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Differential selection and adaptation in different
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in host use traits in the tiger swallowtail butterfly,
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Janice L. Bossart

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DIFFERENTIAL SELECTION AND ADAPTATION IN DIFFERENT HOST ENVIRONMENTS: GENOTYPIC AND PHENOTYPIC VARIATION IN HOST USE TRAITS IN THE TIGER SWALLOWTAIL BUTTERFLY, *PAPILIO GLAUCUS* L.

By

Janice L. Bossart

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ABSTRACT

DIFFERENTIAL SELECTION AND ADAPTATION IN DIFFERENT HOST ENVIRONMENTS: GENOTYPIC AND PHENOTYPIC VARIATION IN HOST USE TRAITS IN THE TIGER SWALLOWTAIL BUTTERFLY, *PAPILIO GLAUCUS* L.

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Host plant use by insect generalists is a function of the insect's ability to behaviorally recognize and physiologically use different host species. Hence, the evolution of host associations in phytophagous insects will depend upon the extent of adaptively significant genetic variation for these behavioral and physiological traits, and the factors maintaining this variation. Evaluating the extent of genetic variation for host preference and larval tolerance in the polyphagous, tree-feeding species, *Papilio glaucus*, the eastern tiger swallowtail butterfly, was the impetus for the research presented in this dissertation. Specifically, I examined whether Ohio, Georgia and Florida *P. glaucus* populations and families within populations responded differently to different host species, and whether the response was genetically based and adaptively significant. I compared patterns observed for this ecologically important variation with those observed for electrophoretically detectable variation.

Adaptively significant genetic variation for host preference and larval tolerance was present among and within *P. glaucus* populations. Geographic populations expressed differing abilities to recognize and use locally abundant hosts, presumably as a result of selective pressures imposed by these hosts. Significant interactions were present between host species and families,

establishing that polyphagy in *P. glaucus* is a function of a collection of differentially adapted genotypes, rather than a single, particularly robust genotype. Physiological tradeoffs in the ability to use *M. virginiana* and *L. tulipifera* may, in part, underlie this differential performance.

The geographic differentiation observed for larval tolerance and host preference contrasted with the lack of any such pattern for electrophoretically detectable, presumably neutral variation. The electrophoretic results indicated that gene flow is common among *P. glaucus* families and populations. The lack of optimization across hosts, despite ample opportunity, suggested that differential selection on ecologically important variation in *P. glaucus* will continue to be countered by gene flow, thereby maintaining genetic variation for host-use traits. In the absence of more substantial barriers to gene flow, the eastern tiger swallowtail will continue to be comprised of a mosaic of genotypes exhibiting differing abilities to use different host species.

ACKNOWLEDGEMENTS

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GENERAL INTRODUCTION

Host plant use by insect generalists is a function of both physiological and behavioral traits, which are, in turn, governed by both genetic and nongenetic components. Ecological factors exerting selective pressure operate only on genetic variance. Yet, little is known regarding the extent of interspecific genetic variation for host preference and larval performance, and even less, regarding intraspecific genetic variation (Futuyma 1991, Futuyma and Peterson 1985).

Genetic variation for host-use traits is generally extensive in generalist herbivore populations. This is not surprising given the multitude of selective factors associated with different host species that shape patterns of host-use in insects. Different hosts present unique selective environments, reflecting differences in physical and chemical composition (Fox and Morrow 1981), associated natural enemies or competitors (Bernays and Graham 1988, Strong et al. 1984), microclimates (Grossmueller and Lederhouse 1985), and abundance and distribution (Gilbert and Singer 1975). The probability that a single genotype is able to respond optimally in all situations seems remote. That different genotypes are optimal in different situations seems much more likely. Such differential selection in an ecologically diverse, stochastically changing environment, is thought to explain the persistence of much of the genetic variation for host-use traits observed in natural populations (Hedrick et al. 1976, Hedrick 1986, Koehn and Hilbish 1987, Powell and Taylor 1979). Genotypes selectively eliminated on one host species or in one population will persist as long as gene flow occurs among populations and mating is mostly random within populations. That differential selection, in part, serves to maintain genetic variation seems even more likely given that most host-use traits are probably polygenic.

The amount of genetic variation harbored by a species determines the ability of the species to adapt to environmental conditions that vary through space and time. Evaluating the extent of genetic variation in traits affecting the use of different hosts and the factors maintaining this variation is a necessary requisite of studies examining the evolution of host associations in phytophagous insects. Such information is especially important with regard to species that use multiple hosts or span a large geographic area, since environmental heterogeneity is greatest for these species.

The tree-feeding *Papilio spp.* group is perhaps the most intensively studied group of generalist herbivores to date. All members of this group, *P. glaucus*, *P. canadensis*, *P. eurymedon*, *P. alexiares*, *P. rutulus* and *P. multicaudatus*, are characterized by their ability to feed on several host families (Scriber et al. 1991). Genetically based variation in host associations appears to be a major factor delimiting the geographic range of this group (Scriber and Lederhouse 1992). The abundance of information gathered on the *Papilio spp.* group far exceeds that for most other generalist insects. Yet even in this well studied group, the extent of genetic variation for host-use traits among populations within a species (although see Scriber 1986), and among individuals within populations remains virtually unknown. Most initial studies focused on interspecific variation and, in particular, on interspecific variation between *P. canadensis* and *P. glaucus*, two closely related sister taxa. The discovery of genetically based differences in host use between these two species (Lindroth et al. 1986, Scriber 1982, 1988, Scriber et al. 1989), in combination with differences in diapause physiology (Rockey et al. 1987a, 1987b), color polymorphisms (Scriber 1988, Hagen and Scriber 1989), wing morphometrics (Luebeke et al. 1988), allozymes (Hagen and Scriber 1991), and mitochondrial restriction sites (Sperling 1991), recently led to the designation of *P. canadensis* and *P. glaucus* as distinct species (Hagen et al. 1991). These sister taxa are prime examples of species that are subjected to extensive environmental heterogeneity. In combination, *P. glaucus* and *P. canadensis* utilize hosts from approximately 9 plant families and their geographic range covers much of North America. The transition zone between the two species occurs through central Wisconsin and Michigan and extends eastward through central New York and southern New England (Scriber 1988), corresponding with the transition between boreal coniferous forests to the north and mixed deciduous forests to the south.

P. glaucus, the eastern tiger swallowtail butterfly, is especially amenable to an analysis of the interplay among environmental heterogeneity, differential selection, and gene flow and random mating within a single species. *P. glaucus* is the most polyphagous member of the *Papilio spp.* group, using hosts from at least 7 different plant families. No single host species is coincident with the geographic range of *P. glaucus*, an area which extends south from Michigan to Florida, west to Texas, then south into Mexico. Although most *P. glaucus* populations encounter multiple hosts species, others are virtually restricted to a single local host.

The issues outlined in the preceding paragraphs served as the impetus for the following dissertation. Specific objectives included establishing whether *P. glaucus* populations and families within populations responded differentially to different host species, whether this response was genetically based and adaptively significant, and whether gene flow was common. These objectives were addressed with regard to two important host use traits, larval tolerance and female preference, for *P. glaucus* populations from southern Ohio, north central Georgia, and southern Florida. Specific populations were selected based on the probability that they represented "pure" *P. glaucus*, their location along a north-south transect, and the local abundance of host species. The dissertation is presented as three separate manuscripts. The first manuscript addresses the hypothesis of differential selection and gene flow, the second, is a comprehensive analysis of the genetic and nongenetic components of female host selection, and the final manuscript examines intrapopulation genetic variation and covariation in larval performance across hosts.

MANUSCRIPT 1

Maintenance of ecologically significant genetic variation in the tiger swallowtail butterfly through differential selection and gene flow

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 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 nutritional physiology) 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 8 polymorphic loci.

Significant genetic differentiation was observed among populations for both oviposition preference and larval performance. This differentiation can be interpreted as the result of selection for enhanced use of *Magnolia*, the prevalent host in Florida, by Florida *P. glaucus*. 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. Results suggest that most of the electrophoretically detectable variation is neutral and that gene flow among *P. glaucus* populations is common. The contrasting patterns of spatial differentiation for ecologically important variation and lack of differentiation for neutral variation implies that differential selection among populations will be conterbalanced by gene flow, thereby maintaining genetic variation for host use traits.

Introduction

Natural insect populations are commonly comprised of an abundance of different genotypes. In many cases, these different genotypes reflect heritable differences for ecologically significant traits (e.g., Gould 1979, Jaenike 1989, Via 1984). It is unclear how such non-neutral variation persists in natural populations. Much of the variation (i.e., that which is nonadaptive) 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 (Gillespie 1973, Gillespie and Turelli 1989, Hedrick et al. 1976, Levene 1953, Nevo 1988, Powell and Taylor 1979, Via and Lande 1985). In part, 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 in a population when it is offset by the homogenizing effects of gene flow and random mating (Futuyma

1986). A genotype selectively eliminated from a population under unfavorable conditions can be reintroduced to the gene pool as a result of gene flow from favorable environments and random mating among individuals (Koehn and Hilbish 1987). As a consequence, a balance may be achieved between selection, which acts to reduce genetic variation, and gene flow and random mating, which serve to counteract this loss (Slatkin 1973).

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 herbivores with a particular genotype are frequently affected differently by individual host species. 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., Futuyma and Peterson 1985, Nitao et al. 1991a, Rausher 1984, Rossiter 1987, Scriber 1986, Via 1984). However, the extent to which this variability might be the consequence of 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 in its breadth of diet, using at least 18 different host species from 7 plant families (Bossart and Scriber 1993, Scriber 1984). However, separate populations and even individuals within a population encounter only a subset of the potential hosts available (Scriber 1983, 1986). No single host has a range which 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 *P. canadensis* (Scriber 1988, Scriber et al. 1989), and in part, lead to these former conspecifics being regarded as distinct species (Hagen et al. 1992). 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 ecologically important traits with those obtained from electrophoretic analyses of loci ostensibly reflecting selectively neutral variation (Karl and Avise 1992). Our specific objectives were to 1) quantify genetic variation in *P. glaucus* for two ecologically important host use traits, 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 if differentiation among populations was the result of local adaptation. Although demonstrating adaptation directly is difficult, it's 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, then development time and pupal mass would be positively correlated on local hosts but negatively correlated or uncorrelated on rare hosts. Such a result would be expected if adapted populations are mostly comprised of uniformly well adapted genotypes, while nonadapted populations are comprised of a mixture of preadapted and nonadapted genotypes.

Materials and Methods

General

Butterfly Sources: Adult *P. glaucus* butterflies were collected from field populations in southern Ohio (Lawrence Co.), north central Georgia (Clarke Co.) and southern Florida (Highland Co.). These populations span 1300 km, each separated from the nearest sampled population by approximately 650 km. Butterflies were collected from multiple sites in each region. Sites were separated by no more than 30 km and no less than 4 km. 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 generate 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 x 20 cm x 27 cm) clear plastic "shoeboxes" (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 physiology 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 performance. 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*.

L. 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. *M. 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 minimize 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 prior to testing. Emergence within populations spanned a three week 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 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 days, 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 nonetheless excluded from our analyses. Preferences were calculated as the percentage of total eggs oviposited on each host over the 4-day trial. (Compared to an analysis of daily percentages, this technique is less sensitive to low daily egg numbers). Only females ovipositing at least 25 eggs were included in the analysis; 39 Ohio females, 40 Georgia females and 32 Florida females met this criteria.

Larval nutritional physiology

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 eclosion every 2-3 h during the day. Resulting neonate larvae were weighed to the nearest mg, then randomly selected and distributed across the three host species. Ten to twelve full-siblings from 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 cm x 25 cm) screened, plastic petri dishes containing the appropriate host foliage (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 eclosion 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 after shedding their larval exoskeleton, then placed in individual screen cages until adult emergence. Relative growth rate (RGR) was calculated as,

$$RGR = (W_P - W_I)/((W_P - W_I/2) \times D)$$, where

(W_I) is initial larval mass,

(W_P) is pupal mass, and

(D) is larval duration.

Electrophoretic analyses

Of the 26 consistently resovable allozyme loci in *P. glaucus* (Hagen and Scriber 1991), twelve are polymorphic and useful for analyzing population substructuring. Loci were examined using electrophoresis on thin-layer cellulose acetate plates (see Table 3). Approximately 1/4 to 1/3 of butterfly abdomen was homogenized in 300 μl of extraction buffer and then centrifuged for 8 minutes at 16,000 x g. The resulting supernatants were electrophoresed on cellulose acetate plates (Helena Laboratories; Beaumont, Texas). Buffer composition, electrophoretic conditions and staining procedures were adapted from Richardson et al. (1986) and Harris and Hopkinson (1978) 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 4 previous plates (3 from each).

