

**DIFFERENCES IN SEARCH BEHAVIOUR IN LARVAE OF THE
TWO MADEIRAN SPECKLED WOOD BUTTERFLIES,
PARARGE AEGERIA AND *PARARGE XIPHIA*
(LEPIDOPTERA: SATYRINAE),
IMPLICATIONS FOR INTERSPECIFIC COMPETITION?**

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With 1 figure and 1 table

ABSTRACT. Two species of speckled wood butterfly on Madeira, *Pararge aegeria* (L., 1758), a recent colonist, and the endemic *P. xiphia* (F., 1775), may compete for resources during their larval stages. This competition may induce *P. aegeria* and *P. xiphia* larvae to disperse to new host-plants. Laboratory experiments were performed to determine how successful the larvae of both species were at locating new hosts. *Pararge xiphia* larvae spent significantly longer searching for plants, and were also significantly less likely to locate plants than *P. aegeria* larvae. This may indicate that *P. aegeria* larvae have a competitive advantage when locating new host-plants.

A detailed analysis of *P. aegeria* larval search behaviour suggested that they are adapted for local searching. Age was not found to be a significant factor in search time, but starvation significantly decreased the time taken to find host-plants. These results suggest that *P. aegeria* are adapted for dispersal and can forage efficiently in areas where their host-plant (*Brachypodium sylvaticum*) is locally dense.

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RESUMO. Durante a fase larvar, duas espécies de borboletas da Madeira, *Pararge aegeria*, uma recente colonização, e *P. xiphia*, endémica, podem competir pelo alimento. Esta competição pode levar a que ambas as espécies procurem novas plantas-hospedeiro. Foram realizadas experiências em laboratório com objectivo de determinar quão bem sucedidas são as larvas destas espécies na localização de novas plantas-hospedeiro. As larvas de *P. xiphia* demoraram significativamente mais tempo e foram significativamente menos bem sucedidas na procura de plantas do que as larvas de *P. aegeria*. Estes resultados parecem indicar que as larvas de *P. aegeria* possuem uma vantagem competitiva na localização de novas plantas-hospedeiro. Uma análise detalhada do comportamento de busca das larvas de *P. aegeria* parece indicar que elas estão adaptadas para a busca local. A idade parece não constituir um factor significativo no tempo de busca, ao contrário do jejum, o qual diminuiu significativamente o tempo para encontrar as plantas-hospedeiro. Estes resultados parecem sugerir que as larvas de *P. aegeria* estão adaptadas para a dispersão e podem alimentar-se eficientemente em áreas onde a sua planta-hospedeiro (*Brachypodium sylvaticum*) é localmente densa.

INTRODUCTION

Madeira is the only place in the world where two species of speckled wood butterfly (Lepidoptera: Satyrinae; *Pararge*) are sympatric (OWEN *et al.*, 1986). One of these, *Pararge xiphia* (F., 1775), is endemic to Madeira, the other *P. aegeria* (L., 1758), is a European mainland species and was first recorded on the island in 1976 (HIGGINS, 1977). Both species are now widespread and common on Madeira (SHREEVE & SMITH, 1992; SHREEVE *et al.*, 1992; GOTTHARD *et al.*, 1999), and have overlapping ranges (SALMON & WAKEHAM-DAWSON, 1999; SWASH & ASKEW, 1981). Both species use the same host-plants and have overlapping life histories (FERNÁNDEZ-RUBIO & GARCÍA-BARROS, 1995; SALMON & WAKEHAM-DAWSON, 1999; JONES *et al.*, 1998; OWEN *et al.*, 1986), and it is possible that *Pararge aegeria* and *P. xiphia* compete for access to resources.

Competitive interactions can play an important part in the dynamics of insect herbivore communities (FISHER *et al.*, 2000), and recent sympatry of the two *Pararge* species offers an almost unique opportunity to critically examine the importance of interspecific competition. The larvae of both species are known to feed on the same food plants in Madeira (FERNÁNDEZ-RUBIO & GARCÍA-BARROS, 1995), and

females of both species have been observed using the same oviposition sites in some areas (SALMON & WAKEHAM-DAWSON, 1999; JONES *et al.*, 1998; OWEN *et al.*, 1986). JONES *et al.* (1998) observed that although the favoured food plant (*Brachypodium sylvaticum*, Beauv., 1762) was very widespread and abundant, the eggs laid by the two species were not uniformly distributed; *P.aegeria* eggs greatly out-numbered those of *P.xiphia* and eggs of both species were seen on the same blade of grass. Upon hatching, *P.xiphia* larvae are almost twice the size of *P.aegeria* but the development time to pupation occurs at a much slower rate for *P.xiphia* (GIBBS, M., unpublished data).

