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Preferential Mating versus Mimicry: Disruptive Selection and Sex-limited Dimorphism in Papilio glaucus

Abstract. *Spermatophore counts in wild females of* Papilio glaucus *show that the monomorphic nonmimetic male mates less frequently with the mimetic female morph than with the nonmimetic female morph. Female dimorphism in this species cannot be maintained by heterozygous advantage. Mating preference for the nonmimetic female may sufficiently counteract avian predation pressure favoring the mimetic female to account for the maintenance of the nonmimetic morph in the population in those areas in which the model is abundant and to account for the reduction in frequency or elimination of the mimetic morph in those areas in which the model is less numerous or absent.*

Sex-limited dimorphism exists in many populations of *Papilio glaucus* Linnaeus, the eastern tiger swallowtail butterfly of North America. Males are striped black on yellow; females are like males or are predominantly black (Fig. 1). The dark female morph mimics *Battus philenor* (Linnaeus), a swallowtail unpalatable to avian predators (*1*).

The occurrence of the mimic (dark morph of *P. glaucus*) is correlated with the distribution and abundance of the model (*B. philenor*). *P. glaucus* ranges from Newfoundland to Alaska, eastern British Columbia, the western Dakotas, and western Nebraska, and thence throughout the eastern United States, mostly east of the hundredth meridian, to extreme northeastern Mexico (*2*). In areas in which the model is abundant, the dark morph of *P. glaucus* comprises a very high proportion of the female population, but apparently it nowhere totally replaces the light morph (Table 1). By contrast, where the range of *P. glaucus* exceeds that of *B. philenor*, the dark morph becomes scarce or disappears altogether, leaving a monomorphic light population. Thus, the dark morph is essentially absent north of southern New England, central New York, southern Michigan, southern Wisconsin, and southern Minnesota; it is uncommon in peninsular Florida, where the model is rare (*3*, *4*). This decline in frequency of the dark morph both northward and southward indicates that it is not an adaptation to a gradient in an abiotic factor such as temperature or humidity (*3*).

The dimorphism apparently is under simple genetic control. Although the dark morph was earlier thought to be inherited as an autosomal dominant, sex-limited in expression (*5*), recent evidence shows that it is Y-linked (the female is the heterogametic sex) or, possibly, cytoplasmically transmitted. It follows that the dimorphism cannot be maintained by heterozygous advantage (*6*).

The geographically varying relative frequencies of the two morphs must reflect antagonistic selective forces. Pressure favoring the dark mimetic morph is exerted through differential predation on adults by birds that have experienced the model. One factor that might select against the dark morph is preferential mating of the monomorphic males with light females. Courtship studies in a number of butterfly species have demonstrated the importance of visual stimuli and the existence of male preference for females with malelike color-patterns (*7*). Although there is little direct evidence that females discriminate among males visually, departures from the primitive color-pattern by males are assumed to be far less acceptable to females than conservative dress because mimetic polymorphism in butterflies is frequently restricted to the female sex (*8*). Such restriction characterizes species emphasizing visual communication rather than those relying on olfactory stimuli, in which both sexes readily become mimetic (*9*). Male preference for primitive color-pattern is suggested for *P. glaucus* by courtship experiments in which light and dark females were tethered outdoors in Utah and Colorado, areas in which *P. glaucus* is replaced by three closely related western species of tiger swallowtails similar in appearance but not dimorphic. Males of two of these western swallowtails, *P. multicaudata* Kirby and *P. rutulus* Lucas, were far more attentive to the light morph of *P. glaucus* than to the dark morph (*10*).

To test for the existence of male mating preference in *P. glaucus*, samples of females were taken from two natural populations (Mt. Lake, Virginia, and Baltimore, Maryland) in which females are dimorphic (*11*); and, since male lepidopterans deposit sperm in membranous sac-like spermatophores rather than in amorphous ejaculates, the bursa copulatrix of each female was dissected and the spermatophores within were counted. For comparison, mating frequency was similarly investigated in *B. philenor* (*12*).

Two assumptions are made in determining mating frequency by counting the number of spermatophores in the bursa copulatrix: (i) the male transfers but one spermatophore per mating, and, (ii) even though the spermatophore commonly empties, collapses, and shrinks with time, its walls are persistent enough to be recognized. Studies on spermatophores and mating frequencies in several species of butterflies and skippers (*13*) and in a gelechiid moth (*14*) support these assumptions, although in some Lepidoptera more than one spermatophore may be introduced during a single mating and the spermatophore may be digested (*15*). However, with respect to the problem of preferential mating in *P. glaucus*, the

Table 1. Frequency, sample size, and date of capture of dark and light female morphs of *Papilio glaucus* from five localities in the southeastern United States.