Wright's F-statistics were estimated for each locus (Wright 1951, Weir and Cockerham 1984, 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*):

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

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 contradict patterns revealed from other molecular techniques (Karl and Avise 1992). Despite these problems, F_{ST} values can 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

Genetic differentiation in preference for *L. tulipifera* and *M. virginiana* was evident among the *P. glaucus* populations (Figure 1). Ohio and Georgia females were not only less willing 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) oviposited greater than 70% of their eggs on *L. tulipifera*, evidenced by distributions skewed to the right. In contrast, few of the Florida females tested (9%) oviposited greater 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 pressence of a significant population effect ($p=0.0048$, df=2), resulting from deviation between the Ohio and Florida distributions, and the Georgia and Florida distributions (Tukey's studentized range test; $p=.05$, df=108). The Ohio and Georgia distributions were not significantly different from each other.

Larval nutritional physiology

1988 Performance Study: Populations were significantly differentiated in their growth responses. There was an overall population effect on relative growth rate and on larval duration (Table 1), as well as a significant interaction

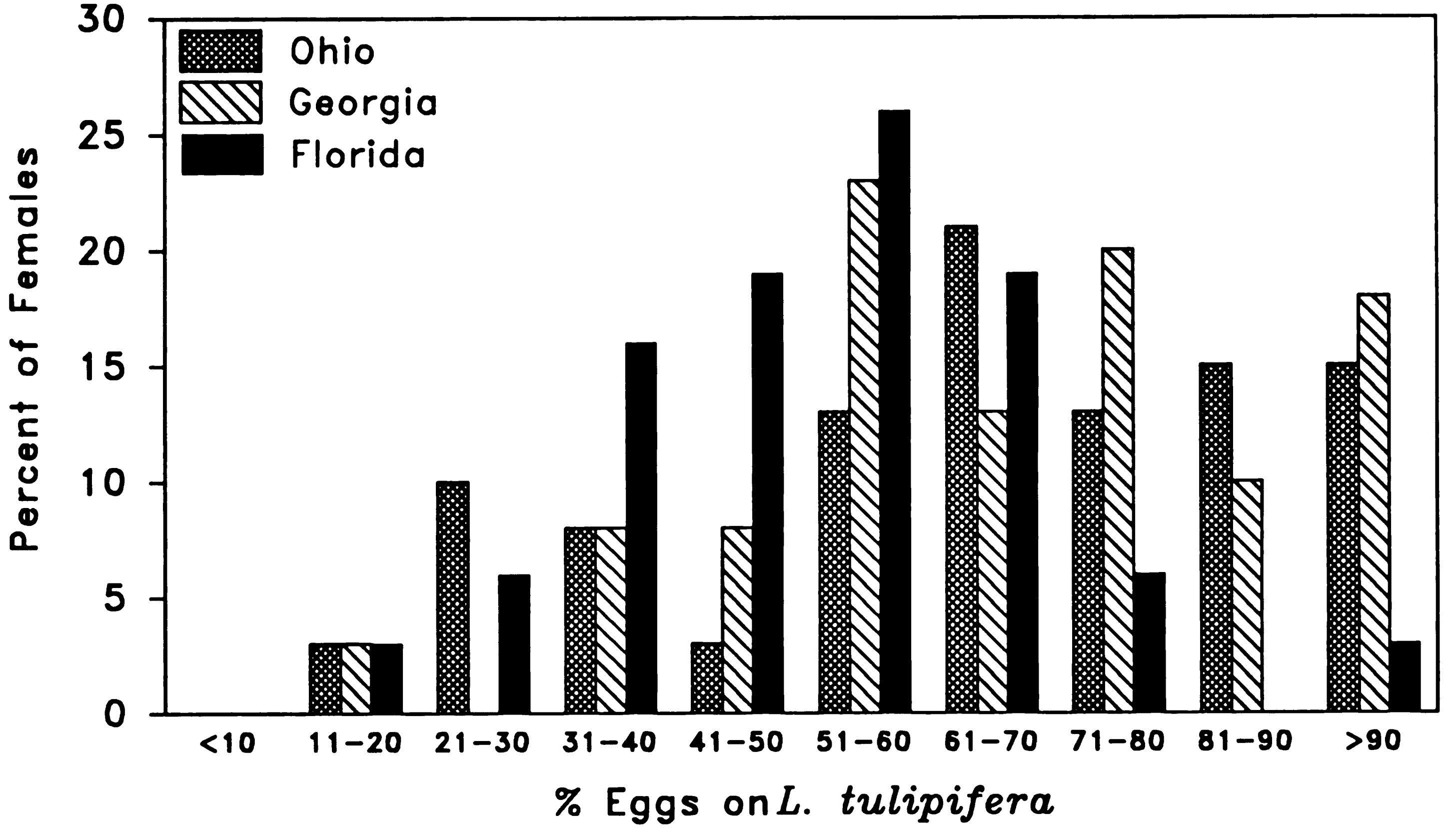

Figure 1. Frequency distributions of *L. tulipifera* preference for Ohio, Georgia and Florida *P. glaucus* females. Preferences were assayed for *L. tulipifera* and *M. virginiana* in 2-choice host trials. Bars to the right on the x-axis indicate increasing *L. tulipifera* preference, bars to the left, indicate increasing *M. virginiana* preference. N = 39 Ohio, 40 Georgia and 32 Florida females tested.

Table 1. Mixed model analysis of variance of 1988 larval performance data comparing *P. glaucus* larvae from Ohio, Georgia and Florida populations on *L. tulipifera*, *M. virginiana* and *P. serotina* foliage; ns = not significant at p = .05.

Trait	Source	df	MS	F^a	p
Relative Growth Rate	Population	2	.0009	10.00	.0001
	Host	2	.0017	30.62	.0001
	Population x Host	4	.0003	4.57	.005
	Family(Pop)	32	.00009	2.36	.0001
	Host x Family(Pop)	64	.00006	1.42	.025
	Error	315	.00003		
Pupal Mass	Population	2	.013	.12	ns
	Host	2	.870	25.59	.0001
	Population x Host	4	.092	2.71	.05
	Mother(Pop)	32	.106	3.12	.0001
	Host x Family(Pop)	64	.034	1.42	.035
	Error	315	.024		
Larval Duration	Population	2	100.84	7.22	.0001
	Host	2	238.42	25.32	.0001
	Population x Host	4	36.32	3.86	.01
	Family(Pop)	32	13.97	2.06	.0001
	Host x Family(Pop)	64	9.41	1.39	.025
	Error	315	6.79		

[a] The F test denominator for Population was MS_{Family}.
The F test denominator for Host and Population x Host was $MS_{Host\ x\ Family}$.
The F test denominator for Family and Host x Family was MS_{Error}.

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; only relative growth rates are depicted since this variable incorporates both larval duration and pupal mass (Figure 2, 1988). 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 *P. serotina*, Ohio larvae tended to grow at the slowest rate, Georgia larvae at the fastest rate and Florida larvae at an intermediate rate (Figure 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 lab-reared parents were assayed in 1989, the patterns of differentiation were similar to those observed in 1988 (Table 2; Figure 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 non-local hosts were consistent with our earlier prediction regarding expected patterns in an adapted versus nonadapted population (Figure 3). In Florida

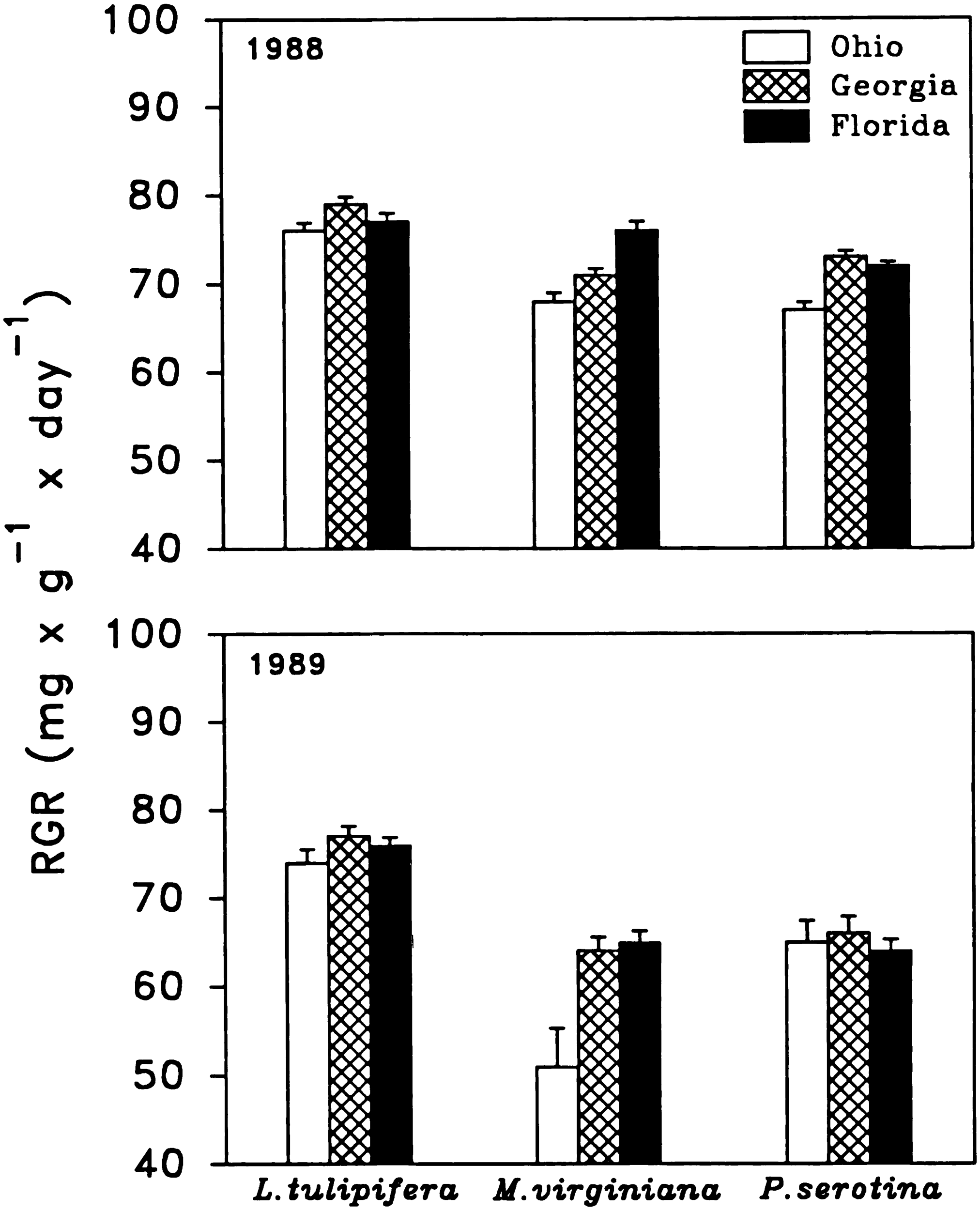

Figure 2. Mean (± SE) relative growth rates (RGR) for Ohio, Georgia and Flordia *P. glaucus* larvae reared on *L. tulipifera*, *M. virginiana* and *P. serotina*. Ten to twelve full-siblings from 13 to 23 families (5 to 16 each year) were allocated to each of the hosts.

