

Does predation maintain eyespot plasticity in *Bicyclus anynana*?

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The butterfly *Bicyclus anynana* exhibits phenotypic plasticity involving the wet-season phenotype, which possesses marginal eyespots on the ventral surface of the wings, and the dry-season form, which lacks these eyespots. We examined the adaptive value of phenotypic plasticity of *B. anynana* in relation to the defence mechanisms of crypsis and deflection. We assessed the visibility differences between spotless and spotted butterflies against backgrounds of brown (dry season) or green (wet season) leaves. Spotless butterflies were highly cryptic and less predated by adult bird predators than were spotted ones when presented against brown leaf litter. However, the advantage of crypsis disappeared in the wet-season habitat as both forms were equally visible. In later experiments, naive birds presented with resting butterflies in the wet-season habitat tended to learn more rapidly to capture spotless butterflies, suggesting a slight selective advantage of possessing eyespots. Moreover, marginal eyespots increased significantly the escape probability of butterflies that were attacked by naive birds compared to those attacked by adult birds, although there were no differences in prey capture success within naive predators. Our results show that natural selection acts against eyespots in the dry season, favouring crypsis, whereas in the wet season it may favour eyespots as deflective patterns.

Keywords: crypsis; defence mechanism; eyespots; naive predator; wing patterns

1. INTRODUCTION

Phenotypic plasticity is exhibited by the African satyrine *Bicyclus anynana* with two alternative seasonal forms, with or without wing eyespots, which occur in the wet and dry seasons, respectively (Brakefield & Larsen 1984; Brakefield & Reitsma 1991; Windig *et al.* 1994; figure 1). The wet-season form (WSF) has ventral eyespots positioned close to the wing margins. When at rest, the wings are kept closed with the forewings fully exposed above the hindwings such that the whole series of marginal eyespots on both forewing and hindwing are exposed. The dry-season butterflies differ in wing pattern as well as in the way resting individuals hold their wings. Eyespots are very small, or completely absent, with the exception of the more posterior part of the large forewing eyespot. A butterfly of the dry-season form (DSF) at rest withdraws the forewings slightly between the hindwings up to the midline of this forewing eyespot, which is thus effectively hidden. The resting butterfly then has a uniform brown coloration. Individuals of similar genotype (e.g. full-sibs) can develop into either of these phenotypes depending mainly on rearing temperature (Brakefield & Larsen 1984; Brakefield & Reitsma 1991), which changes between the seasons when cohorts of larvae are developing in the field (Brakefield & Mazzotta 1995). Wing colour patterns are determined in the last-instar larva and shortly after pupation (Brakefield *et al.* 1996; Kooi & Brakefield 1999), and thus, the ambient temperature is a reliable cue for the adult environment (Kooi & Brakefield 1999). This suggests that seasonal

polyphenism in *B. anynana* is an example of adaptive phenotypic plasticity (cf. Gotthard & Nylin 1995).

To be viewed as adaptive, phenotypic plasticity should confer higher relative fitness to each phenotype in the environment in which it normally occurs. It is assumed that the adaptive role of the wing-pattern polyphenism in *B. anynana* is to produce butterflies that are cryptic (uniform brown wings without eyespots) in the dry season when they rest inactively on a background of dead brown leaves, and in the wet season to produce butterflies that are active among green herbage and have marginal eyespots, which may induce predators to direct attacks to the wings rather than to other body parts, thus allowing a butterfly a better chance of escape (Blest 1957; Brakefield & Larsen 1984; Wourms & Wasserman 1985; Brakefield & Reitsma 1991). However, direct evidence for such a deflective function is weak. In addition, our recent experiment analysing attacks by lizards and adult birds did not support this deflection hypothesis (Lyytinen *et al.* 2003). That is, marginal eyespot patterns on wings did not enhance the butterfly's probability of being released alive once captured by a predator. Furthermore, experienced birds made only a few unsuccessful attacks, giving butterflies, in general, a slim chance of escaping alive. The experiment did not examine the possibility that the deflection function of eyespots might manifest itself when butterflies are attacked by a naive predator that has not yet learnt to grasp the body instead of the fragile wings. This is a specific aspect that we tested in the present study.

