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Use of Multivariate Discriminant Analysis of Male Wing Morphometrics to Delineate a Hybrid Zone for Papilio glaucus glaucus and P. g. canadensis in Wisconsin

HEIDI J. LUEBKE[1,4], J. MARK SCRIBER[1,2], and BRIAN S. YANDELL[3]
University of Wisconsin, Madison, 53706

ABSTRACT: The eastern tiger swallowtail butterfly *Papilio glaucus* L. is distributed throughout Canada and the eastern half of the United States. Two of the three purported subspecies (*Papilio glaucus glaucus* and *Papilio glaucus canadensis*) occur in Wisconsin. In order to determine the extent of hybridization between the two subspecies, 18 wing characters were measured or scored for three reference groups: *canadensis* (from Canada, northern Minnesota and the Upper Peninsula of Michigan), *glaucus* (from southern Ohio and central Illinois) and known laboratory F_1 hybrids. These data were subjected to multivariate discriminant analysis, and the resulting rule was then used to statistically analyze field-captured adults from various counties of Wisconsin.

Individuals collected from Wisconsin were entered as unclassified data and analyzed with regard to the reference groups in two ways. First, general body size (as indicated by forewing length) was included because of long recognized size differences between the subspecies. Second, the data were reanalyzed after body size was removed statistically. The results of the second analysis parallel those of the first, indicating that useful, nonsize related taxonomic characters are included in the measured traits. Of 15 wing traits (and three ratios), three wing characters alone gave an overall classification accuracy of 84%, which is close to the 88.5% correct classification with all traits used.

The southern third of Wisconsin is very different from the northern two-thirds in tiger swallowtail composition. No field-captured individuals (unknowns) were classified as *Papilio glaucus glaucus* in the northern two thirds of the state, while most of the field captures from the southern third of the state were classified as such. Computer-classified hybrids reached their greatest frequency in the S-central part of Wisconsin and support the evidence of a narrow hybrid zone between these *Papilio* subspecies.

INTRODUCTION

Hybrid zones have been defined as "narrow regions in which genetically distinct populations meet, mate, and produce hybrids" (Barton and Hewitt, 1985). Hundreds of suspected hybrid zones have been identified and a recent review concludes that it is difficult to distinguish whether the distinct forms in zones of parapatry are maintained by hybrid unfitness or differential adaptation to environmental factors (Woodruff, 1981; Barton and Hewitt, 1985).

Central Wisconsin is particularly interesting in that it represents a "tension zone" for plant communities (Curtis, 1959) and a zone of overlap and suspected hybridization for many insects (Remington, 1968; Platt and Brower, 1968; Scriber, 1982, 1983). There is great interest in understanding the causal mechanisms and processes promoting or preventing gene exchange among populations and hybrid zones offer a valuable natural laboratory in this regard (*e.g.*, Littlejohn and Watson, 1985; Slatkin, 1985).

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[1]Department of Entomology, University of Wisconsin, Madison 53706.
[2]Current address: Department of Entomology, Michigan State University, East Lansing, 48824.
[3]Departments of Horticulture and Statistics, University of Wisconsin, Madison 53706.
[4]Current address: Department of Medical Genetics, University of Wisconsin, Madison 53706.

The eastern tiger swallowtail butterfly *Papilio glaucus* is believed to be comprised of three subspecies (*P. g. canadensis* R&J, *P. g. glaucus* L. and *P. g. australis* Maynard) (Tyler, 1975; Opler and Krizek, 1984). There is evidence which suggests that *P. g. canadensis* and *P. g. glaucus* meet, (Fig. 1.) overlap and perhaps hybridize in southern Wisconsin, central Michigan (also with a "tension zone" for plants) and throughout central New York/New England southward into the Appalachian Mountains. Significant differences in foodplant detoxication abilities, voltinism patterns, female color polymorphisms and allozyme frequencies between the two subspecies have been described across a narrow latitudinal zone (Scriber, 1982, 1986; Scriber and Hainze, 1987; Scriber *et al.*, 1987; Ritland and Scriber, 1985; Hagen, 1986). This study describes our attempts to locate this zone in Wisconsin, using a multivariate morphometric discriminant analysis of wing patterns with independent reference groups.

Discriminant function analyses have been used in a variety of studies for taxonomic separation and the identification of natural hybrids (Lawrence and Bossert, 1967, 1969; Neff and Smith, 1979; Montanucci, 1978; Miles, 1983; Scott and Shepard, 1976; Atchley, 1970; Hafernik, 1982; Collins, 1984; Daly, 1985). At least two assumptions are implicit in the use of discriminant analysis for hybrid identification: first, that the initial (reference) data can be correctly classified and, second, that the unknowns belong to one of the reference groups (Neff and Smith, 1979; Reyment *et al.*, 1984). If a known hybrid group is included as one of the reference groups, the analysis of hybrid identification becomes more effective. We used hand-paired laboratory-reared hybrids between both sexes of *Papilio glaucus canadensis* and *P. g. glaucus* as our hybrid reference group.