Table 2. Mixed model analysis of variance of 1989 larval performance data comparing *P. glaucus* larvae from Ohio, Georgia and Florida populations on *L. tulipifera*, *M. virginiana* and *P. serotina* foliage; ns = not significant at p = .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	.0004	6.68	.0016
	Host	2	.0034	68.22	.0001
	Population x Host	4	.0002	4.21	.0029
	Error	161	.00005		
Pupal Mass	Population	2	1.427	72.56	.0001
	Host	2	.177	8.99	.0002
	Population x Host	4	.020	1.03	ns
	Error	161	.020		
Larval Duration	Population	2	198.81	8.47	.0003
	Host	2	1012.13	43.14	.0001
	Population x Host	4	163.49	6.97	.0001
	Error	166	23.46		

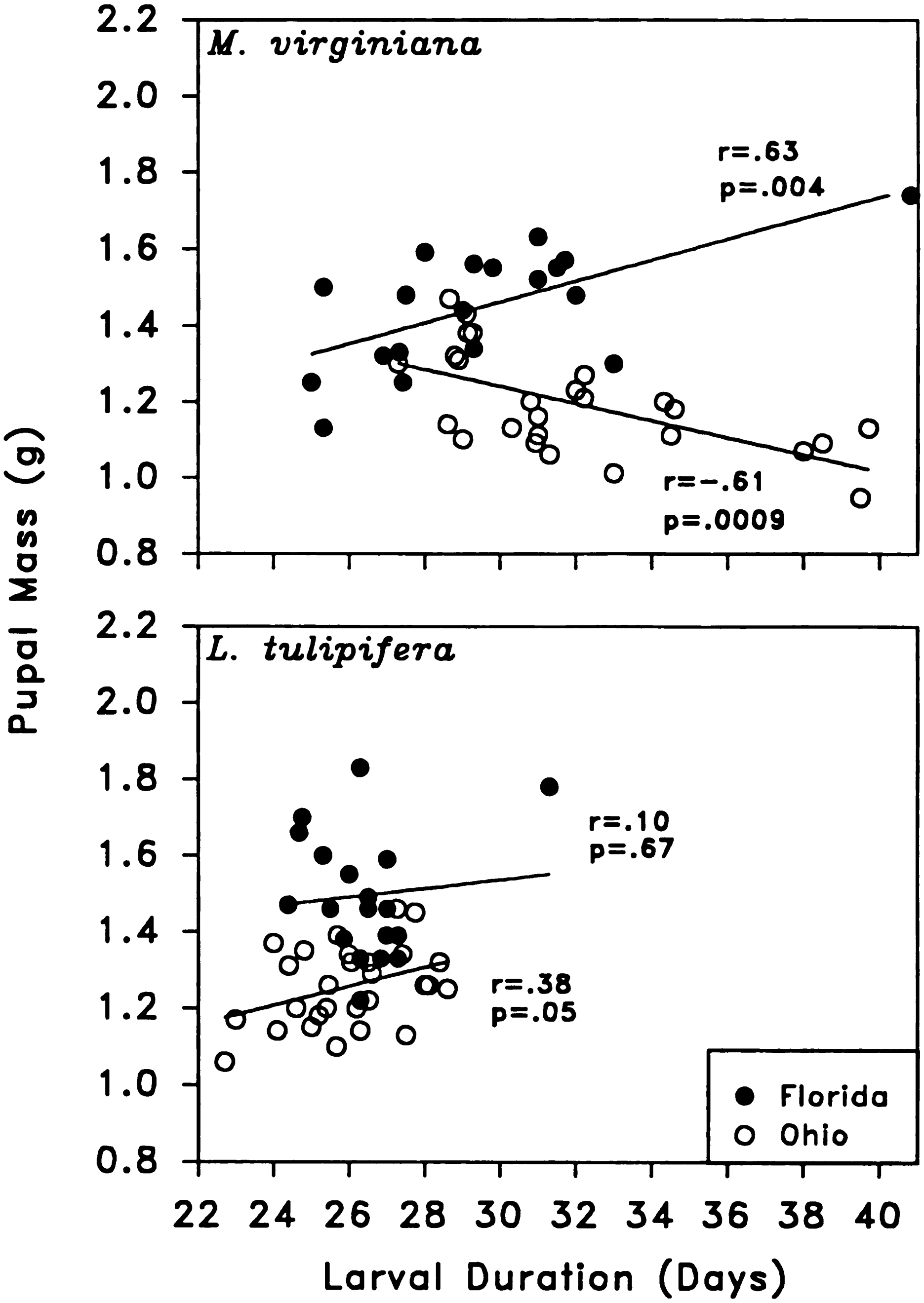

Figure 3. Correlation between pupal mass and larval duration for Ohio and Florida *P. glaucus* larvae reared on *L. tulipifera* and *M. virginiana*. Each point represents the mean of 3 to 30 full-siblings. The analysis was performed on data pooled from 1988 and 1989.

P. glaucus, pupal mass and larval duration were positively correlated on *M. virginiana*, the local host, but uncorrelated on *L. tulipifera*. In contrast, in Ohio *P. glaucus* these traits were positively correlated on *L. tulipifera*, the local host, but negatively correlated on *M. virginiana*, the host not encountered.

Electrophoretic analyses

Allele frequencies for AC-2 and MPI could not be scored consistently and were excluded from further analyses. Two loci, P3GDH and TPI, are X-linked, requiring 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 HBDH and PEP-LA (Table 3). At these two loci, heterozygote frequencies were lower than expected. All F_{ST} values were nonsignificant (Table 3). Negative values of F_{ST} result from sampling error and were interpreted as $F_{ST} = 0$ (Long 1986). The mean jackknife estimate of F_{ST} across loci was 0.00075 when HBDH and PEP-LA were included in the analysis and 0.00091, when excluded, with both estimates indicating a virtual absence of genetic differentiation among populations. The estimate of F_{ST} excluding HBDH and PEP-LA is most appropriate for calculating *Nm* since variation at these loci may not be neutral (this point is addressed in the discussion). *Nm*, using mean $F_{ST} = .00091$ was estimated to be 275.

Table 3. Wright's F-statistics for *P. glaucus* population samples from Ohio, Georgia and Florida. N=48-74, except for the X-linked loci, P3GDH and TPI, where N=23-25. Means and standard errors estimated by jackknife procedure over loci (Sokal and Rohlf 1981). Negative values of F_{ST} interpreted as $F_{ST} = 0$.

LOCUS	F_{IS}	F_{ST}	F_{IT}
AAT-1	-0.0080	-0.0015	-0.0095
AC-1	0.0087	-0.0033	0.0055
GPI	-0.0195	-0.0028	-0.0224
HBDH	0.1112	-0.0052	0.1066
IDH-1	0.0171	-0.0056	0.0116
IDH-2	0.0492	0.0069	0.0558
P3GDH	-0.0175	-0.0119	-0.0295
PEP-LA	0.3299	-0.0012	0.3291
PGM	-0.0046	0.0005	-0.0042
TPI	0.0446	-0.0103	0.0348
Mean	0.0512	-0.00277 0.00091*	0.0466
S.E.	0.0205	0.00169 0.00086*	0.0341

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*The calculation of the jackknife estimate of F_{ST} (± SE) does not include HBDH or PEP-LA.

Discussion

Interpretation of variation in ecological traits

Like many other herbivorous insects, *P. glaucus* exhibits substantial variation for ecologically important host-use traits (Ayres et al. 1991, Bossart and Scriber 1993). At least part of this variation is associated with the differential use of local host species. Genetic differentiation has occurred among *P. glaucus* populations in both oviposition preference and larval performance, apparently as a result of selection for enhanced recognition of and performance on a locally abundant host. The similarity between the 1988 and 1989 assays indicates this differentiation is not simply due to maternal or environmental effects.

Of the three hosts tested, we conclude that selection for enhanced use of *M. virginiana* is largely responsible for much of the spatial 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 the other populations. (It is noteworthy that selection for enhanced use of *M. virginiana* did not decrease performance on *L. tulipifera*, suggesting the absence of a tradeoff between *L. tulipifera* use and *M. virginiana* use). In contrast neither the Ohio nor Georgia *P. glaucus* populations exhibited enhanced performance on *L. tulipifera* or *P. 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 species in which *L. tulipifera* foliage is toxic to developing larvae (Scriber et al. 1991a, 1991b). 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 further support our interpretation of *M. virginiana* as a primary selective agent. In the Florida population, larval duration and pupal mass were positively correlated on *M. virginiana*, reflecting uniform rates of growth among families. Such was not the case in the Ohio populations where these two traits were negatively correlated, indicating variation in rates of growth among families and the presence of genotypes that were not adapted to use this host.

L. tulipifera appears to be a less important selective agent. Only a weak correlation was detected between larval duration and pupal mass in the supposedly adapted Ohio population on *L. tulipifera.* In fact, even a population not sympatric with tuliptree has little problem using *L. tulipifera* and females oviposit willingly on this host. It may be that *L. tulipifera* is a more acceptable host in general. A mixture of neolignans, compounds present in *M. virginiana* foliage, are known to decrease performance in unadapted *P. glaucus* populations, unadapted *Callosamia spp*., the polyphagous *Hyphantria cunea*, *Aedes aegypti* and *Artemia salina* (Nitao et al. 1991b, J. K. Nitao et al. unpubl., K. S. Johnson et al. unpubl.). These same compounds have not been detected in *L. tulipifera* foliage (K. S. Johnson et al. unpubl.).

P. serotina also appears to be less important in effecting adaptive change. Females from all three populations were unwilling to oviposit on *P. serotina* in laboratory choice-tests and larvae from all three populations grew at similar rates on this foliage. These findings need to be interpreted with caution though since larvae are found on *P. serotina* in nature. Moreover, other factors associated with *P. serotina* besides nutritional quality, e.g. enemy free space, may be more important as selective agents (Scriber and Lederhouse 1992).

Interpretation of electrophoretic variation

Variation at all loci examined, excluding HBDH and PEP-LA, is probably neutral and the similarities in frequencies among populations, probably the result of gene flow. This interpretation is based on the fact that F_{IS}, F_{IT} and F_{ST} values were small and nonsignificant at all loci except HBDH and PEP-LA, indicating a lack of departure from Hardy-Weinberg expectations and a lack of differentiation among populations. To invoke a selective interpretation to account for this pattern would require that selection operate in such a way as to mimic Hardy-Weinberg frequencies at these 8 independent, polymorphic loci and simultaneously generate parallel patterns among all 3 *P. glaucus* populations. Such an interpretation seems implausible since selection is much more likely to produce heterogeneity among loci (Slatkin 1987).

This same interpretation is likely not appropriate for explaining variation at HBDH and PEP-LA. While F_{ST} values were again nonsignificant, F_{IS} values were large and positive, indicating a deficit of heterozygotes within subpopulations. Such departures from expectations could result either from selection against heterozygotes or non-random mating among genotypes, but must be indicative of a process that is affecting all 3 populations similarly given the nonsignificant F_{ST} values. A tempting interpretation is to conclude that selection is effecting genotypic frequencies at HBDH and PEP-LA since non-random mating should impact all 10 loci uniformly. However, non-random mate choice on some unidentified trait could correlate with genotype at HBDH and PEP-LA, also resulting in non-uniformity among loci. Determining which mechanism underlies these patterns is impossible without further study.

Scoring error due to non-detectable variation is a caveat that must also be considered when interpreting F_{IS} values. Such error is inherent in most electrophoretic analyzes and may prevent distinquishing 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. With regard to this study, significant F_{IS} values were initially observed at AC-2, HBDH, 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 HBDH and PEP-LA.

Differential selection vs. gene flow

For the 8 allozyme loci exhibiting neutral variation, the absence of substructuring among populations is due either to the mitigating effects of gene flow or to very large effective population size. Mark-recapture studies suggest that *P. glaucus* butterflies are highly mobile (Lederhouse 1982 and R. C. Lederhouse unpubl., J. L. Bossart unpubl.) and capable of sustained flight. 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 patchy and *P. glaucus* populations are reduced (Lederhouse and Scriber 1987, Lederhouse personal communication), gene flow probably occurs frequently. Adequate hosts and nectar sources are scattered throughout most residential areas and around the numerous small lakes, providing sufficient opportunity for butterfly movement among habitat patches. Since only a few migrants are necessary to prevent random drift regardless of population size (Slatkin 1987), we suggest that gene flow resulting from migration among populations is responsible for the lack of differentiation among populations at loci harboring neutral variation. Our estimates of F_{ST}, which are in the range generally observed for other relatively mobile insects (see McCauly and Eanes 1987), further support this interpretation.

Extensive gene flow in *P. glaucus* would necessitate strong selection to permit differentation in oviposition preference and larval physiology. However, the fitness differences documented in this study appear to be more subtle. 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 (Scriber et al., unpublished), but would probably not result in absolute mortality of nonadapted genotypes. A more likely interpretation is that gene flow in *P. glaucus* is sufficiently restricted among populations such that more subtle selective factors are able to operate. Direct observations of *P. glaucus* movement in the field suggest that genetic variation in willingness to leave an area, habitat quality and habitat distribution are each important in determining how readily butterflies move about (R. C. Lederhouse unpubl., J. L. Bossart unpubl.). We hypothesize that such factors tend to restrict migration and gene flow in *P. glaucus*, thereby enabling selection to operate at a more moderate level.

