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MAINTENANCE OF ECOLOGICALLY SIGNIFICANT GENETIC VARIATION IN THE TIGER SWALLOWTAIL BUTTERFLY THROUGH DIFFERENTIAL SELECTION AND GENE FLOW

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Abstract.—Differential selection in a heterogeneous environment is thought to promote the maintenance of ecologically significant genetic variation. Variation is maintained when selection is counterbalanced by the homogenizing effects of gene flow and random mating. In this study, we examine the relative importance of differential selection and gene flow in maintaining genetic variation in *Papilio glaucus*. Differential selection on traits contributing to successful use of host plants (oviposition preference and larval performance) was assessed by comparing the responses of southern Ohio, north central Georgia, and southern Florida populations of *P. glaucus* to three hosts: *Liriodendron tulipifera, Magnolia virginiana,* and *Prunus serotina.* Gene flow among populations was estimated using allozyme frequencies from nine polymorphic loci. Significant genetic differentiation was observed among populations for both oviposition preference and larval performance. This differentiation was interpreted to be the result of selection acting on Florida *P. glaucus* for enhanced use of *Magnolia*, the prevalent host in Florida. In contrast, no evidence of population differentiation was revealed by allozyme frequencies. F_{ST}-values were very small and *Nm,* an estimate of the relative strengths of gene flow and genetic drift, was large, indicating that genetic exchange among *P. glaucus* populations is relatively unrestricted. The contrasting patterns of spatial differentiation for host-use traits and lack of differentiation for electrophoretically detectable variation implies that differential selection among populations will be counterbalanced by gene flow, thereby maintaining genetic variation for host-use traits.

Key words.—Host adaptation, larval performance, local selection, oviposition preference, *Papilio glaucus.*

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Natural insect populations commonly comprise an abundance of different genotypes. Much of this genetic variation is ecologically significant (e.g., Gould 1979; Via 1984; Jaenike 1989) and subject to selection. It is unclear why selectively important variation persists in natural populations, because much of this variation should be selectively eliminated as a population becomes increasingly adapted to local conditions. One hypothesis suggests that differential selection in a heterogeneous environment maintains selectively important genetic variation (Levene 1953; Gillespie 1973; Hedrick et al. 1976; Powell and Taylor 1979; Via and Lande 1985; Nevo 1988; Gillespie and Turelli 1989). This hypothesis is based on the assumption that different genotypes are more or less fit under different environmental conditions and that no single genotype is optimal under all conditions (Powell 1971; Powell and Taylor 1979). Such differential selection can preserve genetic variation when it is offset by the homogenizing effects of gene flow and random mating (Wright 1977; Futuyma 1986; Koehn and Hilbish 1987).

A major determinant of environmental heterogeneity for polyphagous herbivores encountering an array of plant species is host diversity. The physical and chemical attributes of different plant species can be highly variable (Rosenthal and Janzen 1979; Juniper and Southwood 1986) and particular insect genotypes are frequently affected differently by individual host species (e.g., Rausher 1984; Rossiter 1987). As such, different plants can be regarded as separate environments. The extensive variability in host use traits for herbivores feeding on an array of plant species suggests that host diversity is indeed an important determinant of genetic variation (e.g., Rausher 1984; Via 1984; Futuyma and Peterson 1985; Scriber 1986; Rossiter 1987; Nitao et al. 1991a). However, the extent to which variation in host-use traits reflects differential selection on different hosts and gene flow among these different environments remains largely uninvestigated.

Papilio glaucus L., the eastern tiger swallowtail butterfly, is well suited for examining the importance of host diversity and gene flow in maintaining genetic variation in host use traits. It is exceptional among swallowtails in its breadth of diet, using at least 18 different host species from seven plant families (Scriber 1984; Bossart and Scriber 1995). However, separate populations and even individuals within a population encounter only a subset of the potential hosts available (Scriber 1983, 1986). No single host plant has a geographical range that completely overlaps that of the butterfly. Moreover, the relative abundance of each host varies from site to site within its distribution. Previous studies documented variation in response to hosts by *P. glaucus* and *Papilio canadensis* (Scriber 1988; Scriber et al. 1989), and in part, lead to these former conspecifics being regarded as distinct species (Hagen et al. 1991). More recent studies documented variation in host use among populations within *P. glaucus* (Scriber 1986). However, these *P. glaucus* populations were not tested simultaneously, and it is unclear whether observed variation is genetically based or a reflection of environmental effects, such as seasonal variation in host quality.

In the current study, we investigated whether *P. glaucus* populations experience differential selection on locally abundant host species and, if so, whether this differential selection has resulted in genetic divergence among the populations. We contrasted the patterns observed for variation in host-use traits with those obtained from electrophoretic analysis. Our specific objectives were to (1) quantify genetic variation in

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P. glaucus for oviposition preference and larval nutritional physiology; (2) determine whether genetic differentiation for these traits has occurred among three geographically distant *P. glaucus* populations located along a north-south transect; and (3) use electrophoretic data to estimate rates of gene flow and examine population substructuring.