METHODS AND MATERIALS

Field-captured males were used as our *canadensis* (n = 91, from Minnesota, Michigan, and Manitoba) and *glaucus* (n = 72, from Ohio and Illinois) reference groups. In addition, 150 laboratory-reared F_1 hybrid males from hand-paired crosses (both *Papilio*

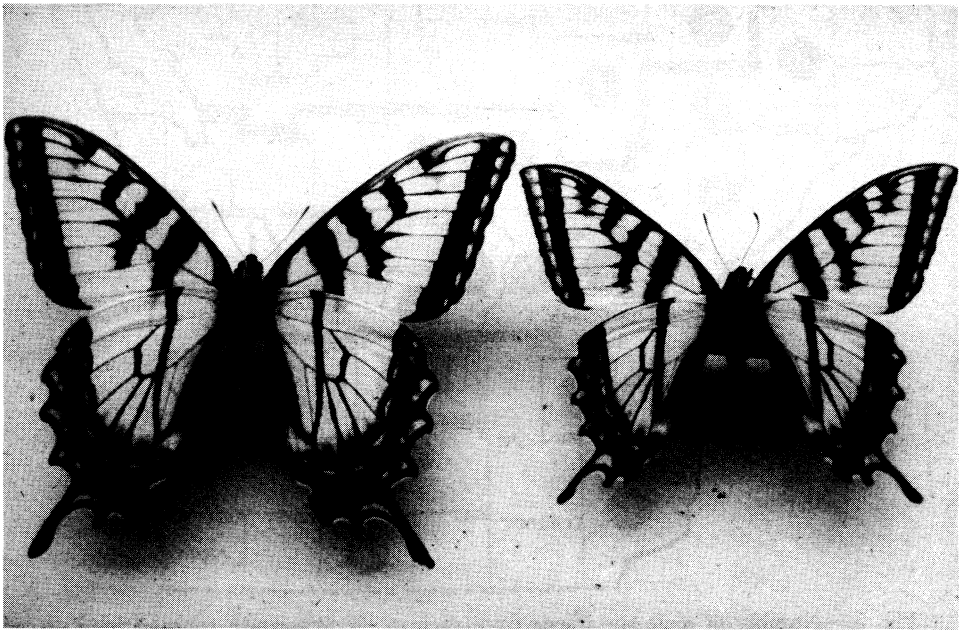

Fig. 1. — Typical *Papilio glaucus glaucus* L. (left) and *P. g. canadensis* R&J (right)

g. c. females x *P.g.g.* males and *P.g.g.* females x *P.g.c.* males) were used as a hybrid reference group. Field-captured males (1982 and 1983) from various counties across the state of Wisconsin were treated as unknowns to be classified (Fig. 2).

We only used males in this study because females were generally less abundant in field collections and were needed for other studies. Furthermore, several of the characters we wished to include in our analyses were obscured in the melanic (dark) morph of *Papilio glaucus glaucus* females.

Our hybrids were produced by hand-pairing (*see* Clarke and Sheppard, 1955, 1956 and 1957) a virgin lab-reared female of known background to a field-collected male. The mated female was then placed in a plastic box measuring 12 x 20 x 30 cm along with an appropriate foodplant on which to oviposit. The plastic box was then placed in front of several 100-w incandescent light bulbs for 1-2 weeks. Females were fed honey