The presence of negative genetic correlations across environments is required for genetic variation to be maintained 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. In light of this prediction, the apparent absence of tradeoffs in performance of *P. glaucus* on different hosts and 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. tulipfera* or *P. serotina*. Moreover, despite the fact that at least 20,000 generations have elapsed since glacial retreat during the Pleistocene, the Ohio population in particular continues to harbor a high frequency of genotypes not adapted to *M. virginiana* (see also Bossart, this volume). We offer three explanations for these contradictory patterns. First, it may be the case that host tradeoffs 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 spatial differentiation in oviposition preference and larval nutritional physiology in *P. glaucus* contrasts with the lack of any such patterns for electrophoretically detectable variation. *P. glaucus* behaves as one large population with regard to neutral variation, but not with regard to ecologically important variation. These contrasting patterns imply that differential selection among populations (also observed within populations; Bossart, this volume) for ecologically important traits will be counteracted by gene flow, thereby maintaining genetic variation for host use 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. We predict that selection on ecologically important variation in *P. glaucus* will continue to be counteracted by gene flow, thereby maintaining genetic variation for host-use traits. In the absence of more substantial barriers to gene flow, *P. glaucus* will continue to be comprised of a mosaic of genotypes exhibiting differing abilities to use different host species.

MANUSCRIPT 2

Genetic and nongenetic components of oviposition preference variation within tiger swallowtail butterfly populations

Abstract

Oviposition preference might be regarded as the major determinant of resource use for insect species which spend the duration of their larval period on the host selected by the ovipositing female. Yet, the extent of genetic variation within local populations, the factors affecting the phenotypic expression of host preference, and the adaptive significance of genetic variation remain relatively undocumented. In *Papilio glaucus*, the expression of preference is a function of both genetic and nongenetic factors. Additive genetic variation for oviposition preference appears to be common and to reflect variation in host specificity, rather than variation in rank order of hosts. Nongenetic factors, such as egg load, prior larval experience, and to a lesser extent age, modify the phenotypic expression of preference. This contribution of nongenetic factors is not sufficient to prevent selection from effecting change however. *P. glaucus* females exhibiting *L. tulipifera* preference produced progeny that grew at a faster rate than progeny of females that did not prefer *L. tulipifera*.

Introduction

The evolution of resource use in a phytophagous insect is a function of the insect's ability to behaviorally recognize and physiologically use a particular host plant. These two traits may not share equal evolutionary importance, however. For those species which spend the duration of their larval period on the host selected by the ovipositing female, oviposition preference might be regarded as the major determinant of resource use, and larval tolerance as the more secondary factor. For these species, variation in oviposition preference (or the lack thereof) will set constraints on future evolutionary trajectories and will determine the direction of these trajectories. Even though larvae might be capable of adapting to a broader array of hosts (Wiklund 1973, 1975), variation for larval tolerance of novel hosts will remain effectively neutral if ovipositing females do not select these hosts.

Despite the significance of oviposition behavior in determining resource use, our knowledge regarding the evolution of host preference is fairly rudimentary in comparison to that of physiological adaptation. A number of basic issues remain inadequately explored. Determining the degree of genetic variation for oviposition preference *within* local populations is a fundamental necessity, since it is intrapopulation variation that delineates evolutionary events. While such variation does not appear to be uncommon (see Jaenike and Holt 1991 for refs.), the current data base needs to be increased substantially before generalizations regarding the extent of genetic variation in host preference will be possible.

A second requisite is characterizing the phenotypic expression of intrapopulation genetic variation. Phenotypic heterogeneity in host choice among individual females may result from genetic variation in willingness to accept less preferred hosts (i.e., host specificity or acceptability), genetic variation in individual preference hierarchies (i.e., the sequence in which particular hosts are ranked by individual females), or some combination of these two factors (definitions of acceptability and preference sensu Singer 1986). Courtney et al. (1989) proposed a unidimensional model of host choice. In this model, each host encountered by a female has an intrinsic acceptability, resulting in a hierarchical ranking of the hosts. The threshold of acceptability may change over time such that less preferred hosts become more acceptable, but not at the expense of hosts initially preferred. An important assumption of the unidimensional model is that genetic variation in the hierarchical ranking of hosts by individual females is not present, i.e., females are not "free to evolve separate affinities" for different host species (Singer et al. 1992). In contrast, the multidimensional model of host choice permits genetic variation among individual females in the hierarchical ranking of hosts. As such, the probabilities of preferring different hosts are independent for individual females (Singer et al. 1992). Until intrapopulation variation is characterized, it will be impossible to evaluate which of these models more closely describes reality.

A third necessity is establishing whether observed genetic variation is adaptively significant. Jaenike (1990) argues convincingly that genetic variation for oviposition preference is effectively neutral, with host choice being determined most frequently by the physiological state and past experiences of ovipositing females. The expression of host preference may be influenced by egg load (Odendaal and Rausher 1990, Minkenberg et al. 1992), age (Jaenike 1990), larval host environment (Hopkins 1917, Jaenike 1983) or previous adult experience (Jaenike 1988). Clearly, if genetic variation is commonly masked by nongenetic factors, then the evolution of host choice will be the result of random processes. A mechanistic exploration of an adaptationist paradigm to explain the evolution of oviposition preference will be of nominal benefit therefore in expanding our understanding of host use patterns in phytophagous insect populations.

As a model system, *Papilio glaucus*, the eastern tiger swallowtail butterfly, provides a unique opportunity for investigating a number of these issues. Techniques are available for mating laboratory reared adults and assessing host preferences. In addition, many of the biological attributes which characterize this species are especially amenable to an investigation of evolutionary aspects of host preference. *P. glaucus* is a polyphagous herbivore using hosts from at least 7 different plant families. Favorite hosts include *Liriodendron tulipifera*, tuliptree; *Magnolia virginiana*, sweetbay; *Ptelea trifoliata*, hoptree; *Prunus serotina*, black cherry and *Fraxinus americana*, white ash. In *P. glaucus*, the ovipositing female determines the host substrate of her progeny since larvae rarely switch hosts. Females will oviposit on a number of different host species, even those that do not support larval development (Berenbaum 1981). Larvae will generally initiate feeding on most plants, even non hosts (Feeny 1991). No single host species is coincident with the geographic range of *P. glaucus,* an area which extends south from Michigan to Florida and west to Texas (Scriber 1983), permitting local host abundance to effect evolutionary change. Earlier studies have described patterns of differentiation in oviposition preference among *P. glaucus* populations based on regional host abundance (Bossart and Scriber 1993). In this study, an analysis of genetic and nongenetic preference variation within populations of *P. glaucus* is presented. Objectives were to 1) determine the extent of phenotypic variation in host preference for an array of hosts, 2) assess what proportion of this variation is genetically based and adaptively significant, and 3) identify potential nongenetic factors affecting preference variation.

Materials and Methods

General

Field collected butterflies were placed in individual glassine envelopes, then transported on ice or shipped using overnight delivery to the laboratory at Michigan State University, East Lansing, Michigan. Females were fed a 20% honey solution immediately upon arrival, then kept at 24°C for 24 h. This procedure extended the lifespan of ovipositing females.

Oviposition preferences were assessed in 2-choice laboratory trials. The initial host comparisons used to assess phenotypic variation included *Liriodendron tulipifera*, *Prunus serotina*, *Fraxinus americana*, *Ptelea trifoliata* and *Magnolia virginiana*. Subsequent genetic analyses concentrated on *L. tulipifera* and *M. virginiana* comparisons. The decision to focus on *L. tulipifera* and *M. virginiana* was based on the extensive phenotypic variation observed in initial 2-choice trials and the fact that both are of the Magnoliaceae family and have non-overlapping αrange distributions; hence, were interesting hosts to compare. *L. tulipifera* and *P. serotina* foliage was collected at least every third day from various areas in the vicinity of the Michigan State University (MSU) campus and stored at 7°C. *M. virginiana* foliage was collected daily from potted trees maintained in a campus greenhouse, and originally purchased as nursery stock in Florida. Although the same host species growing in different geographic regions likely differ morphologically and chemically, relative differences experimentally documented among populations should indicate real variation as long as all sampled populations are assayed simultaneously on foliage collected from the same sources and randomly distributed. Foliage sprigs presented to ovipositing females were placed in water-filled, rubber-capped plastic vials to maintain leaf freshness.

Females were individually placed in clear plastic (10 x 20 x 27 cm) "shoeboxes" (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. Fresh host sprigs of similar leaf surface area were positioned in opposite corners along the long side of the box 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 every 24 h to control for positional effects and foliage was changed as needed. The position of individual boxes in relation to the light source was randomized at each feeding. The few stray eggs that were placed on the paper lining or box were generally adjacent to a particular leaf but nonetheless excluded from the analyses. Eggs were counted and collected daily. Preferences were calculated as the percentage of total eggs oviposited on a given host. Since percentages based on small sample size are not very accurate, only females ovipositing at least 20 eggs were included in the analyses; the majority of females ovipositing at least some eggs, oviposited at least 20.

Laboratory reared butterflies used in preference trials were collected as pupae and placed in individual screen cages until adult emergence. All newly emerged females were fed a 20% honey solution, left for 24 h at 24°C, then stored at 8°C until being mated. Newly emerged males were stored at 18°C and fed daily, alternating a 20% honey solution with male "elixir", an ionic solution that increases male fertility (Lederhouse et al. 1990). Males were fed for at least six days before being mated; females were mated as soon as possible. Butterflies were hand-paired and females set up for oviposition.

Assessment of phenotypic variation

Adult female butterflies were collected from the north-central region of Georgia (Clarke Co.) during August, 1988. Newly captured females were shipped to the laboratories at Michigan State in 4 separate groups at approximately 1 week intervals. Each group of females was used to compare a different 2-choice combination, with each trial including *L. tulipifera* and one of four alternate hosts: *P. serotina*, *M. virginiana*, *P. trifoliata* or *F. americana*. A total of 125 butterflies were tested. Oviposition trials lasted for 4 days. The criterion that at least 20 eggs be oviposited was met by 21 of 22 females assessed in the *L. tulipifera/ P. serotina* comparison, 37 of 41 for *L. tulipifera/M. virginiana*, 21 of 24 for *L. tulipifera/P. trifoliata* and 33 of 38 for *L. tulipifera/F. americana*.

Genetic component of preference variation

The heritability of preference was assessed in 1989 and again in 1991 using data from second generation females collected in late July within a 15km radius in southern Ohio (Lawrence Co.). The same experimental protocol was followed in both years. Relative preferences of mothers were assessed for *L. tulipifera* and *M. virginiana*. Ten to twelve newly eclosed, full-sibling larvae from 7-12 mothers (1991 and 1989, respectively) were reared individually to pupation in (150 cm x 25 cm) screened, plastic petri dishes containing sprigs of *P. serotina*. Larvae were reared on a common host to minimize maternal effects and to control for potential inducible responses resulting from larval host environment. Preferences of mothers were regressed on the average preference of their daughters following methods of Becker (1984) when only one parent is measured and $h^2 = 2\,[(cov_{XZ}/var_X)]$.

Butterflies collected from Ohio in 1989 were also used to examine the relationship between female preference and larval performance. Eight to 30 full-sibling larvae from each of 12 mothers were randomly allocated to *L. tulipifera* and *M. virginiana*. Larvae were reared individually to pupation on the appropriate host foliage. Larval duration, pupal mass and sex were recorded for each individual. 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.

Non-genetic component of preference variation

Female attributes potentially affecting the phenotypic expression of host preference examined in this study include: age, pupal mass and fecundity (as measures of adult vigor), egg load and larval host environment.

Butterfly age was estimated using wing condition class; previous studies have shown that this measure correlates well with adult longevity (Lederhouse 1983). Wing condition class was determined for a total of 88 females collected from Lawrence Co., Ohio in 1991. Females were ranked on a scale of 1-4 (following Lederhouse 1983), with fresh, unworn females being assigned a value of 1 and well worn females, a value of 4. Intermediates were assigned values of either 2 or 3. The majority of the 88 females collected were ranked as either 1 or 2, indicating that sampling must have occurred relatively soon after second generation butterflies began to emerge and become active. Of these 88 females, 50 were set up in *L. tulipifera/M. virginiana* 2-choice trials and the other 38, in *M. virginiana/P. serotina* trials. In each of these 2-choice trials, a more preferred host was compared against a less preferred host. Preference rankings were based on results from the 2-choice comparisons assessing phenotypic variation. Each wear class was split approximately equally between the two preference trials. Preference trials lasted for four days.

The effects of fecundity, egg load and pupal mass on preference were measured on laboratory reared, second generation progeny of butterflies collected from Lawrence Co., Ohio in 1990. First generation neonate larvae were reared individually to pupation on *P. serotina*. Emerging adults were hand paired, and females set up for oviposition. Subsequently, 10 to 15 second generation neonate larvae from each of 12 mothers were reared individually to pupation under diapause conditions (21° C, 12:12 photo:scotophase) on either *P. serotina* or *L. tulipifera*. Pupae were weighed to the nearest mg 24 hours after shedding the larval skin. Diapausing pupae were stored at 8°C. The following year, emerging adults were hand-paired and females tested in *L. tulipifera/M. virginiana* 2-choice trials. Females were allowed to oviposit until death occurred; dead females were stored at 4° C for subsequent dissection. Fecundity was measured as the number of eggs oviposited per day.