Date of capture	No. of specimens	Frequency	
		Dark	Light
Baltimore, Maryland			
24 to 31 Aug. 1965	29	.45	.55
Mt. Lake, Va./W. Va.			
13 to 22 June 1965 26 July to 19 Aug. 1965	84	.86	.14
*Great Smoky Mountains North Carolina/Tennessee**			
11 to 21 Aug. 1954 22 July 1959	15	.93	.07
*Northern Georgia**			
15, 16 Aug. 1959	33	.97	.03
*Central Florida**			
6 Mar. to 13 June 1956	501	.06	.94
13 June to 6 Aug. 1959	517	.08	.92

* Data from Brower and Brower (*3*).

correctness of these assumptions is largely irrelevant. There is no reason to suspect that, if males sometimes pass more than one spermatophore during copulation, the probability of their doing so would be influenced by the particular morph with which they were mating; or that, if spermatophores gradually disintegrate, they would do so more rapidly in one kind of female than in the other. The fact that females are more likely to have mated multiply the older they are (*13*) can be ignored, provided the sample is approximately random and the two morphs emerge and fly more or less synchronously.

A female of either *B. philenor* or *P. glaucus* bears, on an average, about 1.73 spermatophores, but, in the mimetic species, the two morphs contribute unequally (Table 2). In the Mt. Lake and Baltimore samples of *P. glaucus*, the light morph exceeds the dark one by 0.39 and 0.34 spermatophores, respectively, giving the light morph a mean edge of 0.37 spermatophores over the dark morph. Relative to the light morph, the dark morph is mated only 81.3 percent (Mt. Lake) or 81.9 percent (Baltimore) as frequently (*16*).

Table 2. Frequency distribution, percentage occurrence (numbers in parentheses), and mean number of spermatophores in wild-caught females of *Battus philenor* and *Papilio glaucus*.

Species	Morph	Specimens (No.)	Number of spermatophores					Mean number of spermatophores per female
			1	2	3	4	5	
			Mountain Lake Biological Station					
B. philenor		33	17 (52)	11 (33)	3 (9)	1 (3)	1 (3)	1.73
P. glaucus	Dark	72	33 (46)	30 (42)	8 (11)		1 (1)	1.69
P. glaucus	Light	12	6 (50)	2 (17)	2 (17)	1 (8)	1 (8)	2.08
P. glaucus	Total	84	39 (47)	32 (38)	10 (12)	1 (1)	2 (2)	1.75
			Baltimore County, Maryland					
P. glaucus	Dark	13	8 (62)	3 (23)	2 (15)			1.54
P. glaucus	Light	16	4 (25)	10 (63)	2 (12)			1.88
P. glaucus	Total	29	12 (41)	13 (45)	4 (14)			1.72