We also propose an alternative or complementary explanation for the phenotypic plasticity in *B. anynana*, based on the assumption that natural selection is more intense in the dry season because of a low abundance of available

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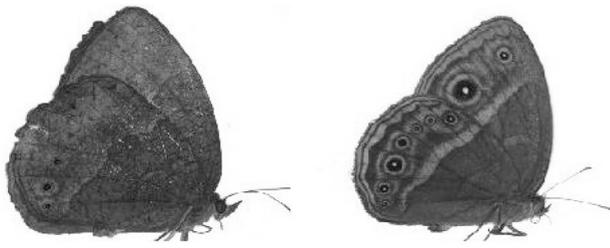


Figure 1. Spotless DSF (left) and spotted WSF (right) of *Bicyclus anynana* used in the present experiment as a model prey. WSF butterflies have a series of eyespots close to the edge of the wings, whereas DSF butterflies lack these wing patterns. Note that DSF butterflies retract their forewings partially between their hindwings when at rest so that the posterior forewing eyespot is not visible (Brakefield & Larsen 1984).

prey to insectivorous predators. There are also only low numbers of naive predators at this time of year (Dowsett & Dowsett-Lemaire 1984) with most adults being experienced in capturing butterflies and unlikely to be decoyed by eyespots (Lyytinen *et al.* 2003). Under these conditions, in the dry season it may be especially disadvantageous to possess conspicuous eyespots, with strong selection in favour of cryptic coloration minimizing the risk of visually mediated predation (Cott 1940; Endler 1978, 1983). Butterflies of the DSF must survive by relying on effective crypsis when at rest for several months before females can oviposit on grass food plants regrowing at the beginning of the next rains. By contrast, in the wet season, butterflies cannot rely on crypsis as the vegetation is green and more complex in structure, and they need to be active and to reproduce in the limited time available before the larval food plants desiccate. Thus, defence mechanisms other than background-matching coloration, including marginal eyespots, may be at a premium for these butterflies (see Brakefield 1997).

We devised an experiment to test background-matching crypsis and the effectiveness of the deflection by eyespots against inexperienced predators under laboratory conditions using *B. anynana*. Butterflies at rest were presented to birds at rest as it has been proposed that marginal eyespots (rather than those associated with startling displays; Blest 1957) are particularly favoured in butterflies when they are feeding or at rest on the ground (Young 1979).

2. MATERIAL AND METHODS

(a) *The experimental room*

All experiments were conducted in an indoor aviary (13.3 m², height of 2.5 m) at Konnevesi Research Station in central Finland. The illumination (four bulbs of Spiral light (20 W) and three True-Lite fluorescent tubes (18 W)) used during the experiment in the aviary corresponded to natural daylight.

(b) *Visibility test against dry- and wet-season backgrounds*

Adult great tits (*Parus major*) ($n = 60$) were caught with traps and housed individually in illuminated plywood cages (65 cm × 80 cm × 65 cm) where they were maintained on a diet of sunflower seeds, peanuts and water, which were available *ad*

libitum. The experiment was conducted in January–March 2002. To motivate the birds to forage, they were deprived of food for 2 h before the experiment. After the experiment all birds were ringed and released.

For the dry-season set-up, the aviary floor was covered with brown dry leaves spread over a thin layer of sand, which was partly visible. The background corresponded as closely as possible to the dry-season landscape in the butterfly's natural habitat in central Africa, where the ground is carpeted with dead foliage and bare soil (Brakefield & Reitsma 1991). We divided the aviary into four equal-sized blocks each having 15 possible sites to place a butterfly. We placed one spotless and one spotted *B. anynana* in each block (eight butterflies in total). The sites within each block used for the butterflies were chosen randomly. Each dead butterfly was glued in a normal resting attitude (see figure 1) on a dry brown leaf attached to a small piece of paper-board sunk into the sand. Great tits ($n = 30$) were released individually into the aviary and then allowed to find and eat all eight butterflies. Observations were made from behind a door with a one-way mirror. The butterfly consumed first received a score of 8, the second one a score of 7, and so on; values were then summed for spotless and for spotted butterflies. The sums reflect the relative detectability risk of the butterfly forms and were analysed as a dependent variable in sign tests.