Generally, egg load is either measured as total eggs oviposited or as total mature eggs produced, i.e., eggs oviposited plus the number of mature eggs dissected from the ovaries of dead females (Minkenberg et al 1992). An analysis using total eggs oviposited provides little information since this estimate may or may not correlate with actual egg load; hence, the better measure of egg load is total mature eggs produced. Egg load was estimated both directly and indirectly to account for an unknown egg maturation rate. Direct estimates of egg load based on total mature eggs are valid only if the majority of eggs mature concurrently or if maturation rate and total egg load are positively correlated. This technique is not legitimate if maturation rate is variable or uncorrelated with total egg load. Direct estimates of egg load were obtained by summing the number of eggs oviposited and the number of mature eggs dissected from the ovaries of dead females to obtain total mature eggs. This number was then divided by pupal mass to determine the number produced per gram weight, a technique that controls for possible correlation between mass and total egg production. Indirect estimates were obtained by analyzing trends in preference over time for each ovipositing female, permitting the detection of any increasing or decreasing patterns of preference associated with changes in egg load from day to day independent of maturation rate.

Effects of larval host substrate on preference were examined using two different host comparisons. In one comparison, relative preferences for *L. tulipifera* and *P. serotina* were compared for a population of females reared on *L. tulipifera* and a population reared on *P. serotina*. In the second comparison, relative preferences for *L. tulipifera* and *M. virginiana* were compared for females reared on *L. tulipifera* and *M. virginiana*. Newly eclosed larvae generated from females collected from Lawrence Co., Ohio were reared individually to pupation on the appropriate host foliage. Newly emerged adults were mated, and females allowed to oviposit until death occurred. Effects of larval host substrate on *L. tulipifera* and *P. serotina* preference were also assessed within a family. Ten to 15 full-sibling larvae from a single Ohio field-collected mother were randomly allocated to either *L. tulipifera* or *P. serotina*. Newly emerged females were mated to field collected males, then allowed to oviposit.

Statistical analyses

All linear regression and correlation analyses were performed using SAS (1985). Correlation analyses were performed on family means unless stated otherwise. Fecundity, pupal mass and egg load were analyzed in terms of a preference index. Preference index measures the degree of preference expressed irrespective of host species and was calculated as the absolute value of the difference between actual percent of eggs oviposited on *L. tulipifera* and 50% of eggs oviposited on *L. tulipifera*. Preference index = 0 for females ovipositing 50% of their eggs on both hosts; preference index = 50 for females ovipositing 100% of their eggs on one particular host species. Trends in preference over time were analyzed for individual females using the nonparametric D-statistic (Lehmann 1975). Statistical significance for the population of females was determined as $Z_i = (D_i - \mu_{Di})/\sigma_i$, where the sum of the Z_i^2's are approximately distributed as Chi-square with *n* degrees of freedom. Effects of larval host environment were analyzed using Chi-square analyses on 2-way frequency data.

Results

Phenotypic variation

Georgia *P. glaucus* females expressed extensive phenotypic variation in host preference in 2-choice trials, and exhibited differing degrees of relative preference for *L. tulipifera* depending on the 2-choice combination offered (Figure 4). In the *L. tulipifera*/*P. trifoliata* comparison, neither host seemed to be clearly preferred over the other. Forty-eight percent of the females oviposited 60% or more of their eggs on *P. trifoliata*, and 38% of the females oviposited 60% or more on *L. tulipifera*. This contrasts with the distinct preference expressed when the choices presented were *L. tulipifera* and *P. serotina*, where 81% of the females oviposited greater than 70% of their eggs on *L. tulipifera*. Females also exhibited preference for *L. tulipifera* (although to a lesser extent) when offered either the *L. tulipifera*/*F. americana* comparison or the *L. tulipifera*/*M. virginiana* comparison. Seventy percent of the females presented with *L. tulipifera* and *F. americana* oviposited 60% or more of their eggs on

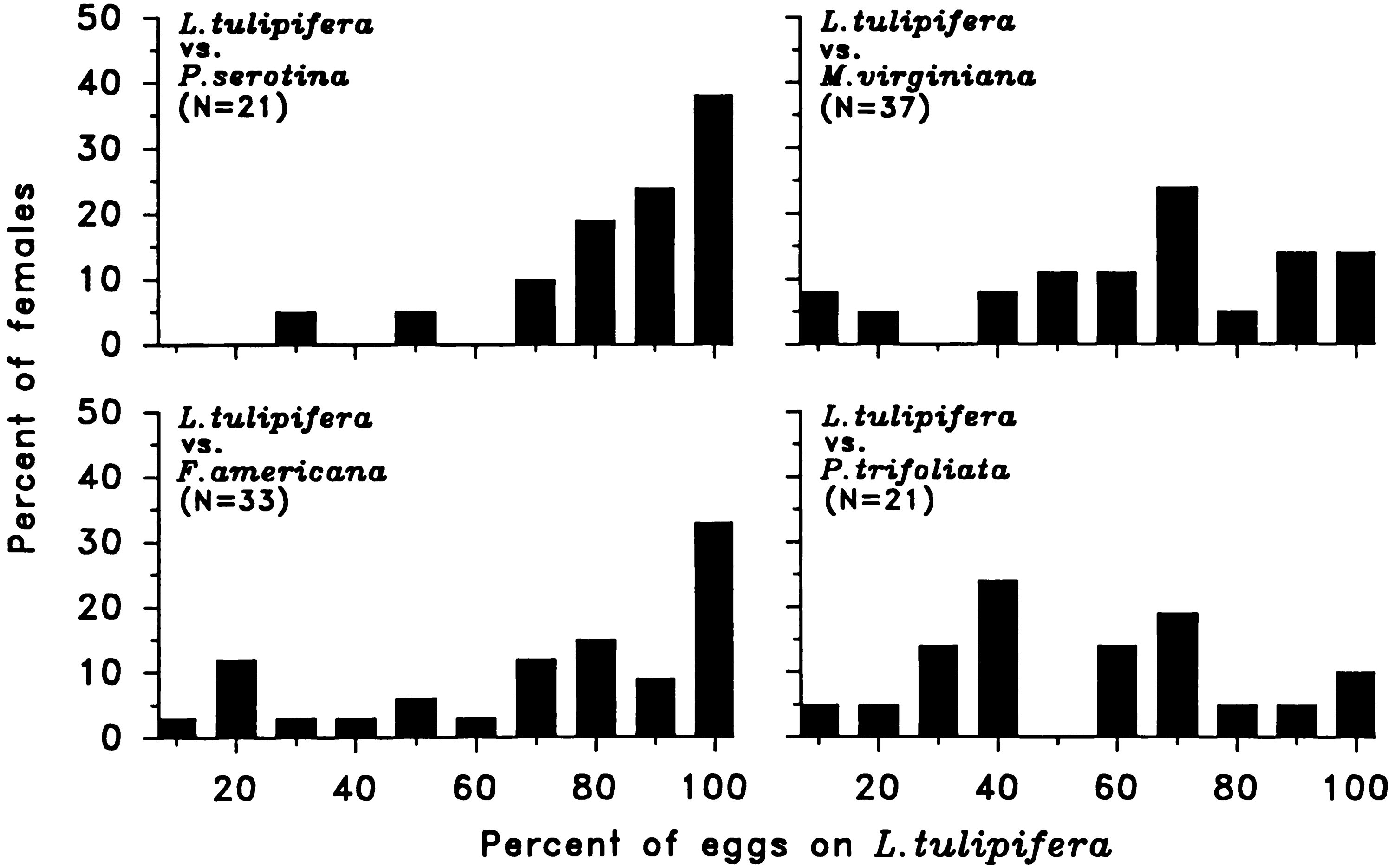

Figure 4. Distribution of preferences expressed by *P. glaucus* females collected from north central Georgia. Distributions reflect relative preference for *L. tulipifera*. Preferences were assessed in 2-choice trials comparing *L. tulipifera* and one of four alternate hosts: *P. serotina*, *M. virginiana*, *F. americana* or *P. trifoliata*.

L. tulipifera; over half (57%) of the females presented with *L. tulipifera* and *M. virginiana* oviposited 60% or more on *L. tulipifera*. When ranked relative to *L. tulipifera* preference, the order of preference expressed by Georgia females for the five hosts was *L. tulipifera* = *P. trifoliata* > *M. virginiana* > *F. americana* > *P. serotina*.

Genetic component of variation

Mother/daughter regressions: The average *L. tulipifera* preferences expressed by mothers when given a choice between *L. tulipifera* and *M. virginiana* was similar to that expressed by their daughters when given a choice between the same two hosts (Figure 5). In general, only two preference states were expressed: no preference and *L. tulipifera* preference. None of the females tested expressed strong *M. virginiana* preference. With data from both years combined, the positive correlation between mothers and daughter-averages was readily apparent ($p<.02$). Only 1 of the 16 families failed to exhibit a corresponding mother/daughter preference. In this particular 1989 family, the mother preferred *M. virginiana*, while the daughters preferred *L. tulipifera*. The lack of agreement between this mother and her daughters in combination with the fact that her daughters exhibited very similar levels of preference, suggests that the preference expressed by this mother was probably not genetically based.

With data from each year viewed separately (as is more appropriate when estimating heritabilities), the positive association between relative preferences of mothers and daughters was significant in 1991 ($p<.02$), but not in 1989 ($p=.35$). The lack of significance in 1989 was due to the one atypical family in which

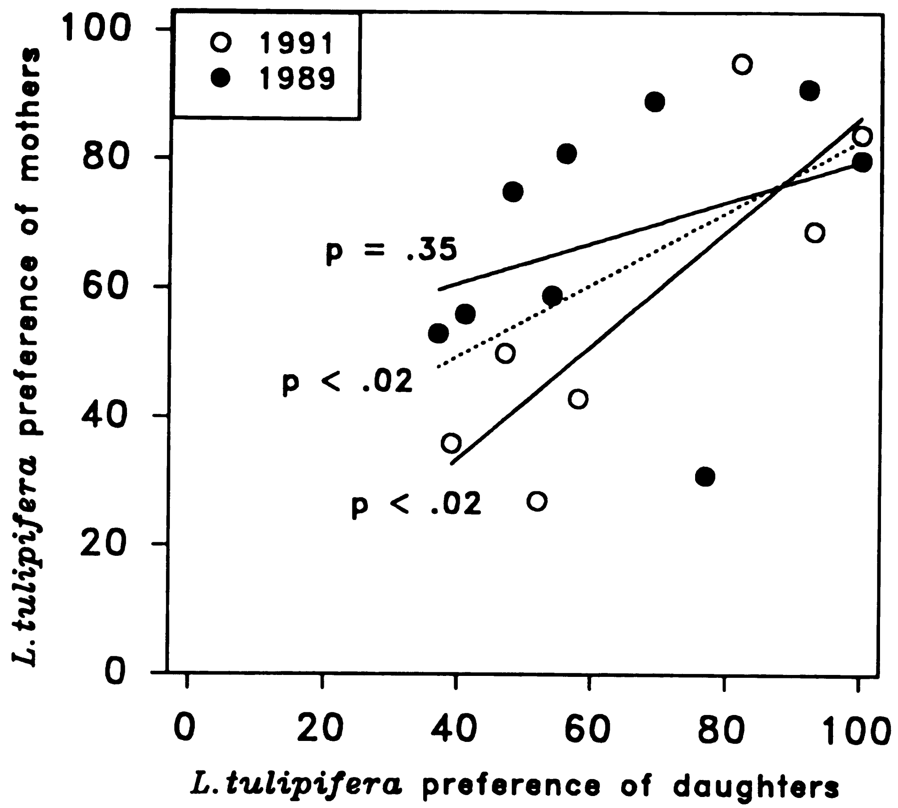

Figure 5. Regression of *L. tulipifera* preference of mothers on mean *L. tulipifera* preference of daughters. Solid lines are regressed through data from different years; the dotted line is regressed through data from both years combined.

daughters did not exhibit the same preference as their mother; the association was significant ($p=.03$) with this family excluded from the analysis. Estimated heritabilities were high in both years ($h^2=.71$ in 1989 and 1.13 in 1991), indicating that expressed variation in relative preference for *L. tulipifera* is at least partially genetically controlled.

Preference/performance correlations: In general, the average relative growth rate of a female's progeny was positively correlated with her preference for *L. tulipifera* (Figure 6). This correlation was significant on *L. tulipifera* ($r=.65$, $p=.02$), and nearly so on *M. virginiana* ($r=.53$, $p=.07$). Relative growth rates of full-siblings were positively correlated across both hosts ($r=.65$, $p=.02$), arguing against ecological specialization on different hosts as the basis for the preference/performance correlation.