A secondary interest was to determine whether differentiation among populations was the result of adaptation for locally occurring hosts. Although demonstrating adaptation directly is difficult, it is possible to make certain predictions regarding the behavior of host-use traits in an adapted versus nonadapted population. We predicted that if locally abundant hosts are selective agents effecting differentiation among *P. glaucus* populations, developmental time and pupal mass would be positively correlated on local hosts but negatively correlated or uncorrelated on novel hosts. Such a result would be expected if adapted populations comprise mostly uniformly well-adapted genotypes expressing similar growth rates, whereas maladapted populations comprise a mixture of preadapted and nonadapted genotypes expressing variable growth rates. In an adapted population, variation for pupation time may be present, but additional time spent on a host should be manifest as additional mass gained. In a maladapted population, extended larval periods will be associated not only with variation in timing of pupation (preadapted genotypes) but also with unadapted genotypes that are unable to detoxify or otherwise use the novel host foliage. For these "sick" or "starving" larvae, additional time spent on a host will either not correlate, or negatively correlate, with additional mass gained.

Materials and Methods

General

Butterfly Sources.—Adult *Papilio glaucus* butterflies were collected from three geographic regions: southern Ohio (Lawrence County), north central Georgia (Clarke County) and southern Florida (Highland County). These regional populations span 1300 km, each separated from the nearest sampled region by approximately 650 km. Butterflies were collected from multiple sites in each region to ensure that samples were representative of variation in the region. Sites were separated by no more than 30 km and no less than 4 km. This distance among sites was small compared with the distance among regions. Collecting spanned a 7-to-10-d period during peak flight activity. The relative age of collected butterflies, as indicated by the wear condition of their wings (Lederhouse 1983), ranged from fresh, young butterflies showing virtually no wing wear, to well worn, older butterflies. The majority were relatively young or intermediate in age. Field-collected butterflies were placed in individual glassine envelopes, then transported on ice or shipped using overnight delivery to our laboratory at Michigan State University. All field-collected butterflies not used for oviposition were frozen at −80°C to preserve tissues for allozyme electrophoresis. Females to be used to produce eggs were fed a 20% honey solution immediately upon arrival, then kept at 24°C for 24 h. This procedure provided for maximum survival of ovipositing females. Females were individually placed in (10 cm × 20 cm × 27 cm) clear plastic "shoe boxes" (Tristate Plastics) with sprigs of appropriate foliage and fed a 20% honey solution daily. Boxes were maintained under artificial illumination, alternating 4h:4h photo:scotophase. This photoperiod permitted maximum oviposition, while preventing high mortality due to overheating. Eggs were collected daily and resulting neonate larvae used for the nutritional physiological studies and to generate adults for the oviposition assays.

Foliage.—Responses to *Magnolia virginiana* L. and *Liriodendron tulipifera* L. (Magnoliaceae), and *Prunus serotina* Ehrhart (Rosaceae), foliage were compared. All three hosts support generally high levels of survival and larval growth. The frequency of use of these three hosts is different among the three *P. glaucus* populations. Florida *P. glaucus* are largely restricted to *M. virginiana,* the only common host throughout much of peninsular Florida (Scriber 1986). In contrast, Georgia and Ohio populations rarely or never encounter *M. virginiana* but frequently encounter *L. tulipifera* and *P. serotina.*

Liriodendron tulipifera and *P. serotina* foliage was collected at least every third day from various areas in the vicinity of the Michigan State University campus and stored at 7°C. *Magnolia virginiana* foliage was collected daily from potted trees maintained on campus. Foliage sprigs presented to developing larvae and ovipositing females were placed in water-filled, rubber-capped, plastic vials to maintain leaf freshness.

Oviposition Preference

Oviposition preferences were assayed using progeny from field-collected females. To standardize for possible maternal effects, larvae were fed a common host (*P. serotina*). After emergence, females were hand-paired with males originating from the same population. Mated females were fed, then kept at 24°C for 24 h before testing. Emergence within populations spanned a 3-wk period from mid-July to mid-August, dictating the period over which the oviposition trials were conducted.

Preferences were assessed for two of the three host species, *M. virginiana* and *L. tulipifera.* Assays comparing *P. serotina* with the other hosts were not included because preliminary studies indicated that females from all populations rarely oviposited on *P. serotina* in choice tests when alternate hosts were available. Fresh host sprigs of similar leaf surface area were positioned in opposite corners along the long side of the box facing towards the lights. Females were free to move within the box and were commonly observed fluttering between host sprigs. The position of each sprig was alternated once a day to control for positional effects, and any wilted foliage was replaced. The position of individual boxes in relation to the light source was randomized at each feeding. After 4 d, the total number of eggs deposited on each host species was counted. The few stray eggs that were placed on the paper lining or box were generally adjacent to a particular leaf but were nonetheless excluded from our analyses. Preferences were calculated as the percentage of total eggs laid on each host over the 4-d trial. (Compared with an analysis of daily percentages, this technique is less sensitive to low daily egg numbers). Only females ovipositing at least 25

times were included in the analysis; 39 Ohio females, 40 Georgia females, and 32 Florida females met this criterion.