To test whether the two *B. anynana* forms are equally visible against green leaves, we made a landscape that resembled the wet-season habitat in the same aviary. We used potted plants because the natural wet-season habitat of *B. anynana* consists of standing mixed herbage rather than of a carpet of green leaves or grass. The area of resting background was equivalent to the brown background in the dry-season environment. The floor of the aviary was covered with sand and potted plants, *Philodendron scandens*, placed in five rows, each with four plants. Thus, in each of four blocks there were five plants out of which two were assigned randomly to the experiment. For a new set of great tits ($n = 30$), the one DSF and one WSF butterfly in resting positions (heads up) were placed individually on randomly chosen green leaves of *P. scandens* within each block (eight butterflies in total), again selected at random. The consumption of butterflies was ranked in the same way as explained above.

To study qualitatively the relative crypsis of butterflies on the two backgrounds, we measured reflection spectra of wings and leaf backgrounds with a USB2000 spectrometer. Reflection spectra of the left hindwing of each butterfly form ($n = 3$) were recorded twice. The light source was a pulsed xenon light PX-2 (Ocean Optics, Inc.). Reflectance is given in proportion to that of a white standard (Labsphere Inc.). Three green and three brown leaves were each measured once.

(c) *Testing the deflection hypothesis with naive birds*

Nestlings of pied flycatchers (*Ficedula hypoleuca*) from five nests were captured in Konnevesi, central Finland, and transferred with their parents to outdoor cages (floor area of 3 m × 3 m, height of 2 m) where they were fed with mealworms (*Tenebrio molitor*). Other insects were also available as the net of the cages (mesh size of 5 mm) allowed the entry of small insects but excluded butterflies. Thus fledglings had had no experience of capturing butterflies at the time of the experiment. Water was available *ad libitum*. The experiment took place in July 2002. Before the experiment, birds were deprived of food for 2 h. All birds were released back to the wild following the experiment and when they were able to forage.

We tested whether marginal eyespots are advantageous to butterflies when encountered by a naive bird. We used the same set-up as in our previous experiment with adult pied flycatchers (Lytinen *et al.* 2003) to be able to compare these two datasets. A pole (height of 103 m, diameter of 10 m) covered with green leaves of *Sorbus aucubaria* was placed in an aviary. At a distance of 2 m from the pole, a perch was available for the bird. One half of the nestlings in each brood were randomly assigned to one of the two treatments: spotless ($n = 10$) or spotted ($n = 12$) butterflies. Thus, an individual bird foraged for only one form of butterfly. Three spotless (DSF) or three spotted (WSF) *B. anynana* that were deprived of food overnight were presented to a bird by placing live butterflies sequentially on a slice of banana on the top of the pole. In this way we were able to conduct the experiment with resting butterflies as adult *B. anynana* feed on fruit. Birds were allowed to attack each butterfly until they either ate the prey or caused wing tears. The outcome of attacks (i.e. escaped with wing damage or killed) and the area of missing wing were recorded.

To test whether marginal eyespots enhance the chance of escape, the proportion of butterflies that escaped was analysed using Mann–Whitney or Fisher’s exact tests. To examine whether the extent of wing damage differed between spotless and spotted butterflies, damage scores were made for all four wings in each insect. A butterfly that escaped without wing damage after initially being caught received a score of 0. For hindwings, if the damage comprised up to 5% of the wing area, the butterfly received a score of 1 per hindwing; loss of 10% of the wing area resulted in a score of 2; 20%, score 3; 30%, score 4; 40%, score 5; 50%, score 6; and greater than 50%, score 7. Since a loss of even a small piece of forewing apparently impairs flight ability more than the equivalent missing fragment on the hindwing (Dennis *et al.* 1984), corresponding scores for forewings were multiplied by two. Those butterflies killed and eaten by a bird received a score of 8 per hindwing and 16 per forewing. Killed butterflies were included in the index because those traits that enhance the survival of the prey are under the strongest selection pressure. The scores of the four wings were summed such that the index ranged from 0 (no wing damage) to 48 (eaten). Because of the ordinal scale, non-parametric tests were used to compare the wing-damage scores between spotless and spotted individuals in three consecutive trials.