Nongenetic component of variation

Female Age: Twenty-three of the 50 females included in the *L. tulipifera/M. virginiana* comparison, and 11 of the 38 included in the *M. virginiana/P. serotina* comparison, oviposited sufficient numbers of eggs to be included in the analysis ($n=20$). These numbers also reflect the fact that generally only a third of field collected females will oviposit under laboratory conditions. In both 2-choice comparisons, the relative preferences expressed by young females (i.e., those with wing condition rankings of 1 or 2) ranged from *L. tulipifera* preference to *M. virginiana* preference (Figure 7). Younger females as a group exhibited all three preference states: preference for the more preferred host of the two hosts offered, preference for the less preferred host and

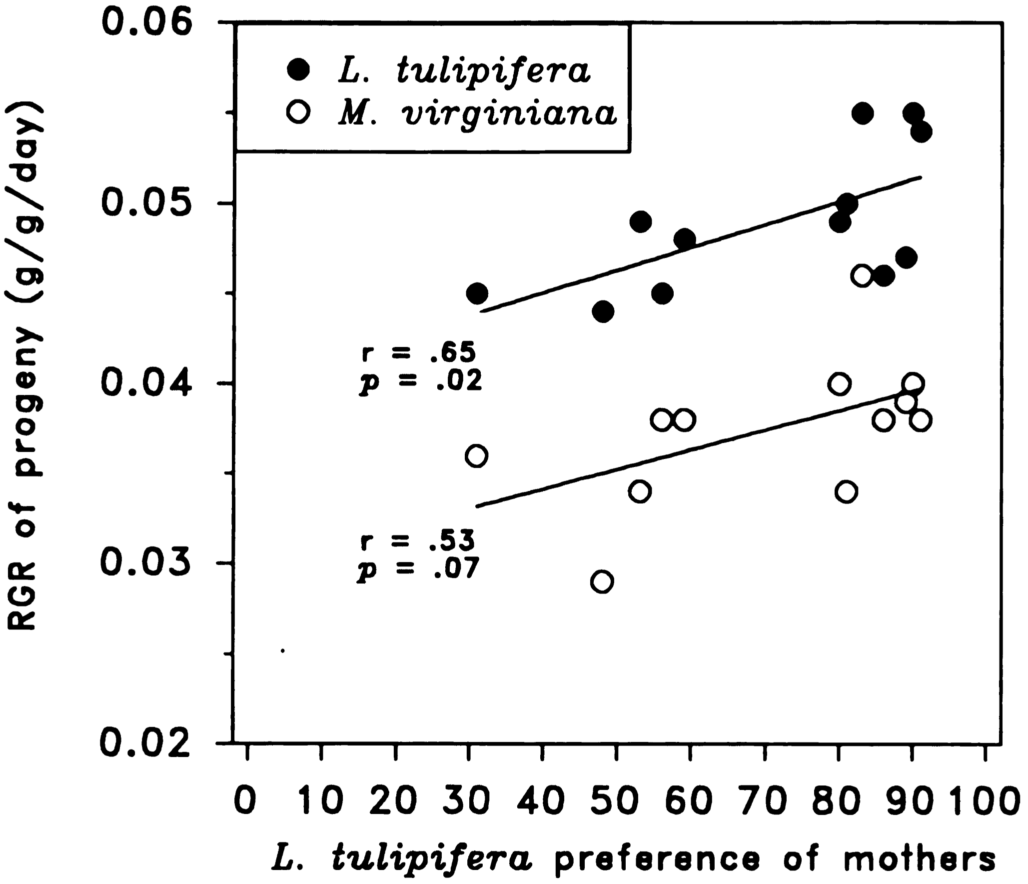

Figure 6. Correlation between *L. tulipifera* preference of mothers and the mean relative growth rate of her progeny on *L. tulipifera* and *M. virginiana*.

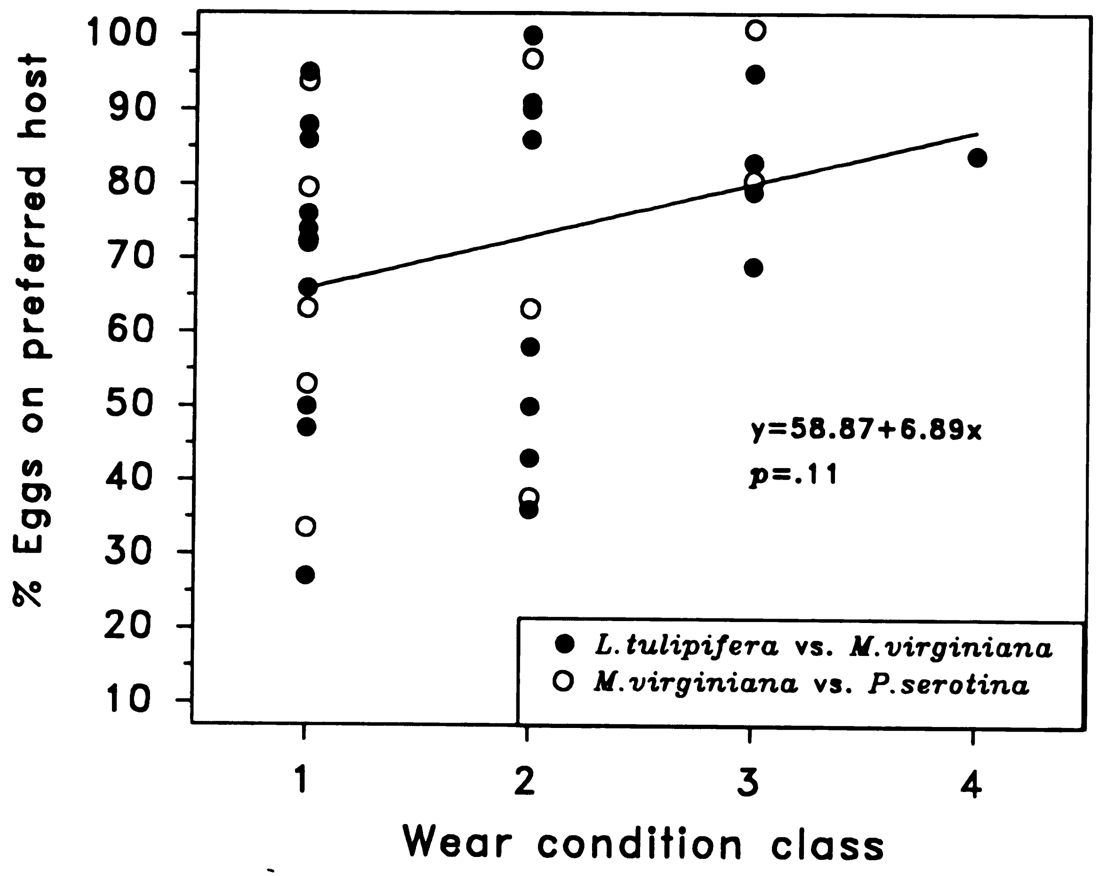

Figure 7. Specificity for preferred hosts when compared against a less preferred host as a function of the wear condition class of females. Wear condition is a reliable indicator of relative age. See text for definition of *preference index*.

no preference. In contrast, all 7 older females (i.e., those ranked as either a 3 or a 4) exhibited only one preference state: a strong preference (70% or greater of deposited eggs) for the higher ranked host of the two hosts offered. Simple linear regressions of wing condition class on relative preferences were not significant however, and did not support this implied pattern of increased specificity in older females.

Female Fecundity and Pupal Mass: The physiological "condition" of *P. glaucus* females in terms of fecundity and pupal mass did not appear to affect female preference. Neither of these traits correlated with preference (Figure 8). Moreover, there were no differences in the distribution of preferences expressed by *L. tulipifera* reared females and those expressed by *P. serotina* reared females towards *L. tulipifera* and *M. virginiana*. This similarity is despite the fact that *L. tulipifera* reared females were significantly heavier on average ($F_{[1,10]}=12.53$, $p=.005$; Figure 8) than *P. serotina* reared females.

Egg Load: The average number of mature eggs produced by *L. tulipifera* reared females per gram of pupal mass was significantly greater than the number produced by *P. serotina* reared females ($F_{[1,10]}=15.47$, $p=.002$; Figure 9). More importantly though, egg load appeared to have a significant impact on the level of preference expressed. Females with greater egg loads tended to be "no preference" females, ovipositing relatively equal numbers of eggs on each host and resulting in low preference indices (Figure 9). Females with smaller egg loads generally exhibited a distinct preference, ovipositing the majority of their eggs on a single host and resulting in high preference indices (Figure 9). The relationship was significant for *P. serotina* reared females; the relationship was not

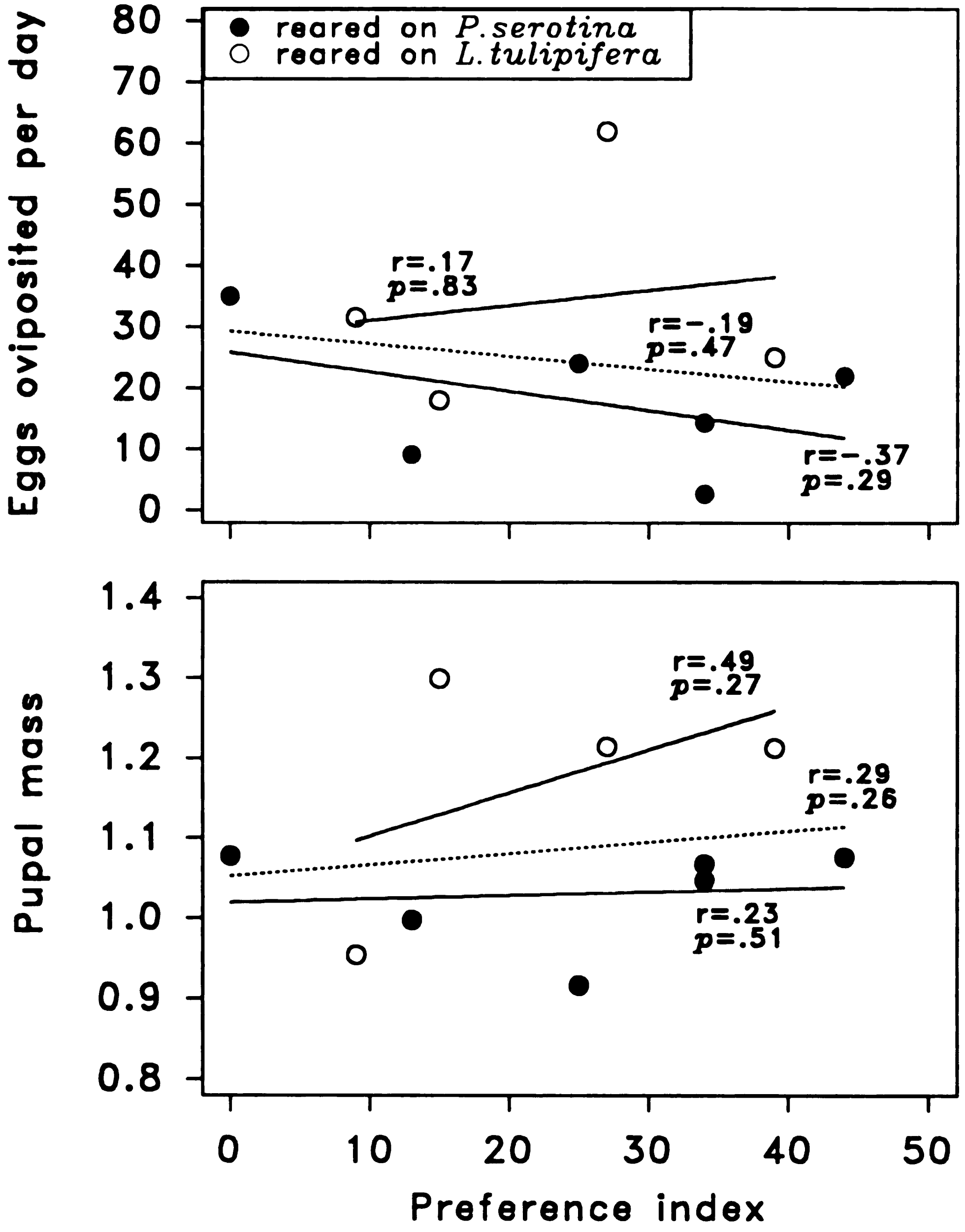

Figure 8. Relative degree of preference exhibited by females as a function of female fecundity and pupal mass. Females were tested in *L. tulipifera/M. virginiana* 2-choice trials. Solid lines are regressed through data from different hosts; the dotted line is regressed through data from both hosts combined. See text for definition of *preference index*.