Larval Performance

The performance of larvae from each of the three populations was compared on each of the three hosts in two separate studies. In 1988, the performance of offspring of field-collected females was assessed. Due to differences in emergence times among populations, Florida larve could not be tested concurrently with Ohio and Georgia larvae. In 1989, we analyzed the performance of progeny generated from laboratory-reared females fed a common host (*P. serotina*) during their development. A similar protocol for testing larvae was followed in both studies. Eggs were checked for hatch every 2–3 h during the day. Resulting neonate larvae were weighed to the nearest milligram, then randomly selected and distributed across the three host species. Ten to 12 full siblings from each of 13 to 23 families from each population (5 to 16 each year) were allocated to each of the hosts. Larvae were reared individually to pupation in (150 mm × 25 mm) screened, plastic petri dishes containing foliage from one of the three host species (24°C, 18h:6h photo:scotophase). Larvae were checked daily. Fresh foliage was provided at least every other day. Larval duration, pupal mass, and sex were recorded for each individual. Larval duration was defined as the period from day of hatch to the prepupal stage, whereupon larvae cease feeding, void gut contents, and undergo a conspicuous color change from green to brown. Pupae were collected and weighed 24 h ecdysis, then placed in individual screen cages until adult emergence. Relative growth rate (RGR) was calculated as,

$$\mathrm{RGR} = (W_P - W_I)/\{([W_P + W_I]/2) \times D\},$$

where (W_I) is initial larval mass, (W_P) is pupal mass, and (D) is larval duration. Ratio-based indices of performance, such as RGR, should be used with caution since results can be misleading (Raubenheimer and Simpson 1992).

Pupal mass and larval duration tend to be sexually dimorphic in *P. glaucus*. Such dimorphism could confound our analyses if, by chance, some families were mostly, or entirely, all male, and others, all female. Sex could not be included as a source of variation in the ANOVA because not all families were represented by both sexes. To control for this potential confounding effect, mean male and female pupal mass and larval duration were compared for all families producing both sexes. Females on average tended to be 100 mg (8.0%) heavier and grew 1.2 d (4.4%) longer than males. Differences in larval duration and pupal mass between males and females did not differ between populations or hosts ($P > 0.3$, df = 2303 for all tests). The following regression equations were developed to adjust male values to those expected for females (Haukioja and Neuvonen 1985; Ayres et al. 1987; Nitao et al. 1991a):

$$\mathrm{mass}_{\mathrm{female}} = 0.151 + 0.937 \times \mathrm{mass}_{\mathrm{male}}$$
$$(P = 0.001;\ \mathrm{df} = 1,32;\ r^2 = 0.60).$$

$$\mathrm{days}_{\mathrm{female}} = 6.861 + 0.778 \times \mathrm{days}_{\mathrm{male}}$$
$$(P = 0.001;\ \mathrm{df} = 1,32;\ r^2 = 0.54).$$

All analyses of larval duration and pupal mass used these adjusted values.

Electrophoretic Analyses

Of the 26 consistently resolvable allozyme loci in *P. glaucus* (Hagen and Scriber 1991), 11 are polymorphic and useful for analyzing population substructuring. Loci were examined by electrophoresis on thin-layer cellulose acetate plates (see table 3). Approximately one-fourth to one-third of an individual butterfly abdomen was homogenized in 300 μl of extraction buffer and then centrifuged for 8 min at 16,000 × g. The resulting supernatants were electrophoresed on cellulose acetate plates (Helena Laboratories; Beaumont, Tex.). Buffer composition, electrophoretic conditions, and staining procedures were adapted from Harris and Hopkinson (1978) and Richardson et al. (1986) and follow those of Hagen and Scriber (1991). Alleles were designated according to their relative mobility; negative numbers were assigned to cathodally migrating allozymes. Three standards (i.e., butterflies previously scored for genotype on earlier runs) were included on subsequent plates to aid in scoring. As an additional check, every fifth plate was a rerun of 12 randomly chosen individuals from the four previous plates (3 from each).

Wright's F-statistics were estimated for each locus (Wright 1951; Weir and Cockerham 1984), using the computational methods of Long (1986). F_{ST} measures variance in allele frequencies among demes relative to the expectation when genes are randomly assorted among populations. F_{IT} measures deviations from homozygote frequencies expected in a panmictic population by estimating inbreeding within individuals relative to Hardy-Weinberg expectations for the total population. F_{IS} measures homozygote frequency relative to Hardy-Weinberg expectations within populations; positive values indicating a deficit of heterozygotes, negative values indicating an excess of heterozygotes. For neutral alleles, F_{ST} provides an indirect mechanism for estimating the degree of subdivision between populations and thus the relative strengths of gene flow and random drift (Nm):

$$(Nm)_{\mathrm{est}} = (1/F_{ST} - 1)/4$$

where N is the effective size of a population, and m is the number of migrants (Slatkin 1985, 1987).

All indirect and direct methods currently available for assessing gene flow have associated assumptions and drawbacks and often generate disparate estimates (Slatkin 1985, 1987; Johnson et al. 1988; Whitlock 1992). To compound the problem, patterns of population substructuring revealed from electrophoretic analyses, the classical and most widely used technique, may not agree with patterns revealed from other molecular techniques (Zink 1991; Karl and Avise 1992). Despite these problems, F_{ST}-values provide a conventional means of estimating gene flow for comparison with published values. It remains the most widely used method for estimating gene flow.