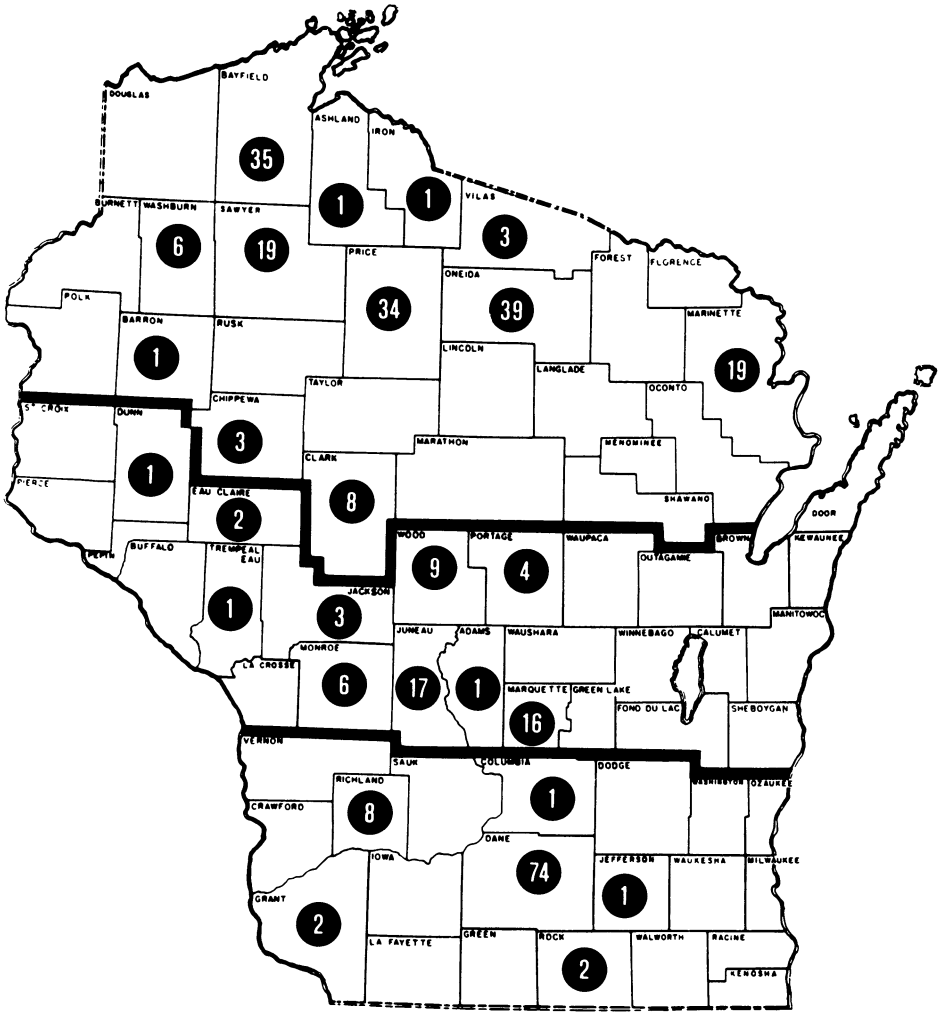

Fig. 2.—Number of male specimens from each of the Wisconsin counties used as unknowns for classification via multivariate morphometric analyses. The lines represent standard crop reporting zones for the state (with the central zone the suspected area of hybridization)

water solution once or twice daily. Every 2-3 days the eggs were removed to petri dishes. First instar larvae were placed upon appropriate host foliage supported in water-filled aquapics (Scriber, 1977) in a petri dish. They were maintained at a 16:8 photoperiod and a temperature of approximately 23 C while reared to pupation and adult eclosion.

Fifteen morphological wing characters were measured (three measurements of wing structure, five measurements of scale pattern features and seven qualitative features of the pattern coded for discrete characters) and three ratio transformations were computed. Both discrete and continuously varying data were included (Pimentel, 1979; Bookstein, 1982; Reyment *et al.*, 1984). Character selection was guided, in part, by avoiding parts of the body that are prone to shrinkage (*i.e.*, the body proper). Characters were also chosen on the basis of the degree of variability among the three groups. Initially, many specimens from all three reference groups were examined closely. Characters that appeared to vary among the three groups were selected. Differences based on wing size and color patterns have been a primary means of distinguishing between *Papilio glaucus glaucus* and *P. g. canadensis* previously (Rothschild and Jordan, 1906; Clark, 1932; Munroe, 1960; Ebner, 1970; Scriber, 1982; *see* Fig. 1). On the dorsal surface of the spread wings, five continuous variables were measured; on the ventral surface, seven discrete and three continuous variables were used. Three ratios (A, B and C) were also included in the data analysis (*see* Table 1). All measurements were made with a transparent plastic ruler in units to the nearest 0.5 mm. Nearly all variables were measured or scored from the right side of the insect's body, except in cases of wing damage. The location of each character is illustrated in Figures 3 (a,b,c), 4 (a,b) and 5 (a-q). The specimens used in these morphometric analyses are deposited as vouchers in Professor J. Mark Scriber's laboratory, Department of Entomology, Michigan State University, East Lansing, Michigan.

Discriminant function analysis computes a linear combination of the measured variables in order to find the maximum distance between the centroids relative to the within-group variance (Neff and Smith, 1979). When an unknown specimen is analyzed, each datum is classified on the basis of the centroid to which it is nearest.

We used stepwise discriminant analysis to obtain maximum separation among the two populations and the hybrids. This is expressed in terms of "canonical variates" which are "linear combinations of the characters which maximize the ratio of the between populations sum of squares to the within-population sum of squares" (Reyment *et al.*, 1984). The first canonical variate provides the "best" separation of the populations.

TABLE 1.—Coded designations of measured wing characters

DORSAL SURFACE

1. FWLN. Forewing length from apex to thoracic attachment at the wing base (Fig. 3a).
2. WDYLSPT. Width of yellow spot within cell M1 (HW) (Fig. 4b).
3. LNYLSPT. Length of the above spot (HW) (Fig. 3b).
4. WDSMBND. Width of black submarginal band of interspace M1 of HW. Measurement taken from median border of the band to the lateral edge of the wing margin, with the line of intersection passing through the junction of vein Ml and the discal cell (Fig. 3c).
5. LNSMBND. Length of interspace M1 of HW; measurement taken from junction of M1 vein and discal cell, to the edge of wing margin at midpoint between interspace M1, M2 (Fig. 3c).

VENTRAL SURFACE

6. WDANLBND. Width of anal black band; measured from the distal edge of the anal margin to the proximal border of the wing margin, with the line of intersection through the junction of vein Cu2 and the discal cell (Fig. 4b).
7. WDANLCL. The combined width of cells 2A and Cu2 measured from the lateral anal margin to the junction of vein Cu2 and the discal cell (Fig. 4b).

TABLE 1—Continued

8. LUNTAIL.
   (1) Lunule descending into tail ≥ 2 mm in length (Fig. 5a).
   (2) Lunule descending into tail 1-1.9 mm in length (Fig. 5b).
   (3) Lunule not descending into tail or descending <1 mm in length (Fig. 5c).

9. CLRHWSPT. Color * of submarginal spots in VHW of interspaces Sc + R1, Rs, M1, and M2:
   (1) All yellow, except an orange spot in interspace Sc + R1.
   (2) Spots mostly yellow, except some spots with a trace of orange (≤ 33% of area within spot) (Fig. 5d).
   (3) Spots mostly orange coloration (>were 33% coloration) (Fig. 5e).
   (4) All spots yellow including interspace Sc + R1.

*The orange coloration may be faded; this should not be confused with the *amount* of coloration occupying the spot.

