species not being used as host plants. As larvae from all three plants survived through to pupation and adulthood it would appear that non-host *Rumex* spp. are able to support larvae successfully to maturity. The significant differences in the ability of the larvae to utilise the different plants efficiently suggest that there are some intrinsic differences between the plant species.

The significantly higher ECI achieved by larvae fed on *R. crispus* suggests that this food plant, although not a natural host-plant for *L. d. batavus*, is utilised more efficiently by the larvae than their natural host plant *R. hydrolapathum*. This is in contrast to a statement by Waldbauer (1968) which suggests that “an insect may utilise a food that it does not normally eat, far less efficiently than its natural food.”

The relative consumption rate (RCR), food consumed (FC) and frass produced (FP) by larvae feeding on *R. crispus* and *R. obtusifolius* was lower than for those feeding on *R. hydrolapathum* and a lower intake of food is usually thought to be indicative of a higher efficiency of utilisation. These indices, together with AD, are often linked to a plant's nutritional status. Mattson (1980) suggests that, in most cases, AD is positively correlated with the nitrogen content of a plant. The fact that *R. obtusifolius* had a significantly higher AD in this study, could indicate that it has the highest nitrogen content of the three plant species. This is in agreement with the findings of Hatcher et al. (1995) from their study of the fungus *Uromyces* attacking *R. obtusifolius* and *R. crispus*. However, despite *R. obtusifolius* having the highest AD it had the lowest ECD of the three groups, indicating that larvae feeding on it are less efficient at the conversion of digested food to body mass.

The dry weight gained (DWG) by the larvae during the experiment was similar in all three groups, which suggests that the larvae are compensating for nutritional differences between the plants by changing consumption rate and staying on their developmental track. It is generally accepted that low dietary protein can cause an increase in the rate at which a larva feeds (Rausher, 1981;

Slansky, 1993) and similarly, a high protein diet can reduce feeding rates (Mattson, 1980).

Oviposition preference on alternative *Rumex* species

The fact that eggs were laid on all three species of *Rumex* by the captive population indicates that *R. obtusifolius* and *R. crispus* are accepted as potential food-plants by *L. d. batavus* females in this situation. A number of arguments can be used to explain the above findings. According to Singer (1982), females in cages do not display natural flight patterns and, in time, this may reduce any discriminatory behaviour. Not all females released into the cage landed immediately on a food-plant, many rested on the side of the cage first and the length of time they remained there was dependent on within-cage temperature and the amount of sunshine. *L. d. batavus* become active at temperatures above 28°C (Bink, 1962), a temperature easily attained in a greenhouse, but they also need sunshine to lay large numbers of eggs. In the wild, female butterflies would use both long and short range cues (e.g. visual and olfactory) to locate a food-plant. In caged conditions the small flight area dispenses with the need to use long range search cues. Once on a potential host, females were observed using tactile mechanisms, e.g. tapping the leaf surface with their antennae and “tasting” with their proboscis. In the second oviposition experiment females tended to lay a batch of eggs on the first plant they landed on suggesting that there is no short-range discrimination.

Oviposition preference in natural habitat

In the wild, females from both populations failed to utilise either *R. obtusifolius* or *R. crispus*, despite both being available. It appears that there is discrimination occurring in the field that is not apparent in captive conditions. This suggests that there may be an interaction between habitat choice and foodplant choice that results in such host plant specialisation. However, there are some limitations to our experiments. In the Woodwalton Fen experiment, the use of greenhouse grown *R. crispus* and *R. obtusifolius* could have had an effect on food plant choice as they may have been better (or poorer) quality host plants than naturally growing plants. In the Weerribben experiment, the disturbance caused by translocating plants, may have altered both their suitability and their exposure or apparency to searching females, although in the latter case, *R. hydrolapathum* plants were similarly affected. More studies are needed in this area. In the case of *Palaechrysophanus (Lycaena) hippothoe* ovipositing on *Rumex acetosa*, the butterfly appears to locate the host plant by their inflorescences which are much taller than the leaves (Wiklund, 1984). However, this does not appear to be the case with *L. d. batavus* as two studies have shown no significant preferences for oviposition on flowering versus non-flowering *R. hydrolapathum* (Nicholls, 2000; Webb & Pullin, 2000).

Habitat specialisation

If it is not the foodplant that confines *L. d. batavus* to fenland habitat then what is it? There are several possible explanations. Specialising on a specific habitat reduces

searching time (Courtney, 1984). If *L. d. batavus* only searches in a fenland habitat, and if searching relies initially on visual cues, females may form images/patterns that preclude other habitats. According to Rausher (1978) leaf shape can be used by butterflies to distinguish plant species and, in a fenland habitat, *R. hydrolapathum* has a broader leaf shape than its surrounding vegetation. Also, females searching in tall vegetation may not search foliage below a threshold height, for example, Van Tweel (1995) found that vegetation cover at 20 cm height was a good indicator for the presence of eggs or larvae of *L. d. batavus*. So, although *R. obtusifolius* and *R. crispus* are available within the Weerribben National Park on road sides, disturbed areas and on surrounding agricultural land which is grazed and cut regularly, females may not search these localities and so will not discover them. In this case it may be argued that *L. d. batavus* has undergone habitat specialisation rather than host plant specialisation.

Evidence from our experiments suggests that *L. d. batavus* is confined to fenland habitat for reasons other than the restriction related to the nutritional suitability of alternative host plants used by other subspecies. An alternative explanation is that each plant species may differ in its ability to provide suitable protection for larvae against the hostilities of their environment at one or more of their life stages (Martin & Pullin, 2004). It appears likely that confinement to fenlands occurred first, and specialisation on *R. hydrolapathum* has been a consequence of its relative abundance in this habitat compared with other *Rumex* species. The subsequent decline of fenland habitats has sentenced this subspecies to a period of rarity and vulnerability from which it cannot escape unless there is large scale fenland restoration.

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