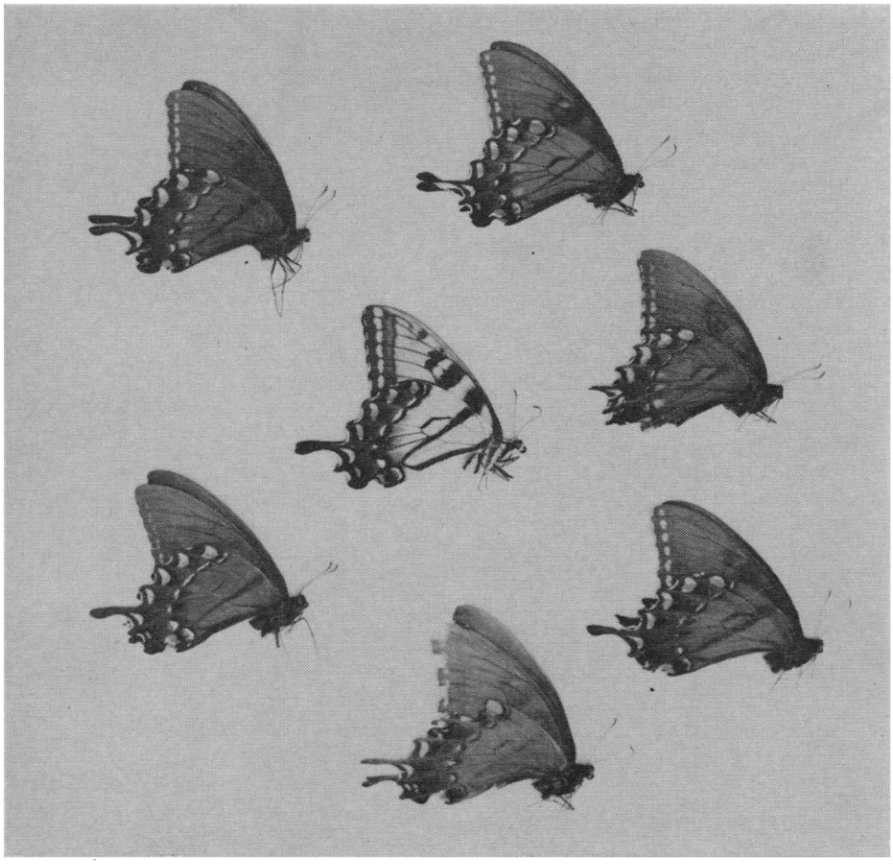

Fig. 1. Dark and light female morphs of *Papilio glaucus* collected 15 August 1965 at Kire, Giles County, Virginia, where only about 1 female in 7 is light. (The distal part of each abdomen has been removed and examined for spermatophores.)

In connection with the suggested reproductive disadvantage of the dark morph, the objection may be raised that none of the females sampled was virgin (Table 2). However, although the sperm of certain insects are long-lived, in some species (including some of those with long-lived sperm) a single insemination is insufficient for the female to produce fertile eggs throughout her egg-laying period (*15*). This may be the situation in the female of *P. glaucus*, which often mates several times, in contrast to that in females of certain other species of butterflies and skippers in eastern North America, which apparently mate but once (*13*). The dark morph of *P. glaucus* is probably not, on an average, fertilized as soon after emergence as the light morph and is thereby significantly handicapped.

Reproductive advantage and mimetic advantage are opposed forces that may sustain female dimorphism in *P. glaucus* in which it cannot be maintained by the familiar mechanism of heterozygous advantage. Male preferential mating may be the general reason that species in which females become mimetic also commonly retain the nonmimetic female morph (with its ancestral color-pattern) in the same population.

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11. Material was collected in the vicinity of Mountain Lake Biological Station, Giles County, Virginia, from mid-June to mid-August, 1965, at elevations ranging from 500 to 1230 m. The "Mt. Lake" sample area

extends from Potts Creek Valley at Waiteville, Monroe County, West Virginia, at the northeast, and Big Stony Creek Valley at Kimballton, Giles County, Virginia, at the northwest, to upper Craigs Creek Valley north of Blacksburg, Montgomery County, Virginia, at the southeast, and Spruce Run Valley at Goodwins Ferry, Giles County, Virginia, at the southwest. In late August 1965, material was collected at "Baltimore" in four woods-bordered fields about evenly spaced along a 6 km stretch of Jones Falls in Green Spring Valley, elevation 90 m, Baltimore County, Maryland. Every effort was made to catch all *P. glaucus* females encountered, and, particularly at Baltimore, very few were missed. Females were not so numerous that the collector was simultaneously presented with a dark and a light one and forced to decide which morph to sample.

12. I was assisted in gathering the Mt. Lake sample of *P. glaucus* by S. N. Burns, M. P. Levin, and the members of my animal speciation class: C. Beirne, S. C. Cornick, E. S. Fouché, D. A. Graham, M. J. Hodge, A. K. Mester, W. H. Rittenhouse, E. Scvortzoff, J. T. Sigros, and D. H. Zimmerman. I collected the Baltimore sample and dissected all specimens of *P. glaucus* from both localities; most of the sample of *B. philenor* was collected by D. A. Graham (from 30 July to 3 August 1965) and dissected by him. I preserved the distal part of the abdomen of 35 Mt. Lake females in Kahle's fluid, stored crude abdominal dissections of all Baltimore females in 80 percent ethanol, and dissected out the spermatophores a few weeks later; both preservatives were satisfactory.
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16. Although the data support this conjecture, their nature precludes rigorous statistical testing by the χ^2 test. Use of an independent 2×3 contingency table for each geographic sample of *P. glaucus* necessitates lumping the data from Mt. Lake in the three right-hand columns of the frequency distribution in Table 2, which results in the loss of some of the information critical in establishing the mating preference. For the data from Baltimore, the contingency table is in the form seen in Table 2. In each contingency table no cell has an expected frequency less than 1, but 33 percent of the cells (rather than the permissible maximum of 20 percent) have expected frequencies less than 5. For Mt. Lake, $\chi^2 = 4.61$, d.f. $= 2$, $P = .10$; for Baltimore, $\chi^2 = 4.84$, d.f. $= 2$, $.10 > P > .05$. Further testing, by adding the values from both samples, yields $\chi^2 = 9.45$, d.f. $= 4$, $.10 > P > .05$ (but with P very close to .05). A final analysis, involving calculation of χ values, their summation, and division by the square root of d.f., yields a standardized normal deviate of 2.18 corresponding to $P = .03$.
17. Supported in part by Mountain Lake Biological Station of the University of Virginia and NSF grant GB 3439. R. K. Selander critically read the manuscript.