10. CONCTMED. Median band of the VHW:
   (1) Converges directly to and intersects the margin of the anal band. No connector bridge present (Fig. 5f).
   (2) Does not directly intersect margin of anal band, but is joined by a connector bridge of 1 mm or less in length (Fig. 5g).
   (3) Does not directly intersect margin of anal band, but instead is joined by a connector bridge of > 1 mm in length (Fig. 5h).

11. BLUDCL. Blue scaling in black region of the discal cell flanked by cell M1:
   (1) Present—3 individual scales or more (Fig. 5i).
   (2) Absent—2 individual scales or less (Fig. 5j).

12. VFWSMBND. Yellow coloration in the submarginal area of interspaces R3, R4, R5, M1 of the VFW, has taken the form of:
   (1) Distinct spots, either oval or half-moon in shape. Spots appear to be separated by black scaling wider than the wing veins (Fig. 5k).
   (2) Nearly contiguous band, separated by distinct black scaling approximately equal to the width of the wing veins (Fig. 5l).
   (3) A solid contiguous band; wing veins are yellow or faintly black, without adjacent medial scalloping between veins (Fig. 5m).

13. SPTINVAG. Yellow spots in submarginal area of interspaces R3, R4, R5, M1 of VFW:
   (1) Appear invaginated on either the lateral or medial wall of the spot (the yellow spot appearing *W* in shape or heart-shaped) (Fig. 5n).
   (2) Show *no* invagination of the lateral or medial walls of the spot (Fig. 5k or 5m).

14. WDVSMBND. Width of VFW submarginal band, measured from medial edge to wing margin between interspace M1 and M2 (Fig. 4a).

15. SMEDGE. Medial margin of submarginal band of interspaces Sc, Rs, M1 is:
   (1) Uneven (Fig. 5o), or scalloped (Fig. 5p).
   (2) More even, often aligned in a linear fashion (Fig. 5q).

RATIOS OF WING CHARACTERS USED:

16. WDYLSPT/LNYLSPT. Ratio (A) of width of the yellow spot in M1 to its length (Fig. 3b).

17. WDSMBND/LNSMBND. Ratio (B) of the width of the black submarginal band to the length of the submarginal interspace (Fig. 3c).

18. WDANLBND/WDANLCL. Ratio (C) of the width of the hindwing and band to the width of the anal cell (Fig. 4b).

The second canonical variate provides the "best" separation of populations from what is left over after the first canonical variate is removed. The second canonical variate is less important than the first in discrimination, and is correlated with it. Only two canonical variates are needed to establish a rule for three groups (Dixon and Brown, 1979). Data were analyzed using Stepwise Discriminant Analysis-BMDP/P7M (Dixon and Brown, 1979) on a Sperry 1108.

The data were log-transformed to remove the possible allometric influence of size on variation (Daly, 1985; Atchley *et al.*, 1976, 1982). The transformation was derived from the power function $Y = aX^b$, where X is the measure of overall size (forewing length, FWLN) used as the predictor for each of the dependent variables. The above power