Dispersal during the larval stage, when larvae are crowded, may help avoid periods of food shortages (ERELLI & ELKINTON, 2000). It is possible therefore, that competition during larval development may stimulate *P. aegeria* and *P. xiphia* larvae to disperse to new host-plants. The timing of this dispersal may prove critical in ensuring their survival. CAIN *et al.* (1985) found that early instar larvae of *Pieris rapae* (L., 1758) were less mobile than later instar larvae, and in the three butterfly species that were examined by JONES (1977), larger larvae travelled faster than small larvae. The success rate of plant location was also greater for larger larvae, particularly if the larvae were starved before searching commenced (JONES, 1977). Therefore, during dispersal to a new host-plant, early instar larvae may be at a disadvantage and have increased vulnerability to ground-dwelling predators (DETHIER, 1959; NICHOLLS & JAMES, 1996).

The dispersal behaviour of *P. xiphia* and *P. aegeria* will be examined experimentally, and the results obtained will be used to predict the outcome of any larval competition that may occur between these two species.

METHODS

Experimental design

To monitor how successful each species was at locating food, we adapted a method described by CAIN *et al.* (1985) carried out under laboratory conditions. On the laboratory floor 25 potted plants of *Brachypodium sylvaticum* were uniformly arranged in five rows to create 16 cells of 0.5 x 0.5 m. Temperature has been shown to affect the searching behaviour of larvae (JONES, 1977). Therefore a temperature of 21 +/- 3° C and a humidity of 55 +/- 10% was maintained throughout the experiment. The light intensity was maintained at 700 Lux, and the laboratory floor provided a uniformly smooth, even surface for the searching larvae. One larva was placed at the centre of each cell so that it was equidistant from each plant. A 1 hour time limit was imposed on searching and larvae that failed to find a plant, or left the area were denoted as failures (as in CAIN *et al.*, 1985).

Comparing the search success of *P. aegeria* and *P. xiphia* larvae

A total of 95 *P. aegeria* larvae, from instars II-IV were observed, and the time taken for each larva to find a plant was recorded. These data were used to determine the effect of age (and hence size) on *P. aegeria* search success. Larvae from instars II and III were classed as young (and small, with weights between 0.03 g and 0.07 g) larvae, and those from instars IV and V were classed as old (large, with weights above 0.07 g) larvae. Additionally, these data were also used to estimate the appropriate numbers of sampling units (*i. e.* number of larvae) required to detect an observable difference in larval search time at a 5% confidence level (for details of this analysis see JONES *et al.*, 1998). This analysis was performed to reduce the number of animals used in the following experimental procedures, and to avoid stressing animals unnecessarily. An estimate of 20 larvae was determined as appropriate.

To compare the search success of *P. aegeria* and *P. xiphia* larvae the experiment described previously was repeated for 21 *P. xiphia* larvae. The *P. xiphia* larvae were weighed and those with weights between 0.04 g and 0.1 g were determined as being from instars II and III. The search success of these larvae was compared with that of *P. aegeria* larvae also from instars II and III (*e. g.* with weights between 0.03 g and 0.07 g).

Pararge xiphia larvae proved to be difficult to rear under laboratory conditions and only 21 larvae were available at the time of these experiments. Thus, further experiments with *P. xiphia* were not possible and the following procedures were performed with only *P. aegeria* larvae.

Detailed analysis of the search behaviour of *P. aegeria* larvae

Using a method similar to that outlined above, a detailed examination of the searching behaviour of an additional 25 unstarved *P. aegeria* larvae was recorded. An experimental plot was set-up as described previously but no plants were placed into the area. The larvae were positioned into the sub-plots as described above. The positions of the larvae were recorded at 5 minute intervals using numbered discs. The markers were placed at the end of the larva's anal claspers, and the larvae were followed around the area for 1 hour. The markers were used to map the movements of the larvae and to record the distance and direction travelled during each 5 minute time interval.

Effect of starvation on *P. aegeria* larval searching success

An experimental plot was set-up as described previously (see Experimental design). Prior to their release into the search arena, 20 *P. aegeria* larvae were starved overnight for a period of approximately 12 hours. The larvae were then released into the experimental plot and allowed to search for a host-plant. The time taken for each

larva to find a plant was recorded. Additionally, whether the larva started feeding immediately on reaching a plant was also recorded.

Statistical analyses

Two-way analysis of variance was used to investigate the effects of starvation, age and species on larval search time. Data on search time were log transformed to meet assumptions of normality. A Pearson chi-square test was used to compare the proportion of larvae that found a host-plant, and to determine whether there were species differences, or any effect of starvation, on host-plant location. All tests were two-tailed. The statistical procedures were performed using Systat 9.0.