Results

Oviposition Preference

The mean number of eggs (±SE) laid by individual Ohio females was 102 ± 8, by Georgia females, 86 ± 8; and by

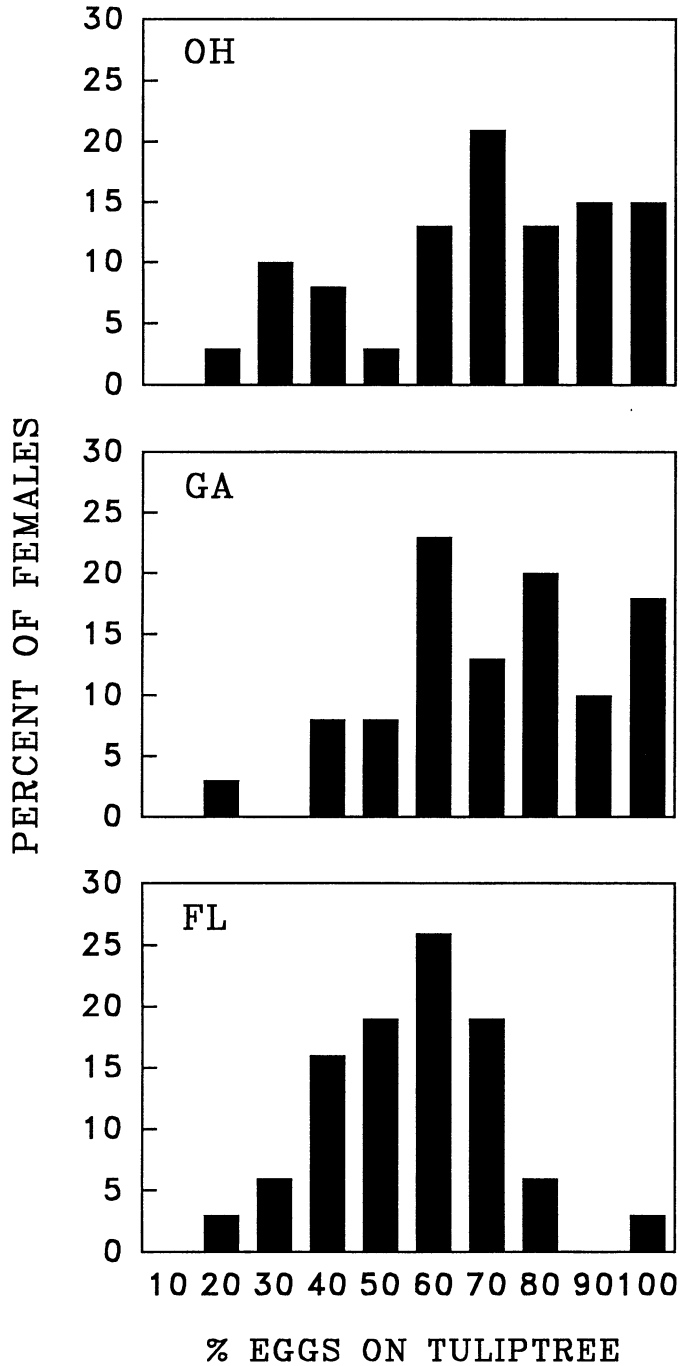

FIG. 1. Frequency distributions of *Liriodendron tulipifera* preference for Ohio (OH), Georgia (GA), and Florida (FL) *Papilo glaucus* females. Preferences were assayed for *L. tulipifera* and *Magnolia virginiana* in two-choice host trials. Bars to the right on the *x*-axis indicate increasing *L. tulipifera* preference, bars to the left, indicate increasing *M. virginiana* preference. $N = 39$ Ohio, 40 Georgia, and 32 Florida females tested.

Florida females, 94 ± 8. Most oviposition occurred during the first 2 d of the preference assays. Differentiation in preference for *Liriodendron tulipifera* and *Magnolia virginiana* was evident among the *Papilio glaucus* populations (fig. 1). Ohio and Georgia females were not only less likely than Florida females to oviposit on *M. virginiana,* the prevalent host in Florida, but clearly preferred *L. tulipifera,* a host commonly encountered in Ohio and Georgia. Approximately half of the Ohio and Georgia females tested (44% and 48%, respectively) laid more than 70% of their eggs on *L. tulipifera,* evidenced by distributions skewed to the right. In contrast, few of the Florida females tested (9%) laid more than 70% of their eggs on *L. tulipifera.* The majority of Florida females oviposited relatively equal numbers of eggs on both hosts, evidenced by a distribution centered around the 51%–60% category. A Kruskal-Wallis analysis on the ranks of these preferences confirmed the presence of a significant population effect ($P = 0.0048$, df = 2), resulting from deviations between the Ohio and Florida distributions, and the Georgia and Florida distributions (Tukey's studentized range test; $P = 0.05$, df = 108). The Ohio and Georgia distributions were not significantly different from each other.

Larval Performance

1988 Performance Study.—Papilio glaucus populations were significantly differentiated in their growth responses on the three hosts. There was an overall population effect on relative growth rate and on larval duration (table 1), as well as a significant interaction between host species and *P. glaucus* population for all three traits measured. For two of these traits, larval duration and relative growth rate, the patterns were identical. Relative growth rates (RGR)s, which incorporate both larval duration and pupal mass, are depicted in figure 2. Although Ohio larvae tended to grow slower than Florida larvae, this difference was most pronounced on *M. virginiana* (the prevalent Florida host) and was negligible on *L. tulipifera.* Georgia larvae exhibited an intermediate rate of growth on *M. virginiana.*

On *L. tulipifera* or *Prunus serotina,* Ohio larvae tended to grow at the slowest rate, Georgia larvae at the fastest rate and Florida larvae at an intermediate rate (fig. 2, 1988). Survival was similar for all three populations on all three hosts, ranging from 71% to 85% on *L. tulipifera,* 65% to 80% on *M. virginiana,* and 70% to 85% on *P. serotina.*