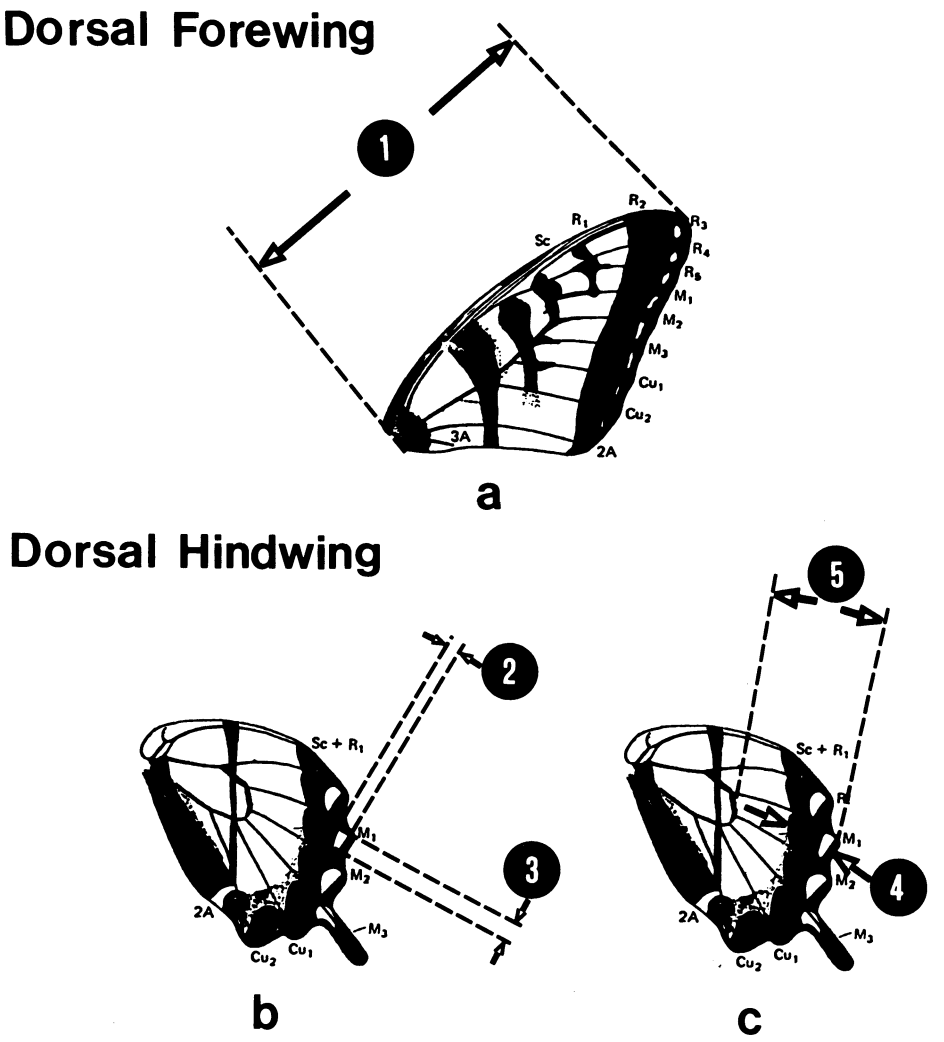

Fig. 3.—Morphological measurements (mm) taken from the dorsal wing surface of *P. glaucus* (refer to Table 1 for further discussion). (a) Length of the dorsal forewing (Table 1:1). (b) Measurement of dorsal hindwing spot (Table 1:2, 1:3). (c) Measurements of dorsal hindwing submarginal band and cell interspace (Table 1:4, 1:5)

function leads to the relationship log Y = log a + B log (FWLN). Each log-transformed dependent variable was then regressed on log (FWLN) and the residuals used as the new data set.

RESULTS

The three reference groups of known specimens (*canadensis, glaucus* and hybrids) were used as standards. The first canonical variate using all of the original data explained 85.6% of the variability among the reference groups, while the second variate explained the rest (14.4%). The variables that provided the most separation based on between-group differences were FWLN, VFWSMBND and SPTINVAG (Table 2a). The percentage of correct classifications for all *glaucus* was 91.7%, for all hybrids, 80.7%, and all *canadensis*, 98.9%. Of 313 individuals, the overall percent correct classification was 88.5%. The second analysis was computed with allometric effects of forewing length removed (Table 2b). Maximum separation was achieved by canonical variates which were essentially variables VFWSMBND and SPTINVAG (Table 2b) with an overall correct classification of 70.3%. The first canonical variate explained 88.9% of the variability among the reference groups.

A total of 290 specimens were analyzed for those 13 Wisconsin counties with at least six specimens (Fig. 2). Each county was treated as a group and a centroid was calculated. The percent classifications for these counties is presented in Table 3. Note that the size variable, FWLN, is included. All but two counties are represented by individuals classified as *canadensis*. The two southern counties, Richland and Dane, are represented primarily by hybrid and *glaucus* specimens, respectively.

Three regions were chosen to represent the northern, central and southern sections of Wisconsin, based on crop reporting districts (Wisconsin Statistical Reporting Service, 1980). A total of 317 specimens were entered as unclassified data; 169 representing the northern region, 60 from the central portion, and 88 from southern Wisconsin. Each region was treated as a single group of unclassified specimens with a single centroid. In both cases (with size included, and with size and allometric effects removed;

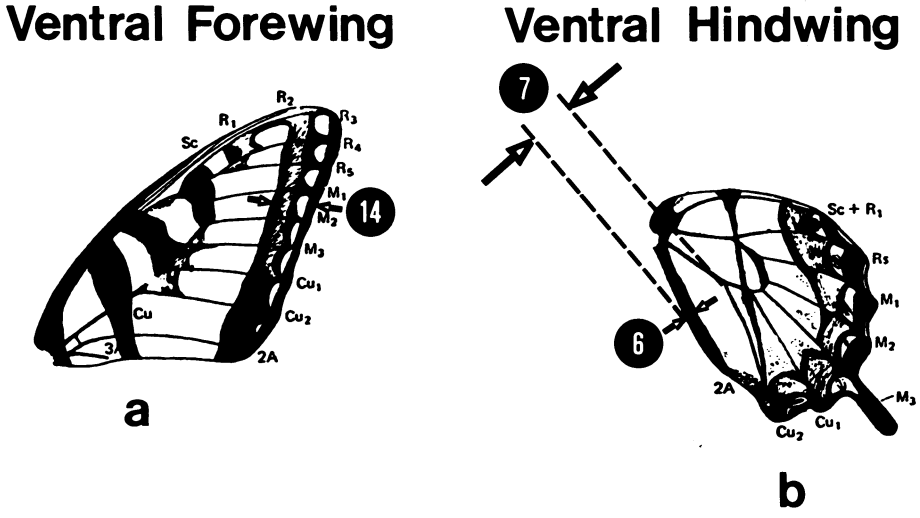

Fig. 4. — Morphological measurements taken from the ventral wing surface of *P. glaucus* (refer to Table 1 for further discussion). (a) Measurement of the ventral forewing submarginal band (Table 1:14). (b) Ventral hindwing measurement of the anal band and cells 2A and Cu2 (Table 1:6, 1:7)