RESULTS

Comparing the search success of *P. aegeria* and *P. xiphia* larvae

Pararge xiphia larvae ($\bar{x} = 30.1 \pm 1.02$ min, $n = 8$) spent significantly longer time searching for plants than *P. aegeria* larvae ($\bar{x} = 17.0 \pm 1.15$ min, $n = 16$; $F = 5.368$, d. f. = 1, $P = 0.030$). There were also significant species differences in plant finding ability, with a significantly larger proportion of *P. xiphia* larvae (9/20) failing to locate a plant compared to *P. aegeria* (16/99; $\chi^2 = 8.090$, d. f. = 1, $P = 0.004$).

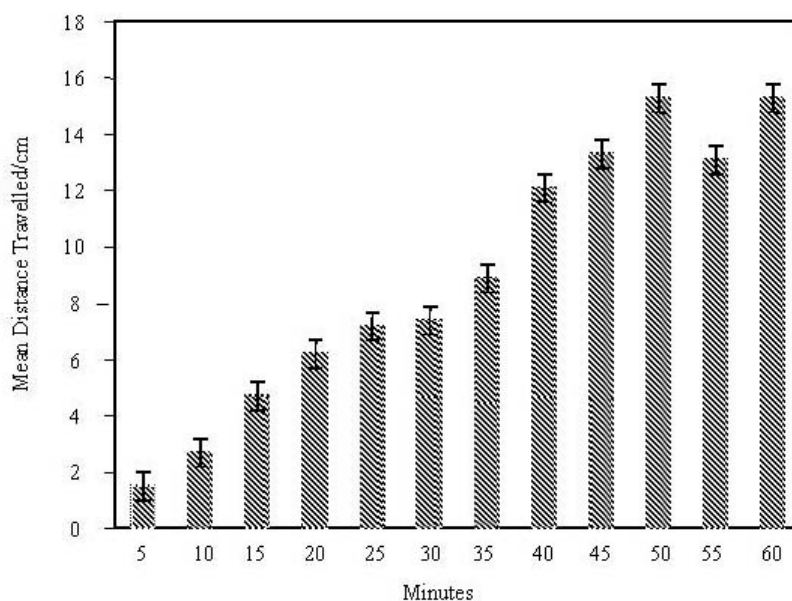


Fig. 1 - Mean distance travelled (cm) per five minute time interval by *P. aegeria* larvae.

Detailed analysis of the search behaviour of *P. aegeria* larvae

The larvae turned frequently, with individuals having a turn bias (*i. e.* with a larger proportion of their total turns) towards either the left or the right, resulting in larvae searching in a circular motion. Overall, larvae did not show a turn bias towards any one direction, as 42% of larvae had a turn bias towards the right and 54% towards the left ($n = 24$).

As the recording period progressed larvae tended to turn less and their movements became straighter and faster. The mean distance travelled per 5 minute time increment increased in a linear fashion over time, with the largest increase in distance travelled occurring after 35 minutes of searching (Fig. 1). Thus, as larvae travelled, their rate of movement increased. Only one larva failed to move for the entirety of the recording period. Scanning for a resource was observed, but was not quantified, scanning took the form of head waving (as described by JONES, 1977), and only occurred when the larvae were stationary (saltatory searching, BELL, 1990).

TABLE 1 - The effect of age and starvation on *P. aegeria* larval search time

Category	Mean time (\pm SE)/ mins	n
Old and Starved	9.87 (1.26)	6
Old and unstarved	18.14 (1.12)	26
Young and starved	9.88 (1.23)	8
Young and unstarved	27.19 (1.21)	9

Effect of larval age and starvation on *P. aegeria* search success

Starved larvae had a mean search time of 9.88 ± 1.15 min ($n = 14$) and unstarved larvae had a mean search time of 19.11 ± 1.06 min ($n = 81$), and this difference was highly significant (ANOVA, $F = 18.055$, d. f. = 1, $P < 0.001$). Food deprivation, however did not increase the likelihood of a larvae finding a plant, 14/17 of the starved larvae and 83/99 of the unstarved larvae were successful at finding a new plant ($\chi^2 = 0.001$, d. f. = 1, $P = 0.976$). Of the larvae that began to feed immediately on reaching a host-plant, 3/43 were from the unstarved group and 2/13 were from the starved group.

Age was not a significant factor in search time (Table 1). ANOVA of search time indicated that older larvae did not reach plants quicker than young

larvae ($F = 1.13$, d. f. = 1, $P = 0.293$). There was not a significant age x starvation interaction ($F = 1.12$, d. f. = 1, $P = 0.296$).