1989 Performance Study.—When progeny from laboratory-reared parents were assayed in 1989, the patterns of differentiation were similar to those observed in 1988 (table 2; fig. 2, 1989). Though not as pronounced, a latitudinal cline in relative growth rate was again observed on *M. virginiana,* but not on *L. tulipifera* or *P. serotina.* Overall survival was much lower in 1989, but again, was similar across hosts for all three populations, ranging from 25% to 40% on *L. tulipfera,* 21% to 40% on *M. virginiana,* and 17% to 23% on *P. serotina.*

Correlations between pupal mass and larval duration on local and nonlocal hosts were consistent with our prediction regarding expected patterns in an adapted versus maladapted population (fig. 3). In Florida *P. glaucus,* pupal mass, and larval duration were positively correlated on *M. virginiana,* the local host, but uncorrelated on *L. tulipifera.* This positive correlation on *M. virginiana* was significant even with the one outlying point eliminated ($r = 0.50$, $P = 0.03$). In contrast, in Ohio *P. glaucus* these traits were positively correlated on *L. tulipifera,* the local host, but negatively correlated on

TABLE 1. Mixed-model ANOVA of 1988 larval performance data comparing *Papilio glaucus* larvae from Ohio, Georgia, and Florida populations on *Liriodendron tulipifera, Magnolia virginiana,* and *Prunus serotina* foliage; NS, not significant at $P = 0.05$.

Trait	Source	df	MS	F^*	P
Relative growth rate	Population	2	0.0009	10.00	0.0001
	Host	2	0.0017	30.62	0.0001
	Population × host	4	0.0003	4.57	0.005
	Family(pop)	32	0.00009	2.36	0.0001
	Host × family(pop)	64	0.00006	1.42	0.025
	Error	315	0.00003		
Pupal mass	Population	2	0.013	0.12	NS
	Host	2	0.870	25.59	0.0001
	Population × host	4	0.092	2.71	0.05
	Family(pop)	32	0.106	3.12	0.0001
	Host × family(pop)	64	0.034	1.42	0.035
	Error	315	0.024		
Larval duration	Population	2	100.84	7.22	0.0001
	Host	2	238.42	25.32	0.0001
	Population × host	4	36.32	3.86	0.01
	Family(pop)	32	13.97	2.06	0.0001
	Host × family(pop)	64	9.41	1.39	0.025
	Error	315	6.79		

* The F-test denominator for population was MS_{family}. The F-test denominator for host and population × host was $MS_{host \times family}$. The F-test denominator for family and host × family was MS_{error}.

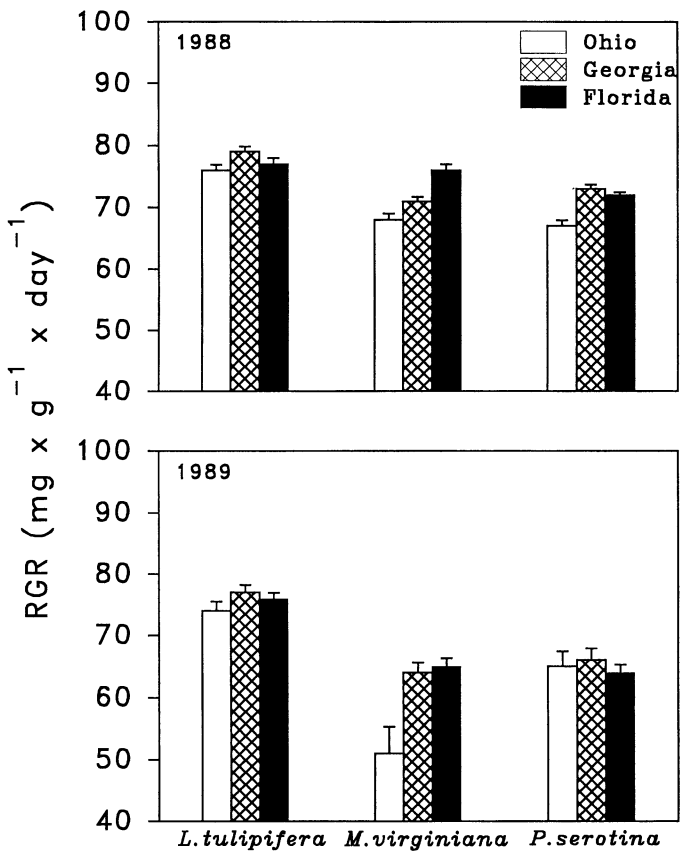

FIG. 2. Mean (± SE) relative growth rates (RGR) for Ohio, Georgia and Florida *P. glaucus* larvae reared on *L. tulipifera, M. virginiana* and *P. serotina.* Ten to 12 full-siblings from 13 to 23 families (5 to 16 each year) were allocated to each of the hosts.

M. virginiana, the novel host. Variances associated with mean growth rate (growth rate = pupal mass/larval duration) on *M. virginiana* also supported the prediction. (Variances among means were multiplied by the corresponding harmonic mean to obtain variance estimates). The variance among Florida families on this host, was less than the variance among Ohio families (120.99 vs. 289.37 for Florida and Ohio, respectively; $P < 0.05$; df = 26, 19). Variances associated with mean growth rate on *L. tulipifera* were not significantly different but were in the appropriate direction (151.08 vs. 237.20 for Ohio and Florida, respectively; $P > 0.1$, df = 19, 28).

