Sex-limited variability and mimicry in the swallowtail butterfly *Papilio polyxenes* Fabr.

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Variation in wing pattern was measured in the swallowtail butterfly *Papilio polyxenes*, a Batesian mimic of the butterfly *Battus philenor*. Males and females differed in the number of yellow spots comprising the proximal spot band on the dorsal surfaces of the wings, with females having fewer spots than do males. This difference results in females being more similar to *B. philenor* than are males. However, females were also more variable in spot number than were males. Full sib analysis of variance of females and regression of daughters on mothers indicates that variation in spot number is heritable. The ventral surfaces of the wings of males and females showed little variability and were similar to those of *B. philenor*, suggesting that the adaptive significance of the dorsal surface wing pattern differs in males and females. Possible reasons for such a difference are discussed and a model based on the genetics of mimicry in *P. polyxenes* and developmental studies of wing pattern formation in butterflies is proposed to account for the suppression of variability in male wing pattern.

INTRODUCTION

Reduced male variability is commonly viewed as a general characteristic of butterflies (Wallace, 1865; Fisher and Ford, 1928), the most striking examples being species of Papilionidae with female-limited Batesian mimetic polymorphisms (e.g., *Papilio dardanus*, Clarke and Sheppard, 1960). Proximate explanations for the phenotypic suppression of genetic variation in males generally assume major regulatory interactions involving one or more X-linked loci which, when present in two doses in males (the homogametic sex in Lepidoptera), affect the suppression of genetic variation at autosomal loci controlling the polymorphism in wing pattern (Stehr, 1959; Johnson and Turner, 1979; Grula and Taylor, 1980a, b). Ultimate explanations for the suppression of male variability generally invoke some form of sexual selection (Turner, 1978; Silberglied, 1984).

To my knowledge, only one study has been specifically designed to address the phenomenon of suppressed male variability (Pearse and Murray, 1982), and sex-limited variation in non-polymorphic Batesian mimics has never been examined.

In this report I present the results of a preliminary study designed to examine the degree to

which sex-limited wing pattern variation exists in the female-limited Batesian mimic *Papilio polyxenes* Fabr. If female-limited variability is a general feature of butterflies, then such variability should be apparent in female *P. polyxenes* even though they are monomorphic, mimicking a single model species.

Papilio polyxenes is well suited for a study of this kind for several reasons. First, the upper surfaces of the wings are sexually dimorphic (Clarke and Sheppard, 1955), suggesting that selection acts differently on wing patterns in males and females. Second, the butterflies are Batesian mimics of the pipevine swallowtail, *Battus philenor* (L.) (Brower, 1958; Jeffords *et al.*, 1979), and the sexual dimorphism makes females better mimics than males (Codella and Lederhouse, 1989). Third, some of the genetic changes associated with the evolution of mimicry in this species are known (Clarke and Sheppard, 1955). And finally, males are territorial, suggesting a potential role for sexual selection on wing pattern (Lederhouse, 1982).

The results, indicating female-limited expression of genetic variation in a wing pattern character affecting the degree of sexual dimorphism and mimicry in *P. polyxenes*, are discussed within the context of theories for the evolutionary significance of reduced male variability in butterflies and the

general tendency of Batesian mimicry to be female limited. In addition, I suggest that the apparent suppression of genetic variation of wing pattern characteristics in males is an illusion arising from slight differences in the development of wing pattern in males and females.

MATERIALS AND METHODS

The wing patterns of Papilio polyxenes and related species

The wings of male and female *P. polyxenes* and *Battus philenor* are illustrated diagrammatically in fig. 1. The dorsal surface is black with two bands of yellow spots (proximal and distal) running from the anterior forewing to the posterior hindwing. In females, the proximal spot band on the fore- and hindwings is reduced. Between the two spot bands on the hindwing lies an area of blue scales that is more prominent in females. These differences between the sexes make the dorsal surfaces of

the wings in females more similar to those of *B. philenor* than are those of males.

For the purposes of comparison and later discussion, the wings of the related, but nonmimetic, western North American swallowtail *P. zelicaon* are also shown in fig. 1. The dorsal and ventral surfaces of the fore- and hindwings of *P. zelicaon* have an enlarged proximal spot band relative to *P. polyxenes*, and a concomitant reduction in the area of black scales near the body. As a result, *P. polyxenes* wings appear black with yellow markings, while *P. zelicaon* wings appear yellow with black markings. The distal spot bands are identical in the two species.