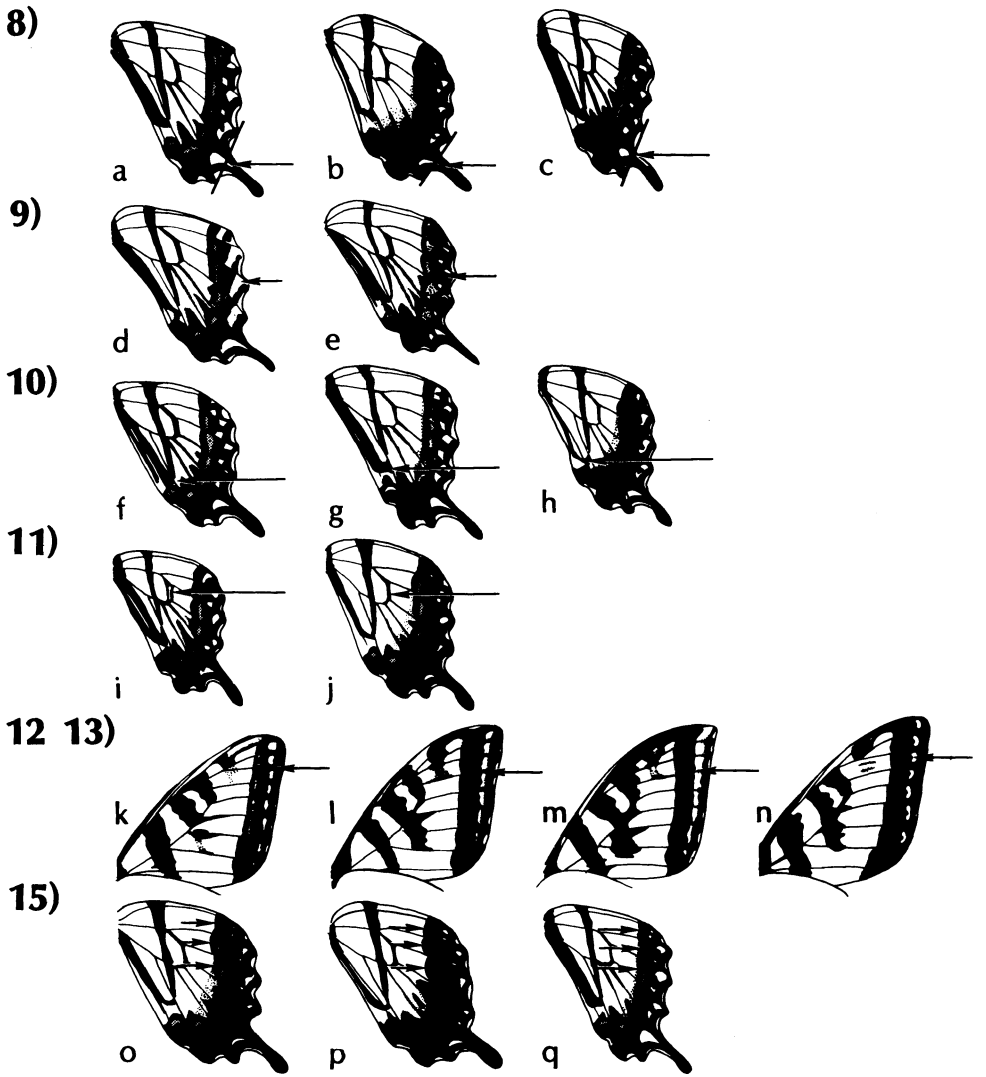

Fig. 5.—For the various wing characters illustrated refer to Table 1. *Character 8*: On the ventral surface of the hindwing, the lunule descends into the tail: (a) greater than or equal to 2 mm in length; (b) 1-1.9 mm in length; (c) does not descend into tail. *Character 9*: Ventral hindwing submarginal spots: (d) are mostly yellow, with a trace of orange; (e) show little or no yellow coloration, are mostly orange. *Character 10*: Median band of the ventral hindwing: (f) converges directly to and intersects the margin of the anal band; (g) does not intersect the margin of the anal band but is joined by a bridge of dark cells of 1 mm or less in length; (h) as above, but bridge of dark cells is greater than 1 mm in length. *Character 11*: Blue scaling in black region of ventral hindwing discal cell: (i) is present, (j) is absent. *Character 12 & 13*: The submarginal area (medial region) of interspaces R3, R4, R5, M1 of the ventral forewing: (k) appears as distinct spots; (l) appears as a nearly contiguous band; (m) appears as a solid contiguous band (refer to Table 1:12); (n) shows yellow spots that appear invaginated on either the lateral or medial wall of the spot (refer to Table 1:13). *Character 15*: The medial margin (ventral hindwing) of the submarginal band of the interspaces S_c, R_s, M1 is: (o) uneven; (p) scalloped, (q) more even, often aligned in a linear fashion. (refer to Table 1:15).

Tables 3 & 4, Fig. 6), the northern and central sections are strongly scored as *canadensis* individuals. *Papilio g. glaucus* specimens and F_1 hybrids dominate the southern section.

DISCUSSION

General size differences between the two swallowtail butterfly subspecies *Papilio glaucus glaucus* and *P. g. canadensis* (*see* Fig. 1) have been noted for at least a century (Rothschild and Jordan, 1906; Edwards, 1884). Size-related characters such as pupal weights and wing lengths are known to be correlated (Scriber, 1982). Furthermore, it is known that in addition to intrinsic size difference between *P. g. glaucus* and *P. g. canadensis*, there is a significant influence of foodplant species and nutritional quality upon larval growth rate, pupal weight and adult wing size (Scriber, 1984; Scriber *et al.*, 1982).