Electrophoretic Analyses

Allele frequencies for *AC-2* and *MPI* could not be scored consistently and were excluded from the F-statistic analyses. Two loci, *P3GDH* and *TPI,* are X-linked, necessitating that females (the heterogametic sex in *Papilio*) be omitted from F-statistic calculations.

F_{IS} and F_{IT} values were small and nonsignificant at all loci except PEP-LA (table 3). At this locus, heterozygote frequencies were lower than expected. All F_{ST}-values were nonsignificant (table 3). The mean jackknife estimate of F_{ST} across loci was small and negative, indicating a virtual absence of genetic differentiation among populations (table 4). Negative values of F_{ST} were interpreted as $F_{ST} = 0$ (Long 1986) for the purpose of determining the relative strengths of gene flow and random drift (Nm). With negative values set equal to zero, mean $F_{ST} = 0.0017$, and $Nm = 147$.

When estimates of Nm are large, as is the case in this study, values will differ substantially depending on whether the estimator of F_{ST} employed is G_{ST} or theta (Slatkin and Barton 1989). Our estimate of $Nm = 147$ was calculated based on an F_{ST}-value using theta as the estimator. For comparative purposes, we also calculated Nm using an F_{ST} based on G_{ST} as the estimator. This mean jackknife estimate of F_{ST} across loci was determined to be 0.0025 (± 0.0013), generating an Nm value of 100.

TABLE 2. Mixed-model ANOVA of 1989 larval performance data comparing *Papilio glaucus* larvae from Ohio, Georgia, and Florida populations on *Liriodendron tulipifera, Magnolia virginiana,* and *Prunus serotina* foliage; NS, not significant at $P = 0.05$. The family(pop) component could not be estimated because not all families were represented across all hosts.

Trait	Source	df	MS	*F*	*P*
Relative growth rate	Population	2	0.0004	6.68	0.0016
	Host	2	0.0034	68.22	0.0001
	Population × host	4	0.0002	4.21	0.0029
	Error	161	0.00005		
Pupal mass	Population	2	1.427	72.56	0.0001
	Host	2	0.177	8.99	0.0002
	Population × host	4	0.020	1.03	NS
	Error	161	0.020		
Larval duration	Population	2	198.81	8.47	0.0003
	Host	2	1012.13	43.14	0.0001
	Population × host	4	163.49	6.97	0.0001
	Error	166	23.46		

DISCUSSION

Interpretation of Variation in Host-Use Traits

Like many herbivorous insects, *Papilio glaucus* exhibits substantial variation for ecologically important host-use traits (Ayres et al. 1991; Bossart and Scriber 1995). At least part of this variation is associated with the differential use of local host species. We believe the differences among populations are genetically based. Because the preference assays and 1989 performance assay tested the three populations simultaneously, using adults reared in the lab on a common host, the differences observed are likely not simply due to maternal or environmental effects. We suggest that differentiation has occurred among *P. glaucus* populations in both oviposition preference and larval performance, as a result of selection for enhanced recognition of and performance on a locally abundant host.

Of the three hosts tested, we conclude that selection acting on the Florida population for enhanced use of *Magnolia virginiana* is largely responsible for much of the geographic population differentiation. We draw this conclusion based on three results. First, Florida *P. glaucus* grew at a faster rate on *M. virginiana* (the prevalent Florida host) than either of

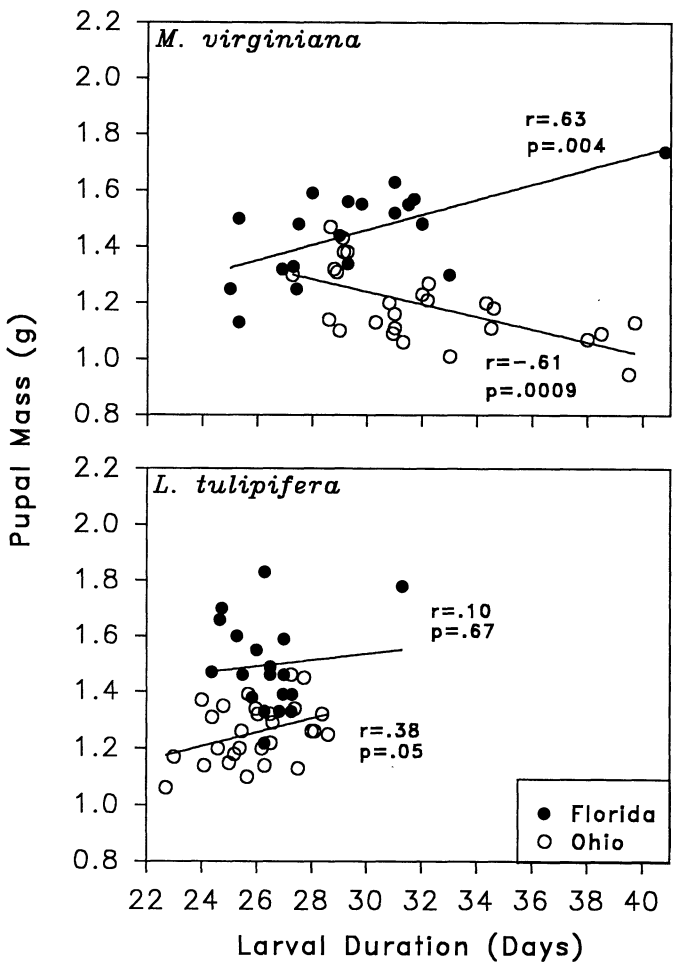

FIG. 3. Correlation between pupal mass and larval duration for Ohio and Florida *P. glaucus* larvae reared on *L. tulipifera* and *M. virginiana.* Each point represents the mean of 3 to 30 full-siblings. The analysis was performed on data pooled from 1988 and 1989.