3. RESULTS

(a) *Visibility test against dry- and wet-season backgrounds*

When butterflies were presented against dead brown leaves, great tits ($n = 30$ in both treatments) consumed spotted wet-season butterflies earlier in the course of the experiment than spotless dry-season ones (sign test: $z = -2.079$, $p = 0.038$; figure 2), whereas on the green vegetation no such difference occurred (sign test: $z = -0.567$, $p = 0.571$). Since the only difference between these two butterfly forms is their eyespots, this suggests that eyespots on the wings increase conspicuousness on the brown background, spotless butterflies being highly cryptic against their semi-natural background (brown leaves). Reflectance spectra of the background brown wing colour of spotless (DSF) and spotted (WSF) butterflies matched similarly against brown leaves but were highly visible when seen against green leaves (figure 3). This also

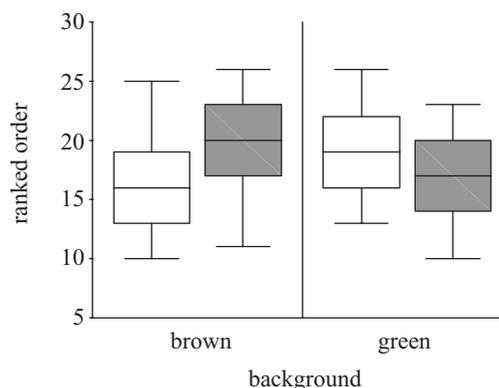


Figure 2. The summed consumption scores of butterflies with (WSF) and without (DSF) eyespots presented to birds on the brown (left) and green (right) leaf backgrounds. Each bird ($n = 30$) preyed upon four spotless (open blocks) and four spotted (shaded blocks) butterflies. The higher the rank order, the earlier a bird consumed the butterfly. A maximum of 26 would indicate that the individuals of this prey type were always taken before the alternative. Error bars show the extreme values within a prey type.

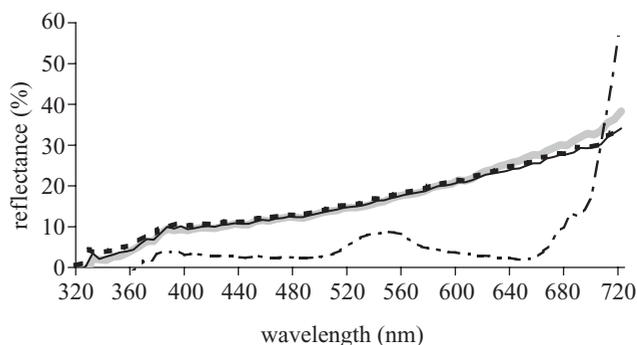


Figure 3. Mean reflectance spectra of the underside hindwings of three spotless (DSF; dashed line) and spotted (WSF; solid line) butterflies. Reflectance spectra of brown (grey line) and green (dotted and dashed line) leaves are also shown.

suggests that it is the wing eyespots that reduce the effectiveness of crypsis against brown dry leaf litter.

(b) *Testing the deflection hypothesis with naive birds*

The total number of birds that made missed attacks (i.e. a butterfly either made an evasive movement or flew off without contact) versus those that grabbed (i.e. killed or damaged) the butterfly at the first attempt did not differ between butterfly morphs in any trial (Fisher’s exact test: all p -values greater than 0.074). However, the total number of missed attacks before the one that resulted in wing tear or death of the butterfly was higher for spotless than for spotted butterflies in the first trial (Mann–Whitney test: $z = -2.172$, $p = 0.030$) but not in the later encounters (second trial: $z = -0.846$, $p = 0.398$; third trial: $z = -1.373$, $p = 0.170$). These tests indicate that the majority of the individual birds managed to obtain contact with the butterfly at the first capture attempt but those birds that missed the butterfly made several mistakes. Furthermore, the average proportion of butterflies that escaped with wing damage once captured did not differ