Morphometrics

The proximal and distal bands of yellow spots on the dorsal and ventral forewings and hindwings of male and female *P. polyxenes* were the subjects of this study. On the forewings there are eight potential positions for spots in each spot band (the

Figure 1 Wing patterns of female (upper left) and male (upper right) *Papilio polyxenes*, *Battus philenor* (lower left) and *P. zelicaon* (lower right). For each drawing the dorsal wing surface is depicted on the left and the ventral surface is depicted on the right. Cross hatching indicates black, striped areas are blue, stippled areas are orange and open areas are yellow. All drawings were made from mounted specimens.

double spot in the posterior cell of the forewing in both spot bands was treated as a single spot), while on the hindwings there are six potential positions for spots in the distal band and eight potential positions for spots in the proximal band. Butterflies were examined for the presence or absence of spots at each of these positions, and the total number of spots present in each band was noted.

Rearing and analysis

The wings of both wild caught males and females and lab-reared female butterflies were examined. The wild butterflies (59 males and 41 females) were sampled in 1984 from a single population in Putnam Co., Indiana. Three hundred and sixty-four lab-reared females were obtained from the eggs of 27 of the wild females. The number of female offspring per family varied from five to 32, with an average of 13.48. Because of sperm precedence (Clarke and Sheppard, 1955), offspring sharing the same mother were assumed to be full sibs.

Larvae were reared in round plastic dishes on a 12L:12D photoperiod at room temperature (approx. 30°C). Larvae were fed daily on fresh food plant (*Daucus carota*). Only butterflies eclosing from pupae that failed to enter diapause, as evidenced by eclosion within six weeks of pupation, were used in this study.

The data were analysed in several ways. First, the number of spots per spot band was compared

in the wild caught males and females. After finding large differences in both mean and variance of spot number for the proximal band on the dorsal surfaces, but only slight differences for other spot bands on the dorsal or ventral surfaces, subsequent analysis concentrated on variation among females from the lab-reared broods in the number of spots in the proximal band on the dorsal surfaces. Variation in spot number for these females was partitioned by one way analysis of variance into among families and within families components so that the genetic component of variation in spot number could be estimated. To obtain a second estimate of genetic variation in spot number, average spot numbers in the 27 female sibships were regressed on the spot numbers of their wild caught female parents.

RESULTS

Only slight differences between male and female wild caught and lab reared butterflies were found for spots of the distal spot band on the dorsal and ventral wing surfaces or for the spots of the proximal spot band on the ventral surfaces of the hind wings. However, females had fewer and more variable numbers of spots in the proximal band on the dorsal surfaces of the wings than did males (fig. 2).

The results of one way analysis of variance of spot numbers for the 364 lab-reared females indi-

Figure 2 Variation in proximal band spot number in male and female *Papilio polyxenes* (N = 100, 59 males and 41 females).

cate that there are significant differences between families in average spot number (table 1). These data provide an estimate of the heritability of variation in spot number of 0.6 with a standard error of 0.14. Because the among families component of variance includes half of the additive genetic variance, one quarter of the dominance variance and any variance due to common environment, this estimate is biased upwards unless the dominance and common environment variances are zero (Falconer 1981).

Table 1 One-way analysis of variance proximal band spot numbers in 364 females representing 27 full sib families (average number of females per family = 13.48)

Source	df	MS	F
Among families	26	49.09	6.92*
Within families	337	7.10	

* $P = 0.001$.

Regression of mean spot number of daughters on spot number in wild caught female parents provides a heritability estimate of 0.46 with a standard error of 0.18. Since the rearing environment of the wild female parents was most likely more variable than that of their lab-reared daughters, and because the heritability estimates based on parent-offspring regression are not biased by dominance variance, this estimate should be biased downward relative to the heritability based on full sib analysis of variance (Falconer, 1981).

DISCUSSION

These results indicate that the sexual dimorphism in *P. polyxenes* is the result of differences in the number of spots comprising the proximal band on the dorsal surfaces of the wings. Females not only have fewer spots than males, but show more variation in the spot number than do males (fig. 2). Moreover, full sib analysis of variance and parent offspring regression provide independent evidence that variation in spot number in females is heritable. This suggests that, at least at the level of spot number, genetic variation is being suppressed in males (but see below). Therefore, these results provide additional evidence for the phenomenon of female-limited variability in the butterflies.

The proximal band of spots defines the sexual dimorphism in *P. polyxenes* and would appear to be important in determining the degree to which *P. polyxenes* is able to mimic *B. philenor*. Since *B.*

philenor lacks a proximal spot band on the dorsal surfaces of its wings, *P. polyxenes* females will, in general, be better mimics than males. However variation among females in the number of spots comprising the proximal band suggests that the effectiveness of mimicry varies. Since variation in spot number is heritable, selection for mimicry should result in a decrease in spot number in populations where *Battus philenor* is common. Thus, spot number in natural populations should be inversely related to the relative abundance of *Battus philenor*. Although geographic variation in spot number has never been examined in *P. polyxenes*, differences between *P. polyxenes* and its close relative, *P. brevicauda*, are consistent with this prediction. *P. brevicauda*'s range lies outside that of *B. philenor*, and *P. brevicauda* males and females are nonmimetic, closely resembling male *P. polyxenes* (Clarke and Sheppard, 1955).