TABLE 3. Wright's *F*-statistics for *Papilio glaucus* population samples from Ohio, Georgia, and Florida. $N = 48–74$, except for the X-linked loci, *P3GDH* and *TPI,* where $N = 23–25$. Numbers in parentheses are standard deviations and were obtained by jackknifing over populations.

Locus	F_{IS}	F_{ST}	F_{IT}
AC-1	−0.0263	−0.0057	−0.0321
	(0.0481)	(0.0014)	(0.0472)
GPI	−0.0768	−0.0039	−0.0809
	(0.0210)	(0.0011)	(0.0223)
HBDH	−0.0056	−0.0031	−0.0088
	(0.0233)	(0.0010)	(0.0242)
IDH-1	0.0517	−0.0040	0.0479
	(0.1053)	(0.0035)	(0.1035)
IDH-2	0.0231	−0.0014	0.0218
	(0.0154)	(0.0046)	(0.0165)
P3GDH	0.1156	−0.0172	0.1004
	(0.1517)	(0.0078)	(0.1535)
PEP-LA	0.3051	0.0155	0.3158
	(0.0992)	(0.0149)	(0.0908)
PGM	0.0302	−0.0037	0.0266
	(0.0361)	(0.0022)	(0.0373)
TPI	0.1886	−0.0007	0.1880
	(0.1884)	(0.0156)	(0.1960)

TABLE 4. Means and 95% confidence intervals (CI) for Wright's *F*-statistics. Confidence intervals were obtained by bootstrapping over loci (Weir 1990).

	Arithmetic means	Jackknife means	95% CI	
F_{IS}	0.0356	0.0327	−0.0096	0.1007
F_{ST}	−0.0032	−0.0033	−0.0056	0.0002
F_{IT}	0.0325	0.0275	−0.0145	0.1001

the other populations. (It is noteworthy that selection for enhanced use of *M. virginiana* did not decrease performance on *Liriodendron tulipifera,* suggesting the absence of a trade-off between use of *L. tulipifera* and use of *M. virginiana*). In contrast, neither the Ohio nor Georgia *P. glaucus* populations exhibited enhanced performance on *L. tulipifera* or *Prunus serotina,* their locally abundant hosts. Second, Georgia larvae tended to grow at an intermediate rate on *M. virginiana.* This pattern is consistent with what would be expected if a genotypic cline has formed as the result of gene flow between an adapted Florida population and a nonadapted Ohio population. Third, Florida *P. glaucus* exhibited an increased rate of oviposition on *M. virginiana.* This pattern is counter to what has normally been observed in *Papilio* spp. In general, *L. tulipifera* is a preferred oviposition substrate, even for those *Papilio* species in which *L. tulipifera* foliage is toxic to developing larvae (Scriber et al. 1991a,b). The oviposition patterns exhibited by Ohio and Georgia females seem to reflect this same general willingness to oviposit on *L. tulipifera.*

The correlations observed between pupal mass and larval duration and associated variances on *M. virginiana* further support our interpretation of *M. virginiana* as an important selective agent. In the Florida population, larval duration and pupal mass were positively correlated on *M. virginiana,* and the variance was low, reflecting uniform rates of growth among families. Such was not the case in the Ohio population in which these two traits were negatively correlated and the variance was high, indicating variable rates of growth among families and the presence of genotypes that were not adapted to use *M. virginiana.*

Liriodendron tulipifera and *P. serotina* appear to be less important in effecting genetic differentiation among populations. All three populations grew at relatively equal rates on these two hosts. In fact, the Florida population, a population not sympatric with tuliptree or black cherry, and rarely, if ever, encountering these hosts, had little problem feeding on either tuliptree or black cherry. It may be that *L. tulipifera* and *P. serotina* are more acceptable hosts in general. A mixture of neolignans, compounds present in *M. virginiana* foliage, is known to decrease performance in unadapted Ohio *P. glaucus* populations (J. K. Nitao et al. unpubl. data), as well as unadapted *Callosamia* spp., the polyphagous *Hyphantria cunea, Aedes aegypti, Artemia salina,* and other *Papilio* spp. (Nitao et al. 1991b, 1992; Johnson 1993; J. K. Nitao et al. unpubl. data). This pattern of differential use of neolignans is consistent with our performance studies.

Interpretation of Electrophoretic Variation

The lack of differentiation in allele frequencies among populations results either from selective pressures that maintain these frequencies, gene flow among populations, or some combination of gene flow and selection. To invoke an overall selective interpretation to account for the pattern observed would require that selection operate in such a way as to generate parallel patterns among all three *P. glaucus* populations at nine presumably independent, polymorphic loci. Such an interpretation is statistically unlikely. Moreover, selection should produce heterogeneity among loci (Slatkin 1987). Given the improbability that selection accounts for the lack of differentiation of allele frequencies across all nine loci, then at least part of this genetic homogeneity must be due to gene flow among populations. That our F_{ST}-values are comparable with those observed for other highly mobile insects (see McCauley and Eanes 1987) supports this interpretation.