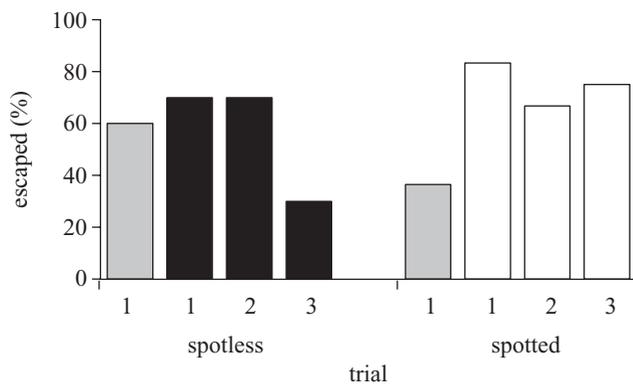


Figure 4. The proportion of butterflies grabbed by birds, but which then escaped with wing damage, in trials with adult birds (grey bars; data for first trial from Lyytinen *et al.* 2003) and with naive birds (three trials; black bars, spotless butterflies; open bars, spotted butterflies).

between the butterfly forms (spotted versus spotless) over the three trials (Mann–Whitney test: $z = -1.146$, $p = 0.252$, $n(\text{spotless}) = 10$, $n(\text{spotted}) = 12$; figure 4). There was, however, a trend towards more escapes for spotted than for spotless butterflies within the third trial. Nine out of 12 spotted butterflies survived compared with three out of 10 spotless ones. Thus, 2.5 times as many spotted butterflies escaped with wing damage as did spotless ones (Fisher's exact test: $p = 0.084$), suggesting some selective advantage to bearing eyespots. When we compare the number of escapes in the first trial with capture success by adult birds, as we reported in our previous paper (Lyytinen *et al.* 2003), additional differences are observed. Naive birds caught and ate fewer spotted butterflies than did adult birds (Fisher's exact test: $p = 0.036$; figure 4), but success rates did not differ when birds attacked spotless butterflies (Fisher's exact test: $p = 1.000$). This shows clearly that the success rate of predation depends on the experience of the predator, but only when birds attack spotted butterflies.

According to the deflection or decoying hypothesis (Blest 1957; Wourms & Wasserman 1985; Brakefield & Reitsma 1991), wing spotting should misdirect attacks by predators resulting in less severe wing damage compared with attacks on spotless butterflies. However, the degree of wing damage (i.e. wing-damage scores) did not differ between spotted and spotless butterflies (Mann–Whitney test: $z = -0.792$, $p = 0.428$) and, moreover, it did not change within the treatment (spotless or spotted) across the three trials (Friedman's test: spotless: $\chi^2 = 2.214$, d.f. = 2, $p = 0.331$; spotted: $\chi^2 = 3.767$, d.f. = 2, $p = 0.152$; figure 5). Thus, the overall results of analysis of wing damage provided no support for a confusing or misdirecting role of marginal eyespots in relation to bird attacks.

4. DISCUSSION

The spotted WSF of *B. anynana* is more prone to predation than are spotless butterflies when presented against brown leaves that mimic the dry-season habitat. Spotless DSF butterflies, in turn, closely matched the dead-leaf background in terms of reflectance patterns, and thus were effectively concealed, avoiding the attention of birds.

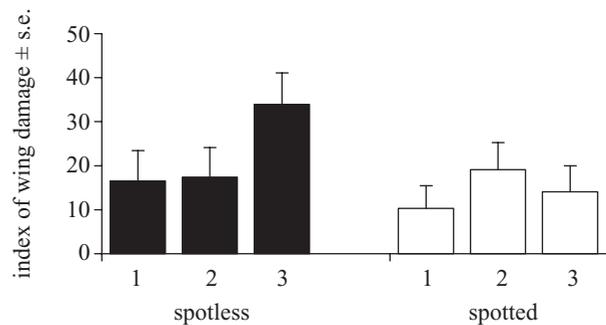


Figure 5. Scores for damage to wings resulting from attacks by naive birds. Each bird received three spotless (DSF) ($n = 10$) or three spotted (WSF) ($n = 12$) butterflies sequentially. The scores ranged from 0 (without damage) to 48 (eaten).