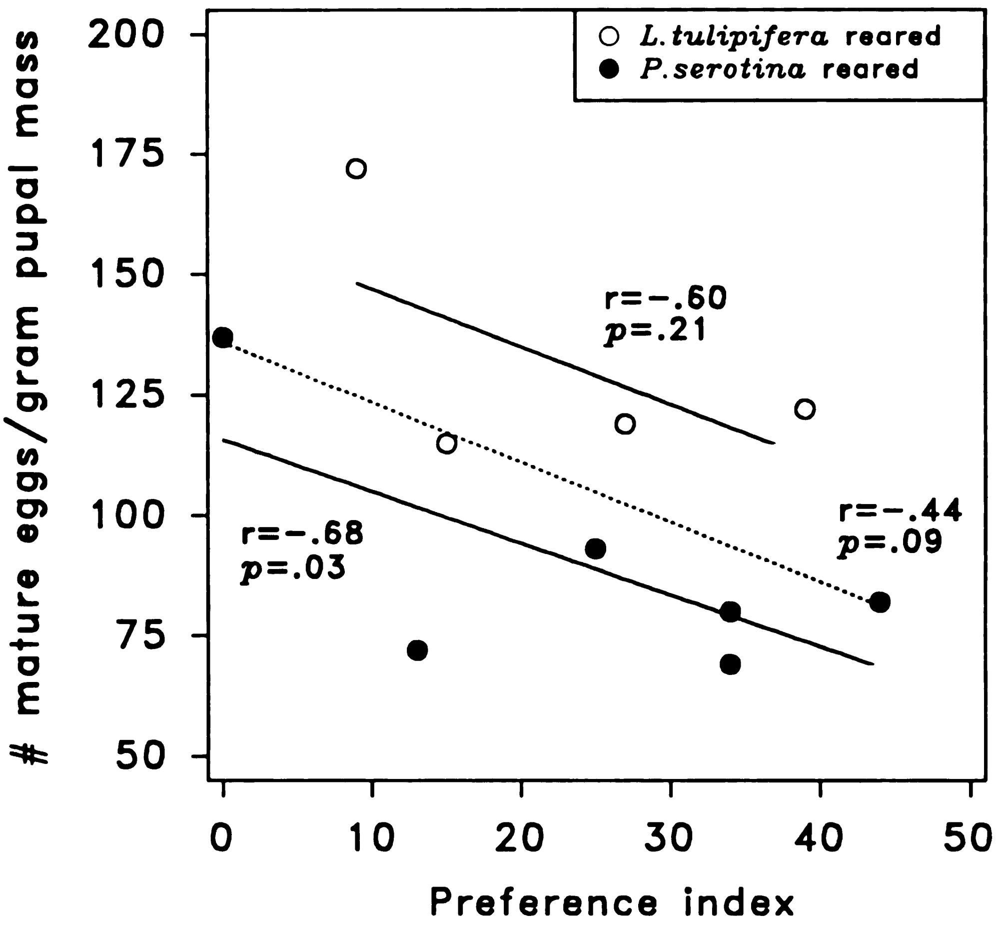

Figure 9. Relative degree of preference exhibited by females as a function of egg load. Females were tested in *L. tulipifera*/ *M. virginiana* 2-choice trials. Solid lines are regressed through data from different hosts; the dotted line is regressed through data from both hosts combined. See text for definition of *preference index*.

significant for *L. tulipifera* reared females although a similar trend was observed.

When viewed as day to day change in individual females, egg load did not appear to affect expressed levels of preference ($X^2_{[21]}=22.1$, $p>.3$). For most females, relative preference remained similar from day to day. Only three of the 21 females tested exhibited a significant change in preference over time. For two of these females, preferences declined; for the other, preference increased.

Larval Host Environment: The relative distribution of eggs on *M. virginiana* and *L. tulipifera* was similar for both the *M. virginiana* reared population of females and the *L. tulipifera* reared population of females ($X^2_{[1]}=1.77$, $p>.1$). However, the relative distribution of eggs on *P. serotina* and *M. virginiana* differed between *P. serotina* reared females and *L. tulipifera* reared females ($X^2_{[1]}=68.88$, $p<<.001$). Three of 5 (60%) *L. tulipifera* reared females oviposited greater than 70% of their eggs on *L. tulipifera*, while only 3 of 11 (27%) *P. serotina* reared females did (Figure 10). Within a single family, *P. serotina* reared females were also more willing to oviposit on *P. serotina* than their *L. tulipifera* reared sisters ($X^2_{[1]}=29$, $p<.001$; Figure 11).

Discussion

Three general conclusions regarding the evolution of oviposition preference in *P. glaucus* are evident from this study: 1) females exhibit genetic variation for host preference, 2) nongenetic factors contribute to phenotypic heterogeneity in oviposition preference, and 3) genetic variation for preference is adaptively significant despite the contribution of these nongenetic factors.

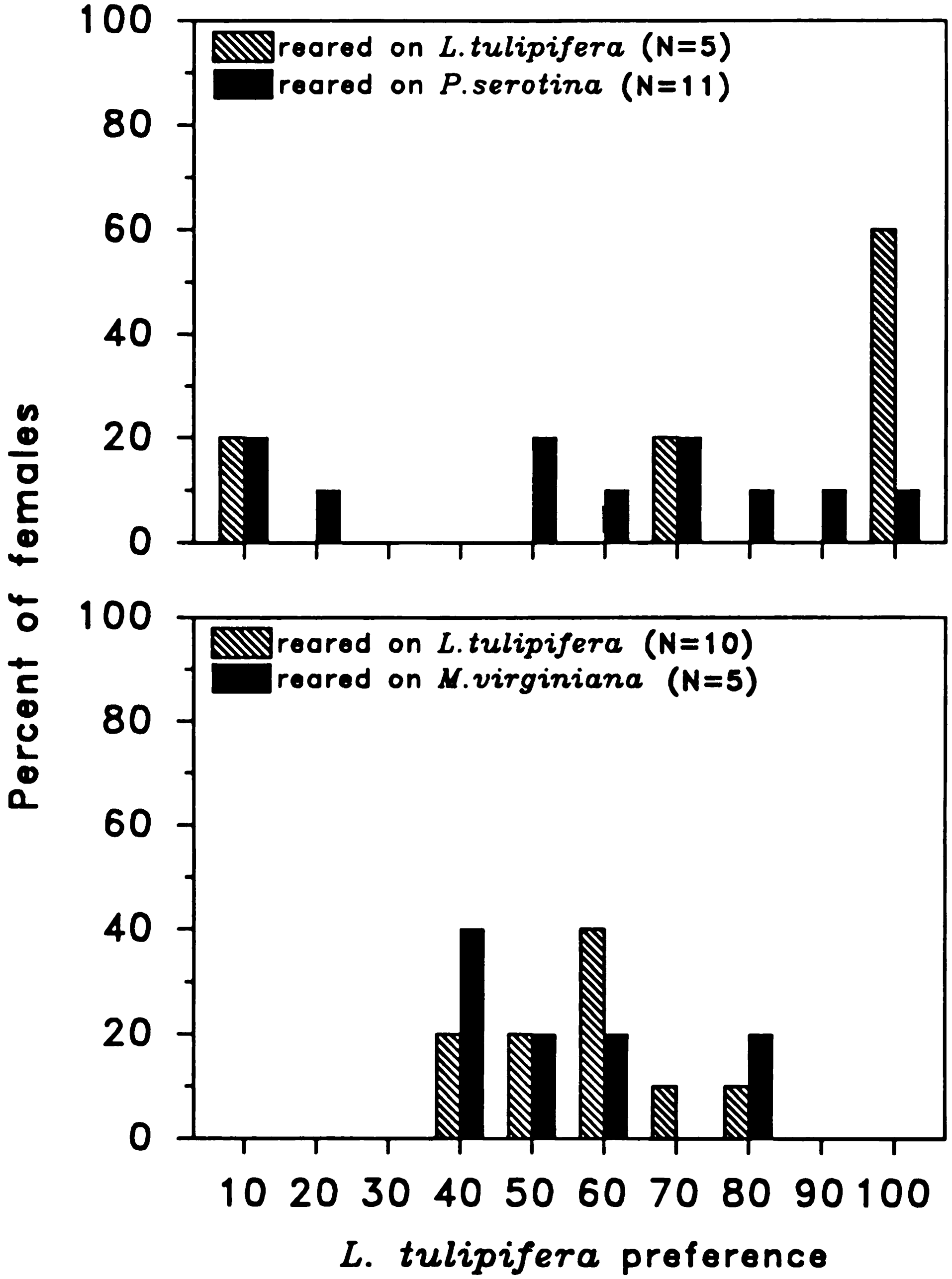

Figure 10. Distribution of preferences for *L. tulipifera* expressed by a population of *P. glaucus* females as a function of larval host substrate.

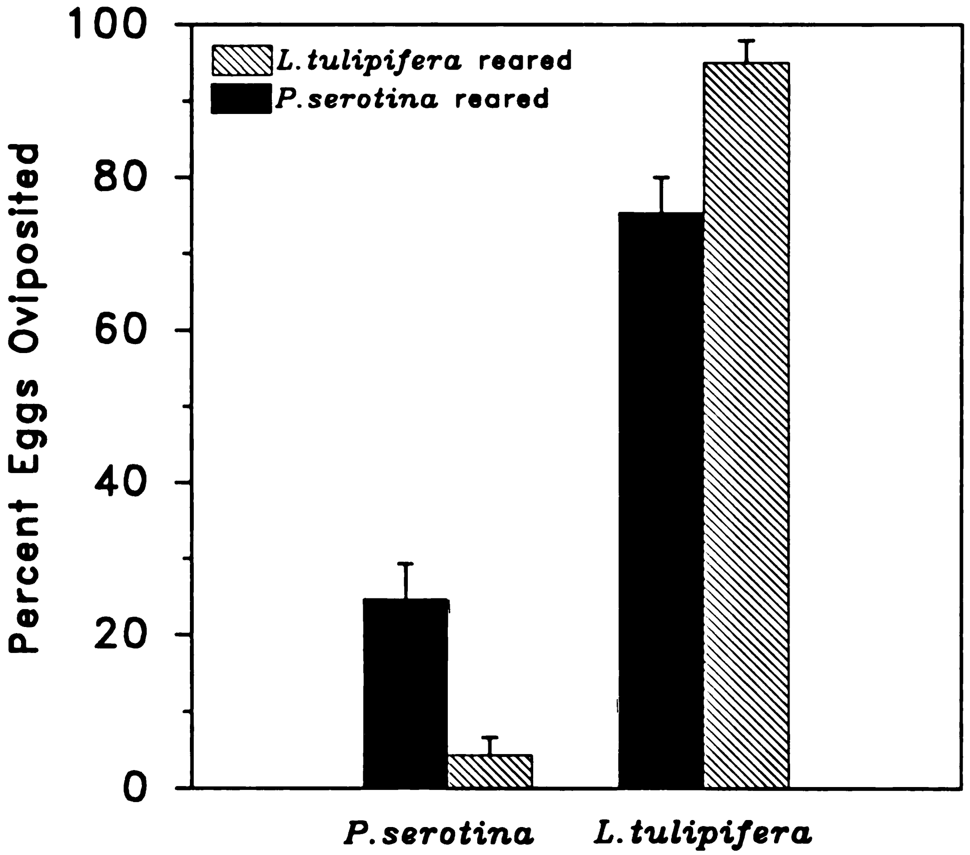

Figure 11. Mean (± SE) preferences for *L. tulipifera* and *P. serotina* expressed by full-siblings as a function of larval host substrate.

Genetic factors contributing to variation in host preference: The high heritabilities estimated in this study reflect the close resemblance between mother and daughter preferences expressed for *L. tulipifera* and indicate a genetic component to preference variation. However, since only females were tested, maternal effects can not be separated from genetic effects. If maternal effects contribute to mother-daughter resemblance, then actual heritabilities may be lower than the estimated values. Maternal effects are known to influence larval performance (Rossiter 1991a, 1991b), but whether such effects influence preferences has not been established. The use of parent-offspring regressions to estimate heritabilities does not permit additive genetic variation (i.e., selectively important variation) to be partitioned from other sources of genetic variation. Hence, these estimates are of heritability in the broad sense. The similarity among the *P. glaucus* estimates and those obtained for *Colias eurytheme* (Tabashnik et al. 1981) and *Euphydryas editha* (Singer et al. 1988), two other lepidopteran species, lend confidence to these values. That genetic variation is present in *P. glaucus* is not surprising given that genetic variation for oviposition preference has been demonstrated for other phytophagous insects (reviewed by Jaenike and Holt 1991).