Scoring error due to nondetectable variation is a caveat that must be considered when interpreting F_{IS}-values. Such error is inherent in most electrophoretic analyzes and may prevent distinguishing heterozygotes from homozygotes if different alleles migrate to very similar regions. Erroneously scoring heterozygotes as homozygotes (or vice versa) would generate inaccurate estimates of genotypic frequencies and inflated F_{IS}-values. In this study, significant F_{IS}-values were initially observed at AC-2, MPI and PEP-LA. Because scoring inconsistencies could not be eliminated as the basis for the significant F_{IS}-values at *AC-2* and *MPI,* these loci were excluded from the *F*-statistic analysis. Scoring inconsistencies were not a problem with regard to *PEP-LA.*

Differential Selection versus Gene Flow

For neutral alleles, the absence of substructuring among *P. glaucus* populations is due to some combination of gene flow or effective population size. However, determining the relative importance of either factor is difficult because effective population size is largely a function of gene flow. Mark-recapture studies suggest that *P. glaucus* butterflies are capable of high mobility and sustained flight in New York (Lederhouse 1983), Florida (R. C. Lederhouse and J. M. Scriber unpubl. data) and Ohio (J. L. Bossart unpubl. data). Suitable habitat occurs continuously throughout much of the butterfly's range, and major ecological constraints seem unlikely. Even in southern Florida where suitable habitat is very patchy (Lederhouse and Scriber 1987) and large citrus orchards predominate, adequate hosts and nectar sources for *P. glaucus* are scattered throughout most residential areas and around the numerous small lakes, providing sufficient opportunity for butterfly movement among adjacent habitat patches. Only a few migrants are necessary to prevent random drift irrespective of population size (Slatkin 1987).

Our electorphoretic analysis indicates that genetic exchange occurs among *P. glaucus* populations. However, completely unrestricted gene flow seems unlikely because extensive gene flow would necessitate strong selection to permit differentation in oviposition preference and larval physiology. The fitness differences documented in this study appear to be more subtle and not sufficiently strong to counter unrestricted gene flow. Mortality rates were relatively equal across hosts, and differences in fitness were due to differences in relative rates of growth. Such differences would likely

translate into an increased probablity of death through attack by predators or parasitoids (Feeny 1976) but would probably not result in absolute mortality of nonadapted genotypes. That these more subtle selective factors have been able to effect genetic differentiation in host-use traits, implies that gene flow among *P. glaucus* populations is, at least, partially constrained. Our direct observations of *P. glaucus* movement in the field suggest that variation in propensity to leave an area, habitat quality, and habitat patchiness are each important in determining how readily butterflies move about. We hypothesize that such factors preclude unlimited migration and gene flow among *P. glaucus* populations, thereby enabling selection to operate at a more moderate level. Of course, this interpretation assumes that rates of mortality observed in the laboratory, are reflective of those occurring in nature.

The presence of negative genetic correlations across environments is required for the maintenance of genetic variation if gene flow is equal between environments. In their absence, optimization across hosts will eventually evolve such that genotypes with high fitness on all hosts will predominate throughout all populations. In light of this prediction, the apparent absence of trade-offs in performance of *P. glaucus* on different hosts, combined with the lack of optimization across hosts must be addressed. Selection on Florida *P. glaucus* for enhanced use of *M. virginiana* did not appear to be associated with a decreased ability to use either *L. tulipifera* or *P. serotina.* Moreover, even though at least 20,000 generations have elapsed since glacial retreat during the Pleistocene and current host distributions were established, the Ohio population in particular continues to harbor a high frequency of genotypes not adapted to *M. virginiana* (see also Bossart 1993); presumably, ample time for selection to optimize performance. We offer three explanations for these contradictory patterns. First, it may be the case that host trade-offs do exist but are associated with hosts not examined here or are unrelated to nutritional physiology per se (e.g., the ability to tolerate or minimize attack by natural enemies associated with different hosts). Second, there may not be a single, optimum genotype, such that a state of equilibrium is never obtained. Rather, the optimum genotype may be dynamic, reflecting a stochastic, constantly changing environment. Third, gene flow may not be balanced between environments.

The geographic population differentiation in oviposition preference and larval performance in *P. glaucus* contrasts with the lack of any such patterns for electrophoretically detectable variation. *Papilio glaucus* behaves as one large population with regard to allozyme variation, but not with regard to variation in host use traits. These contrasting patterns imply that differential selection among populations (also observed within populations; Bossart 1993) for host preference and larval performance will be counteracted by gene flow, thereby maintaining genetic variation for these ecologically important traits. Key to this argument are environmental heterogeneity, genetic variation for traits associated with fitness, and genotype-by-environment interaction (Mitchell-Olds 1992). These three criteria are satisfied by *P. glaucus.* Genetic variation was documented for both oviposition preference and larval physiology. More importantly, this variation was associated with differential recognition of and performance on different host species. In the absence of more substantial barriers to gene flow, we predict that *P. glaucus* will continue to be comprised of a mosaic of genotypes exhibiting differing abilities to use different host species.

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