Since butterflies were dead and had otherwise similar brown wing coloration, we can attribute the observed difference in the visibility directly to the wing patterns. Thus, a uniform brown coloration protects a butterfly against avian predators when at rest on a brown background, such as dead leaves, which is a typical landscape in the natural dry-season habitat of *B. anynana*. It follows that in nature these butterflies with greatly reduced ventral wing markings should choose a microhabitat that matches their coloration as closely as possible to maximize their camouflage. Field observations have shown that the spotless dry-season forms of *B. safitza*, indeed, prefer to perch on brown rather than on green foliage (Brakefield & Reitsma 1991). Comparable observations have recently been made for *B. anynana* butterflies of each seasonal form in flight cages with different resting backgrounds in controlled-temperature rooms (S. M. Dijkstra and P. M. Brakefield, unpublished data).

Owing to the lack of alternative more preferred prey, the selection pressure may be more severe during the dry season than the wet season. If we assume such a difference in the intensity of selection across seasons, *B. anynana* butterflies during the dry season are likely to experience stronger selection for protective coloration (see Cott 1940; Endler 1978, 1983). It would then follow that it is relatively more important for butterflies to adapt the defence mechanism that protects them most effectively against visually hunting predators. As this experiment showed, spotless butterflies are clearly more cryptic against a dead-leaf background and, therefore, better defended than the spotted form. This result corresponds entirely with cohort analyses of *Bicychus* butterflies in the field in Malawi (N. Reitsma, unpublished data). The observations demonstrate that selection favours the evolution of effective cryptic wing coloration in the dry season.

Contrary to the results in the dry-season set-up, both butterfly forms were predated in random order when the background consisted of green plants (wet-season habitat). It is, therefore, unlikely that cryptic or disruptive coloration could explain the occurrence of spotted and the absence of spotless butterflies in the wet season. The alternative, and more usual, explanation of the phenotypic plasticity is that eyespots can decoy the attacks of birds. The average number of missed attack attempts that did not result in wing tear or death of the butterfly was higher when birds encountered spotless than spotted butterflies

for the very first time. The variation between bird individuals was huge and the occurrence of these attacks was independent of butterfly morph in the later encounters. This implies that the observed difference might be caused by a few birds that were poor at capturing prey. The proportion of attacks that resulted in wing damage over the three trials by naive birds was, however, equal in both treatments (spotless versus spotted), although there seems to be some advantage of exposed eyespots in the final trial of the experiment. That is, there was a tendency for more spotted than spotless individuals to escape once captured by a bird (cf. Wourms & Wasserman 1985). This suggests that eyespots impaired predator learning to some extent. Thus, overall the results did not strongly support a hypothesis that wing spots decoy attacking predators leading to a decreased overall probability of being instantly killed (Blest 1957; Wourms & Wasserman 1985; Brakefield & Reitsma 1991). We must interpret the results with caution since the sample size was small. In addition, the wing area loss did not differ between spotless and spotted butterflies as might be expected if eyespots directed the pecks of attacking birds towards the wing margins.

Naive pied flycatchers were, however, much less successful in catching butterflies than were adult birds. Whereas 62% and 71% of adults were successful in capturing and eating spotless and spotted butterflies, respectively (Lyytinen *et al.* 2003), naive birds killed and ate, on average, only 43% of spotless and 25% of spotted butterflies attacked. Thus, the mean success rate depends on the experience of the bird predator when birds attack spotted butterflies. A higher number of naive birds than adult individuals in the wet season (Dowsett & Dowsett-Lemaire 1984) would create the conditions favouring wing eyespots in the wet season. At least there is no selection by predators acting against spotting, as there is in the dry season, since eyespots did not increase the probability of being detected. Thus, although the anti-predatory benefit for spotted individuals is relatively small in the wet-season habitat, when the experience of predators is taken into account, spotting may be favoured because it does not involve anti-predator costs (unlike in the dry season). This again parallels a much smaller advantage to WSF butterflies over those of the DSF found in cohort analyses in the rainy season in Malawi than that which occurred in the reversed direction in the dry season (N. Reitsma, unpublished data).