DISCUSSION

BELL *et al.* (1985) suggested that food deprivation increases an animal's responsiveness to resource-related cues. Our results support this hypothesis in part. *Pararge aegeria* larvae that were deprived of food found host-plants significantly faster than non-starved larvae, but equivalent numbers of larvae from each group were successful at locating food. Therefore, starvation would appear to increase the responsiveness of *P. aegeria* larvae without improving their chance of host location. Starved larvae were twice as likely to begin feeding immediately on reaching a host-plant than non-starved larvae. Overall, however, small percentages of larvae actually began to feed on reaching a plant. This may be due to the need for a rest period to allow recovery after searching, or may indicate that a larva's need for camouflage is a large motivational force behind host-plant location.

As a result of competitive interactions it is possible that larvae may experience food shortage/deprivation prior to their dispersal. *Pararge aegeria* larvae, however, will not be disadvantaged if such a situation were to arise because starvation appears to increase their motivation for host location. Age-related differences in searching success were not observed for *P. aegeria* larvae, suggesting that old and young larvae are equally successful at locating new plants, this may be expected, however, as there is the potential for food to become depleted at any time during larval development. These results would suggest that *P. aegeria* larvae are adapted for dispersal. The high success rate of *P. aegeria* larval dispersal may partly be explained by adult ecology. *Pararge aegeria* females oviposit on small *Brachypodium sylvaticum* plants in areas where the host-plant is locally dense (GIBBS, M., *unpublished data*). Given that *P. aegeria* larvae search intensively, in a manner characteristic of that described by BELL (1990) and STANTON (1986) for animals that restrict their foraging to locally dense areas, oviposition on small plants in dense areas will increase the amount of food available to larvae by facilitating dispersal. LAWRENCE (1990) suggested that local movement of larvae reduced predation and resulted in higher survival rates because moving groups had fewer predators. It is possible, therefore, that the oviposition behaviour of females has evolved to 'encourage' local larval dispersal to increase the survival of *P. aegeria* larvae.

Larger larvae have a longer stride than small larvae and are therefore able to move faster (REAVEY, 1993). *Pararge xiphia* larvae have a larger body size (at all instars) than *P. aegeria*, it could be expected, therefore, that they would move quicker. However, *P. xiphia* larvae both took significantly longer to locate a new host-plant, and were less successful at finding plants than *P. aegeria* larvae. It is possible that *P. xiphia* larvae have different periods of inactivity than *P. aegeria* larvae, and they may not forage

during these times even if they perceive resource-specific cues. They may also have different search tactics. Some large caterpillars do not move if it increases their vulnerability to predators (REAVEY, 1993). Differences in the visual and olfactory sensitivity of *P. xiphia* and *P. aegeria* larvae could account for the differences in their searching success. *Pararge xiphia* larvae may be more dependent on environmental cues (*e. g.* orientation of the sun) than *P. aegeria* larvae, and the artificial conditions of the laboratory may have hindered their searching.

Pararge xiphia larvae are larger than *P. aegeria* larvae (FERNÁNDEZ-RUBIO & GARCÍA-BARROS, 1995), and there may be less urgency for these larvae to find a new plant since their large size decreases their physiological vulnerability (*e. g.* they are less vulnerable to starvation because they have larger energy reserves and lower metabolic rates (ALJETLAWI & LEONARDSSON, 2003; REAVEY, 1993). However, the risks from predation will still exist for *P. xiphia* larvae. Longer search times increases the vulnerability of larvae to predation (DETHIER, 1959; NICHOLLS & JAMES, 1996), indicating that *P. xiphia* larvae may be more vulnerable than *P. aegeria* larvae. SOKOLOWSKI (1985) found that for *Drosophila melanogaster* (MEIGEN, 1830) intraspecific variation in larval foraging behaviour was under genetic control. Genetic differences may, therefore, also be responsible for the intra- and interspecific variation in foraging behaviour observed for *P. aegeria* and *P. xiphia* larvae.

Adult ecology may also help to explain why *P. xiphia* are less successful at locating new food plants. Female *P. xiphia* lay fewer eggs (than *P. aegeria*) and distribute them more widely over host-plants (M. JONES, *pers. com.*), suggesting that larval competition may have occurred less frequently for this species (before the colonisation of *P. aegeria*) and therefore, the larvae may not be adapted for dispersal. This suggests that larval displacement due to interspecific competition for food may be more detrimental to *P. xiphia* larvae than *P. aegeria* larvae.

Differences in the larval searching success of *P. aegeria* and *P. xiphia* larvae may indicate that the recent colonists (*P. aegeria*) are at a competitive advantage during host-location. Such a situation would be predicted by the Taxon cycle hypothesis (WILSON, 1959, 1961; RICKLEFS & COX, 1972, 1978), which suggests that recent colonists are at a competitive advantage (JONES *et al.*, 1998). Therefore, further work would be valuable to assess the impact of interspecific competition on the *P. aegeria* and *P. xiphia* ecology.

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