There would seem to be no *a priori* reason why selection should not favour mimicry in both *P. polyxenes* males and females. However, the differences between males and females in mean and variance in spot number specific to the spots comprising the proximal band on the dorsal fore and hindwings suggest that this particular aspect of wing pattern has been subjected to very different selection in the two sexes. This is apparently not the case for the ventral surfaces of *P. polyxenes* wings, which are identical in males and females, and which resemble the ventral surfaces of the wings of *B. philenor*. As a result, caged jays that have experienced *B. philenor* will reject both male and female *P. polyxenes* when these butterflies are offered with their wings folded so that only their ventral surfaces are visible, but are more likely to attack males when the dorsal surface is visible (Brower, 1958; Codella and Lederhouse, 1989).

The idea that the wing patterns of males and females might be subject to differing selection has been proposed to account for the scarcity of Batesian mimicry in male swallowtail butterflies, as well as the tendency for polymorphisms in mimicry to be female limited. Most explanations for female-limited mimicry and suppressed male variability invoke some form of sexual selection. For example, female choice based on visual cues could exert strong stabilizing selection on male wing patterns, resulting in the evolution of a genetic mechanism for the suppression of wing pattern variation in males but not in females (Turner, 1978). The disproportionate frequency of sex-limited genetic polymorphisms in butterflies as opposed to moths, which usually rely on olfactory cues for mate selection, has been interpreted by

Sheppard (1965) as supporting this explanation. However, Pearse and Murray (1982) suggest a number of reasons why Sheppard's interpretation may not be valid.

Pearse and Murray (1982) have strongly criticized the female choice hypothesis, suggesting that male-male competition could just as readily exert the selection pressure necessary for the evolution of a mechanism for suppression of variation in male wing pattern. The male-male competition hypothesis is based on studies of territoriality in butterflies, where prior ownership determines the outcome of male-male encounters, thus providing evidence of male-male recognition (Silbergield, 1984).

The results of this study do not allow a test of these hypotheses, but they do suggest clear directions for future research by indicating the precise aspect of male wing pattern in *P. polyxenes* that should be the focus of sexual selection. Since *P. polyxenes* males are territorial, experimental elimination of the proximal band of spots in males should either affect their ability to hold territories or decrease their attractiveness to females.

What genetic and developmental mechanisms can account for female limited mimicry and variability in *P. polyxenes*? Some members of the *machaon* group of swallowtails, to which *P. polyxenes* belongs, are clearly nonmimetic (e.g., *P. zelicaon*). With respect to the areas of their wings distal to the proximal band of yellow spots, the species are almost identical (see fig. 1). However, the nonmimetic species differ from *P. polyxenes* by having a broader proximal band of yellow spots on both the dorsal and ventral fore- and hindwings, and a narrow area of black proximate to the yellow spot band, giving their wings the appearance of being yellow with black areas near the body and along the outer edges, as opposed to appearing black with a narrow yellow band of spots as in *P. polyxenes*. The difference between male and female *P. polyxenes* is similar to that between male *P. polyxenes* and the nonmimetic members of the *machaon* group. In *P. polyxenes* females the proximal spot band is reduced relative to males, while in *P. polyxenes* males the proximal spot band is reduced relative to nonmimetic member of the *machaon* group. Since the difference between *P. polyxenes* and the nonmimetic species in the size of the proximal spot band results from the presence of a dominant autosomal allele (*B*) in *P. polyxenes* (Clarke and Sheppard, 1955), it seems likely that the sexual dimorphism in *P. polyxenes* is the result of sex-limited modifiers which either enhance the

effect of the *B* allele in females or suppress its effect in males.

Developmental studies of wing pattern determination in butterflies suggest that pigmentation patterns are induced by morphogens that diffuse outward in a concentration gradient from their points of origin (foci) and initiate pigment production in adjacent cells that are competent to respond (Nijhout, 1986, and references therein). According to this model, the effect of the *B* allele in *P. polyxenes* could either be to increase the concentration of the morphogen that initiates the production of black pigmented wing scales, or to increase the competence of wing cells to respond to the morphogen. Since the morphogen would be expected to be produced from foci proximal to the yellow spot band and diffuse outward, sex limited differences in either morphogen concentration or cellular competence would account for both the sexual dimorphism and the variable presence of spots in females. For example, if one assumes that the distal margin of the proximal spot band is fixed, then if females only produce slightly more morphogen than do males, females will have the same number of spots as males, but the spots will be smaller than those of males. If, however, females produce considerably more morphogen than males, individuals spots will be eliminated depending their distance from the source of the morphogen. As a result, females would vary in spot number and males would vary only in spot size. If this hypothesis is correct, then the apparent suppression of genetic variation in male *P. polyxenes* is illusory, simply being a consequence of the developmental mechanisms underlying wing pattern formation in butterflies, and not the result of major regulatory interactions suppressing variation at gene loci.

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