Since size of any individual is variable with foodplant species and/or nutritional quality of the larval food, it would be inaccurate to base taxonomic inferences about a field-captured specimen on size or size-related characters alone. For example, we know that sizes of tiger swallowtails of particular subspecies or their hybrids will be smaller on *Populus tremuloides* Michx. (quaking aspen) and *Fraxinus americana* L. (white ash) than on *Prunus serotina* Ehrh. (black cherry) or *Liriodendron tulipfera* L. (tulip tree) (Scriber, 1982). In order to encompass such potential natural size variation, we included in our F_1 hybrid reference group, individuals which were laboratory-reared from foods at both ends of this quality spectrum. We believe that these reference hybrids represented the range of size variability that might be encountered in hybrid zones (*see* Fig. 7 for the wing length distribution of the three reference groups).

Forewing length is the best single character for discriminating between the reference groups. However, because of allometry (Atchley, 1983; Daly, 1985), we have the likelihood of size bias and confounding of the intrinsic value of certain other character traits.

TABLE 2a. — Standardized coefficients for the most significant canonical variates*

Variable	Standardized coefficients for canonical variates 1	2	Length = $\sqrt{C1^2 + C2^2}$
FWLN	−0.3352	−0.8911	0.9521
VFWSMBND	0.7190	−0.2742	0.7695
SPTINVAG	−0.1389	−0.5496	0.5669
LNYLSPT	0.1443	0.4686	0.4903
B = (ratio)	−0.2967	−0.3636	0.4693
CONCTMED	−0.2573	−0.1928	0.3215
BLUDCL	−0.2271	−0.0470	0.2319

TABLE 2b. — Log-transformed data with size (LOG FWLN) removed

Variable	Standardized coefficients for canonical variates 1	2	Length = $\sqrt{C1^2 + C2^2}$
VFWSMBND	0.6229	0.4179	0.7501
SPTINVAG	0.3627	−0.6414	0.7368
LNYLSPT	−0.2590	0.5823	0.6373
B = (ratio)	−0.5073	0.0661	0.5116
BLUDCL	−0.0858	−0.2466	0.2611
CONCTMED	−0.2314	−0.0150	0.2319
WDANLCL	−0.1900	0.0970	0.2133

*The standardization of these variables was done to better show the relative importance of these predictors of separation.

TABLE 3.—*A posteriori* classification (%) of Wisconsin male wing characters including size as a variable

County	*Canadensis*	Hybrid	*Glaucus*	(n)
Bayfield	100	0	0	35
Washburn	100	0	0	6
Sawyer	100	0	0	19
Price	97.1	2.9	0	34
Oneida	100	0	0	39
Marinette	100	0	0	19
Clark	100	0	0	8
Wood	100	0	0	9
Monroe	100	0	0	6
Juneau	94.1	5.9	0	17
Marquette	100	0	0	16
Richland	25	62.5	12.5	8
Dane	2.7	12.2	85.1	74

TABLE 4.—*A posteriori* classification (%) of Wisconsin male wing characters with size (FWLN) removed

County	*Canadensis*	Hybrid	*Glaucus*	(n)
Bayfield	80	8.6	11.4	35
Washburn	100	0	0	6
Sawyer	78.9	5.3	15.8	19
Price	64.7	17.7	17.6	34
Oneida	76.9	10.3	12.8	39
Marinette	94.7	5.3	0	19
Clark	100	0	0	8
Wood	88.9	11.1	0	9
Monroe	100	0	0	6
Juneau	70.6	23.5	5.9	17
Marquette	87.5	0	12.5	16
Richland	25	25	50	8
Dane	14.9	17.6	67.6	74

TABLE 5.—Classification of reference (known) specimens of *Papilio glaucus glaucus, P. g. canadensis,* and F_1 hybrids using only two characters (VFWSMBND and SPTINVAG; *see* characters 12 and 13 of Table 1 and Fig. 6k-6n)[1]

	VFWSBND						
	Spots		Intermediate		Band		% Correct
SPTINVAG	Yes	No	Yes	No	Yes	No	
P. g. glaucus	7	62*	1	2	0	0	62/72 = 86
P. g. canadensis	0	1	1	11	3*	75*	78/91 = 86
F_1 hybrid	60*	52	8*	27*	0	3	95/150 = 63
							235/313 = 75 Overall

[1]When a third character (FWLN) was added, the overall accuracy of classification was increased from 75%-84%, which is close to the 88.5% accuracy using discriminant analysis with FWLN included (*see* text). The correct classification is indicated by asterisks (*)

After analyzing the data without size, the two most important variables that maximize separation are VFWSMBND and SPTINVAG.

Without the aid of multivariate methods, these two characters alone can give excellent discriminatory results in classifying reference *Papilio glaucus glaucus*, *P. g. canadensis* and their hybrids (Table 5). Using categorical (noncontinuous) classification (*i.e.*, ventral forewing submarginal banding character (VFWSMBND) and spot invagination (SPTINVAG)), we can achieve an overall accuracy of 75% for all of the 313 reference