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Glial Responses to Degenerating Cerebellar Cortico-nuclear Pathways in the Cat

Abstract. *Astrocytic changes in regions of long-term, cerebellar corticonuclear degeneration are characterized by large increases in cytoplasmic volume, as well as by formation of vacuoles and fibrils. Lipids are demonstrable in the vacuoles with oil red-O staining.*

Glial reactions to degenerative processes in the central nervous system have been described in a number of recent investigations, which have focused some attention on changes occurring in astrocytes in response to the degeneration of neural elements (*1–5*). We have observed reactive astrocytes in areas of the cerebellar nuclei of the cat in which large-scale degeneration of cerebellar cortico-nuclear fibers occurred after extensive destruction of the overlying cerebellar cortex.

Unilateral cortical lesions were made under aseptic conditions on the cerebellums of six adult cats by scratching the cortical surface with a needle. Subcortical white matter was largely spared. Each lesion, involving major portions of the cerebellar cortex on one side, resulted in degeneration of fibers to all three homolateral cerebellar nuclei (*6*).

All experimental animals were killed 3½ months after operation. Four were perfused with 5 percent glutaraldehyde made up in phosphate buffer (*7*). Small blocks of cerebellar nuclei (fastigius, interpositus, dentate) and adjacent white matter were removed from operated and control sides, treated with osmic acid, dehydrated, and embedded in capsules of Maraglas. Sections were cut on an LKB Ultratome, mounted on unsupported 200-mesh copper grids, stained with lead citrate (*8*), and viewed with an RCA EMU-3 electron microscope. Two animals were perfused with 10 percent formol-saline (0.9 percent NaCl); their cerebellums were cut into 20-μ frozen sections, and the sections were stained either with oil red-O (*9*), for the demonstration of lipid, or with the Nauta-Laidlaw silver method (*10, 11*), for the demonstration of degenerated fibers.

The morphology of normal macroglia found in areas of the cerebellar nuclei and adjacent white matter corresponded well with previous descriptions of these cells in other parts of the central nervous system (*4, 5, 12*). Oligodendrocytes were readily identifiable by the dense chromatin of their nuclei and by the relatively greater density of their cytoplasm. Normal astrocytes had large, round nuclei with dispersed chromatin and a cytoplasm less rich in ribosomes. Fibrous astrocytic processes were seen only rarely in normal cerebellar nuclear regions. Cells identifiable as microglia were never observed.

The responses of glial elements to long-term degeneration of cerebellar cortico-nuclear fibers was confined to cells which could be, in most cases, identified as astrocytes. There was no evidence of non-glial cellular infiltration which characterizes other degenerating systems (*2, 13*), at least not at this stage of degeneration, nor were microglia ever seen. This was a surprising observation since it is commonly held that the microglia participate in phagocytosis of degenerating neural elements.

The cytoplasmic volume of reactive astrocytes increased greatly, and numerous vacuoles and fibrils were formed. Increases in volume and vacuolation were particularly marked in perivascular astrocytic processes (Fig. 1) which are normally so attenuated that they are not easily identifiable. The membrane-bound vacuoles occupy most of the cytoplasm of the reactive cell, especially in perinuclear regions (Fig. 2). Most of them appear empty in electron micrographs, although dense material is often found in the cytoplasm in regions containing many vacuoles (Fig. 2). The remaining cytoplasm contains mitochondria and elements of granular and agranular endoplasmic reticulum (Fig. 2).

Fibrils, although not a common feature of normal astrocytes in the neuropil of deep cerebellar regions, are a distinct characteristic of the reactive astrocyte in these specimens and are easily discernible with both the light and electron microscopes (Figs. 1–3). The presence of fibrillar material, vacuoles, and glycogen (*5*) in the same cell (Fig. 3) indicates that the reactive glial cells in our specimens are astrocytes.

Frozen sections of degenerated regions, stained with oil red-O, reveal the presence of much reactive lipid; none is found in normal nuclear areas of the cerebellum. The stained lipid occupies the enlarged perivascular processes, as well as the cytoplasm of other reactive astrocytes, and its distribution corresponds well with the distribution of cytoplasmic vacuoles, indicating that most, if not all, of the lipid available for oil red-O reaction resides at these sites. Adjacent frozen sections prepared