The results of our experiment show one possible mechanism that can maintain the phenotypic plasticity in *B. anynana*. They clearly demonstrate the advantage of a uniform brown wing coloration in the dry season. The unmarked spotless form that occurs during the dry season appeared highly cryptic against its semi-natural background, brown dead leaf litter. It, therefore, experienced a lower predation pressure in terms of consumption order than butterflies with marginal eyespots (WSF). In the wet-season habitat, eyespots did not lead to a higher incidence of escape of live butterflies once captured by a naive bird, but there was some indication that eyespots could function effectively as decoys for naive but not for adult birds. Further work will be necessary to determine whether other advantages, such as mate choice (Breuker & Brakefield 2002), may also favour eyespots in the wet season.

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REFERENCES

- Blest, A. D. 1957 The function of eyespot patterns in the Lepidoptera. *Behaviour* **11**, 209–255.
- Brakefield, P. M. 1997 Phenotypic plasticity and fluctuating asymmetry as responses to environmental stress in the butterfly *Bicyclus anynana*. In *Environmental stress, adaptation and evolution* (ed. R. Bijlsma & V. Loeschke), pp. 65–78. Basel: Birkhäuser.
- Brakefield, P. M. & Larsen, T. B. 1984 The evolutionary significance of dry and wet season forms in some tropical butterflies. *Biol. J. Linn. Soc.* **22**, 1–12.
- Brakefield, P. M. & Mazzotta, V. 1995 Matching field and laboratory environments: effects of neglecting daily temperature variation on insect reaction norms. *J. Evol. Biol.* **8**, 559–573.
- Brakefield, P. M. & Reitsma, N. 1991 Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies. *Ecol. Entomol.* **16**, 291–303.
- Brakefield, P. M., Gates, J., Keys, D., Kesbeke, F., Wijngaarden, P. J., Monteiro, A., French, V. & Carroll, S. B. 1996 Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**, 236–242.
- Breuker, C. J. & Brakefield, P. M. 2002 Female choice depends on size but not symmetry of dorsal eyespots in the butterfly *Bicyclus anynana*. *Proc. R. Soc. Lond. B* **269**, 1233–1239. (DOI 10.1098/rspb.2002.2005.)
- Cott, H. B. 1940 *Adaptive colouration in animals*. London: Methuen.
- Dennis, R. L. H., Porter, K. & Williams, W. R. 1984 Ocellation in *Coenonympha tullia* (Müller) (Lepidoptera: Satyridae). I. Structures in correlation matrices. *Nota Lepidopterologica* **7**, 199–219.
- Dowsett, R. J. & Dowsett-Lemaire, F. 1984 Breeding and moult cycles of some montane forest birds in south-central Africa. *Rev. Ecol.* **39**, 89–112.
- Endler, J. A. 1978 A predator's view of animal color patterns. *Evol. Biol.* **11**, 319–393.
- Endler, J. A. 1983 Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fish.* **9**, 173–190.
- Gotthard, K. & Nylin, S. 1995 Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74**, 3–17.
- Kooi, R. E. & Brakefield, P. M. 1999 The critical period for wing pattern induction in the polyphenic tropical butterfly *Bicyclus anynana* (Satyridae). *J. Insect Physiol.* **45**, 201–212.
- Lyytinen, A., Brakefield, P. M. & Mappes, J. 2003 Significance of butterfly eyespots as an anti-predator device in ground-based and aerial attacks. *Oikos* **100**, 373–379.
- Windig, J. J., Brakefield, P. M., Reitsma, N. & Wilson, J. G. M. 1994 Seasonal polyphenism in the wild: survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. *Ecol. Entomol.* **19**, 285–298.
- Wourms, M. K. & Wasserman, F. E. 1985 Butterfly wing markings are more advantageous during handling than during the initial strike of an avian predator. *Evolution* **39**, 845–851.
- Young, A. M. 1979 The evolution of eyespots in tropical butterflies in response to feeding on rotting fruit: a hypothesis. *J. N. Y. Entomol. Soc.* **87**, 66–77.