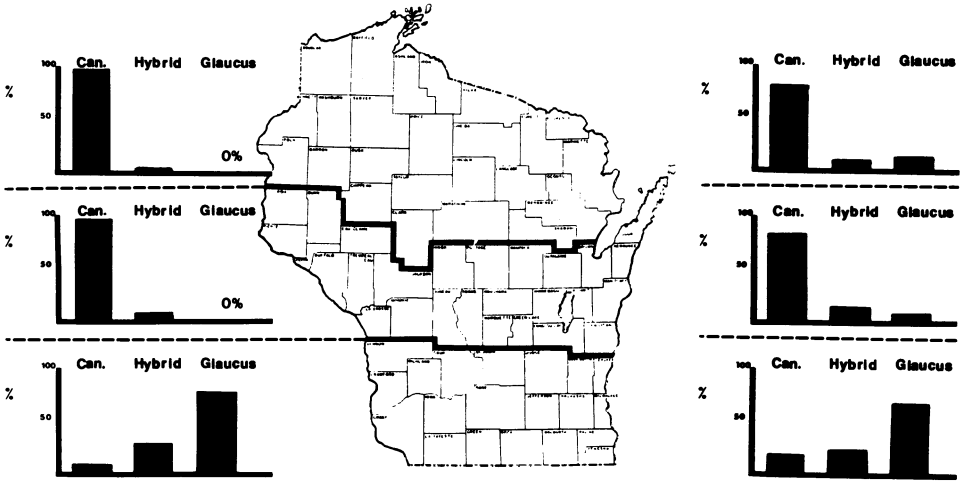

Fig. 6.—Regional multivariate analysis of unclassified data. Overall body size, FWLN, was included in the multivariate statistics (left). The histograms represent the proportion of each group (Northern region, n = 169; Central region, n = 60; Southern region, n = 88) classified as *P. g. canadensis*, *P. g. glaucus* or as "hybrid." Regional multivariate analysis of unclassified data with body size, FWLN, and allometric effects removed (right)

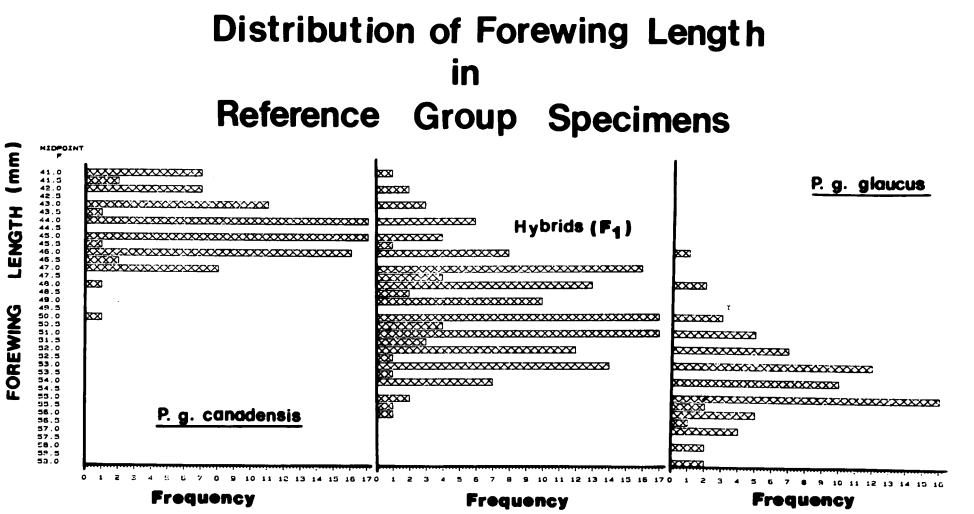

Fig. 7.—The distribution of forewing lengths (mm) of *P. g. canadensis*, F_1 hybrid, and *P. g. glaucus* reference groups

specimens (Table 5). The greatest error is with the hybrids where only 63% are correctly classified (Table 5). By using a third character, however (forewing length, FWLN) we achieved an overall accuracy of 84.3%, which is nearly as good as the multivariate technique.

When body size is removed from the data, the most obvious result is the decreased discriminatory abilities among the three reference groups. In general, however, for regional analysis the results give the same overall picture as those with body size included in the analysis (*see* Fig. 6). Research in quantitative genetics has shown body size to be moderately to highly heritable, often as much as 50% (Atchley, 1983). We recognize the nonheritable component of body size variation (*e.g.*, Scriber, 1982; Scriber *et al.*, 1982); however, because of the large number of genes that usually control body size, Atchley (1983) feels that it is generally a good character to include in morphometric analysis (Fig. 7; Atchley, 1983; Leamy and Thorpe, 1984).

Our study with adult wing morphometrics suggests that a significant change in taxonomic composition of tiger swallowtail butterflies is maintained with evidence of hybridization across a narrow zone in S-central Wisconsin. Data from this study are compatible with several other independent taxonomic/systematic techniques that we have used to elucidate the nature of this zone of overlap and suspected hybridization. It is intriguing that the ability of larvae to detoxify and grow upon tulip tree leaves, the genetic capacity for dark morph females, and the capacity for direct development (facultative diapause) all end abruptly in the area of Wisconsin which delineates the northernmost limits of *Papilio glaucus glaucus* as seen here in this morphometric study (Scriber, 1986a, 1988b; Scriber *et al.*, 1986; Rockey *et al.*, 1987).

Hagen (1986) showed, using protein electrophoresis, that there is also apparent hybridization occurring between *Papilio glaucus glaucus* and *P. g. canadensis* in northern Pennsylvania and central New York. His results indicate a potentially much broader hybrid zone through the Appalachian region than shown in this Wisconsin study. Further studies combining morphometrics with protein electrophoresis (*see* Berlocher, 1984) across this eastern and midwestern hybrid zone are in progress in our laboratory. We will specifically address the occurrence of the "spring form" of *P. g. glaucus* which more typically resembles the *P. g. canadensis* subspecies (Clark, 1932) and the environmental vs. genetic effects influencing its expression.

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