When given a choice between *L. tulipifera* and *M. virginiana*, the relative preferences of Ohio daughters ranged from no preference for either host to strong *L. tulipifera* preference. None of the daughters tested expressed a preference for *M. virginiana*, even the daughters from the *M. virginiana* preferring mother. The most parsimonius conclusion is that only mothers preferring *L. tulipifera* and mothers with no apparent preference, were expressing a genetically based phenotype. The observation that *M. virginiana* preference does not have an apparent genetic basis suggests an absence of genetic variation for the order in which different hosts are ranked by individual *P. glaucus* females. Rather, variation in host preference seems to be due to genetic variation in host specificity. Some females are inherently more willing than others to oviposit on a less acceptable host (in this case, *M. virginiana*), while other females oviposit exclusively on the more acceptable host (*L. tulipifera*).

Whether this pattern of genetic variation for host specificity, but not rank order, hold ups to further scrutiny remains to be confirmed. Some of the mothers exhibiting *M. virginiana* preference could not be included in the analysis because daughters either were not obtained or did not oviposit. Hence, it could not be determined whether these particular mothers were expressing a genetically-based preference. Many of the females tested in the other 2-choice comparisons expressed preferences for the alternate (non-*L. tulipifera*) hosts (Figure 4). At least some of these females were potentially expressing a genetically based preference; an analysis of daughters from mothers tested in one of these other comparisons might have detected additive genetic variation for rank order. At least one mother preferring *F. americana* in a separate 2-choice trial gave rise to daughters preferring *F. americana* (Bossart, personal observation).

Historically, genetic variation for host specificity, like that shown here, has been more easily demonstrated than genetic variation in the hierarchical ordering of different hosts (Wasserman 1986, Thompson 1988, Jaenike and Holt 1991). This absence of variation in rank order among *P. glaucus* females is predicted by the unidimensional model of host choice, whereby females do not evolve separate affinities for different hosts species (Courtney et al. 1989). Other results of this study however are more compatible with a multidimensional model of host choice. The observation that three of the mothers tested in this study exhibited a nongenetically based preference for *M. virginiana* over *L. tulipifera*, the more acceptable host, is counter to the unidimensional model. The unidimensional model predicts that even when lower ranked hosts become acceptable, they are not preferred to the exclusion of higher ranked hosts. Hence, in a population of females we might expect to detect females that prefer the most preferred host (in this case, *L. tulipifera*) and "no preference" females, but should not observe females that prefer the less preferred host (in this case, *M. virginiana*). Results from the other 2-choice comparisons also contradict the predictions of the unidimensional model. None of the females in these comparisons should have expressed a preference for the less preferred host, regardless of whether this variation was genetically based or not. If genetically based, then females are exhibiting genetic variation in the hierarchical ranking of different hosts. If not genetically based, then "motivated" females are preferring a less acceptable host to the exclusion of a more acceptable host. Neither scenario is consistent with a unidimensional model. Clearly, additional study will be required before the issue of dimensionality of host preference variation can be resolved.

Nongenetic factors contributing to variation in host preference: The presence of substantial additive genetic variation for host preference in *P. glaucus* indicates that evolutionary events, such as host shifts and host range expansion, should not be impeded by a lack of suitable genetic variation. However, for selection to effect change in patterns of host preference requires that this variation not be negated by the contribution of nongenetic factors. Of the factors measured in *P. glaucus*, both egg load and prior larval experience, and to a lesser extent age, appear to contribute to the phenotypic expression of preference and may serve to constrain the evolution of this trait.

Physiological factors such as egg load and age can modify the expression of oviposition preference by altering the motivational state of the female (Courtney et al. 1989, Jaenike and Holt 1991, Minkenberg et al. 1992). Presumably, proprioceptors detect abdominal distension caused by increased egg load, stimulating a higher state of motivation. Highly motivated females are expected to exhibit reduced host specificity and oviposit more willingly on less acceptable, lower ranked hosts (Mangel 1989, Courtney et al. 1989). Both young females and females deprived of an oviposition site for some length of time should have higher egg loads and therefore, higher motivational states, than older females and females ovipositing at a normal rate (Jaenike 1990, Minkenberg et al. 1992). The negative association between egg load and preference index documented for *P. glaucus*, and positive trend between age and host specificity support this prediction. Such relationships have also been documented for certain dipteran and other lepidopteran species (see Minkenberg et al. 1992). In most of these other studies however, egg load was confounded by female mass. Since female size and number of eggs produced are generally, positively correlated (e.g., Blau 1981, Haukioja and Neuvonen 1985), analyzing egg load irrespective of body size is not very useful. The egg load of large females producing a greater number of eggs may be no different than that of small females producing a smaller number of eggs. Without controlling for female body weight, the patterns observed in these other studies are difficult to interpret, and provide little useful insight with regard to the interpretation of the results presented here.

The lack of a time effect in day to day preferences is likely a function of the experimental technique rather than the absence of a relationship between preference and egg load. Only 33% of the *P. glaucus* females tested lived longer than five days and over half of the females oviposited a majority of their eggs on the first two days. Given these conditions, detecting any type of statistically significant pattern over this short period would have been unlikely. In fact, if total mature eggs was largely a function of the number of eggs oviposited on the first two days, this "dumping" of eggs early in the study may explain why the direct analysis detected a correlation between egg load and preference that was not detectable from the indirect/time analysis.

The general health or vigor of a female might also modify the expression of host preference by altering a female's motivational state. Healthier, more robust females might be expected to express a higher degree of specificity than less robust females. Presumably, stronger, more vigorous females should be more likely to search for an extended period for preferred hosts than their less vigorous counterparts. When measured in terms of pupal mass and fecundity, the general vigor of *P. glaucus* females did not correlate with degree of preference. Despite a significant difference in pupal mass between *L. tulipifera* and *P. serotina* reared females, the average preference for *L. tulipifera* and *M. virginiana* was no different between these two groups. Correlations were also absent when individual females were analyzed as separate data points.

The effect of larval host substrate on subsequent host preference by an ovipositing female continues to be a topic of discussion (Jaenike and Holt 1991). In part, interest in this issue has remained high because of its ecological and evolutionary importance as a mechanism whereby learned behaviors reinforce genetically based differences in preference. Such associations if they occur can promote sympatric, host associated divergence (Maynard Smith 1966, Rausher 1983, Jaenike and Papaj 1992). To date, little evidence has accumulated to support the hypothesis that larval host environment modifies ovipositional preferences in adult females (Rausher 1983, Wcislo 1989, Jaenike 1990). Hence, the observation that larval substrate apparently influences subsequent preferences of *P. glaucus* females is especially intriguing. In general, when presented a choice between *L. tulipifera* and *P. serotina*, the distribution of preferences expressed by *P. glaucus* females tends to be skewed towards *L. tulipifera* preference (as depicted in Figure 4), a pattern that has been observed more than once in the laboratory. This unwillingness to oviposit more than a few eggs on *P. serotina* by *P. glaucus* females has been observed in multi-choice host trials as well (Scriber 1993). *L. tulipifera* reared females exhibited the beginnings of this same general distribution of preferences skewed towards *L. tulipifera* preference, when presented with an *L. tulipifera/P. serotina* 2-choice combination (Figure 10). This pattern was observed despite the fact that only five females were tested; three of the five females oviposited 90% or more of their eggs on *L. tulipifera*. In contrast, the distribution of preferences of *P. serotina* reared females when presented with this same 2-choice combination was not skewed towards *L. tulipifera* preference, but rather spanned from *P. serotina* preferring females to

L. tulipifera preferring females. In fact, only one of eleven females oviposited at least 90% of her eggs on *L. tulipifera*. In general, *P. serotina* reared females were more willing to oviposit on *P. serotina*, a host typically not preferred. These population distributions are based on small sample size and, as such, are not amenable to direct statistical analysis; however, the fact that a similar link between larval host substrate and adult females preference was observed within a single family supports the interpretation that larval environment can modify ovipositional preference in *P. glaucus*.

Only certain larval host environments appear to influence subsequent preference in adult females. In contrast to results from the *L. tulipifera/ P. serotina* assay, *L. tulipifera* reared females behaved no differently than *M. virginiana* reared females when presented with an *L. tulipifera/M. virginiana* 2-choice combination (Figure 10). The similarity in preferences expressed between these two groups of females may reflect the fact that *L. tulipifera* and *M. virginiana* are both Magnoliaceae. The suite of chemical cues perceived by larvae and ovipositing females may be very similar for these two hosts.

Larval environment is thought to modify adult chemosensory responses in one of two ways. The link may result because females retain some "memory" of their larval host via an induced response. Sensory receptors may become "locked in on" stimuli experienced during early stages of development to the exclusion of stimuli experienced at a later stage (=Hopkins host selection principle; Hopkins 1917). Alternatively, this link may depend not on memory, but rather on chemical cues that are retained in the insect's cuticle or other tissues and perceived during subsequent stages of development (=chemical legacy hypothesis;

Corbet 1985). Such trace chemicals may be especially important during susceptible periods of development, e.g., chemical stimuli incorporated into the pupal case may be perceived by a female as she emergences. The causal mechanism underlying the link between larval host substrate and female preference in *P. glaucus* is unknown. However, determining the physiological basis of this link is secondary to the fact that such a link apparently exists for certain host species. The functional outcome will be the same regardless of the mechanism involved.

Adaptive significance of host preference variation: For genetic variation in host preference to be effectively neutral in *P. glaucus* would require that the nongenetic factors modify behavior to the extent that selection is unable to "recognize" an optimum genotype (Michaud 1990). Although nongenetic factors do alter the behavioral responses of ovipositing *P. glaucus* females, this contribution to phenotypic variation is apparently not sufficient to prevent selection from effecting change. When tested in an *L. tulipifera/M. virginiana* 2-choice array, *P. glaucus* females exhibiting *L. tulipifera* preference produced progeny that grew at the fastest rate on both *L. tulipifera* and *M. virginiana*. Since the preferences exhibited by these mothers had a significant genetic component (Figure 5), the existence of these positive correlations indicates a selective advantage. The interpretation that genetic variation is adaptively significant in *P. glaucus* is consistent with results that indicate that genetic variation in oviposition preference has lead to differentiation among geographic populations, presumably as a result of selection for increased recognition of locally abundant hosts (Bossart and Scriber 1993, Bossart, this volume).

The observation that relative preferences for *L. tulipifera* were positively correlated with relative growth rate on both *L. tulipifera* and *M. virginiana* constrasts with the response typically observed. In general, correlations between female preference and offspring performance arise because females choose the plant species (Via 1986, Singer et al. 1988) or plant type within a species (Ng 1988) most suitable for offspring performance among the choices presented. Such correlations are purported to be a mechanism whereby sympatric divergence can result (Smith 1966). The results presented here differ from these other studies in that preference for a single host (*L. tulipifera*) correlated with performance on multiple hosts. Since the *P. glaucus* females used in this experiment were collected from Ohio and never actually encounter *M. virginiana*, selection for *M. virginiana* tolerance has not occurred. That selection has increased recognition and tolerance of *L. tulipifera* without a corresponding loss of *M. virginiana* use, indicates the lack of a tradeoff between *M. virginiana* use and *L. tulipifera* use. Despite the fact that *M. virginiana* is a less suitable host in general (the fastest growing families on *M. virginiana* still grew at a slower rate than the slowest growing families on *L. tulipifera*), the significant positive correlation between relative growth rates of full-siblings on the two hosts suggests that larval tolerance on *L. tulipifera* and *M. virginiana* is controlled by many of the same genes.

MANUSCRIPT III

Intrapopulation genetic variation and covariation in larval performance across hosts in the polyphyagous eastern tiger swallowtail

Abstract

Full-sibling *Papilio glaucus* larvae from different geographic populations and broods expressed differential abilities to use three different host species: *Prunus serotina*, *Liriodendron tulipifera* and *Magnolia virginiana*. The significant host x family interaction resulted from changes in rank order of family means on the different hosts, indicating the *P. glaucus* is a "composite" generalist rather than a "true" generalist. This is despite the presence of homeostatic genotypes. Host use patterns were variable among populations and broods, arguing for the importance of analyzing intrapopulation variation for addressing evolutionary questions. Indirect evidence of tradeoffs in host use from multiple sources suggests that negative genetic correlations underlie host use patterns in *P. glaucus*.

Introduction

Falconer (1952) viewed a character expressed in two environments as two separate, genetically correlated traits. Such correlations among traits expressed in different host environments are purported to be a major determinant of diet breadth in herbivorous insects. Host generalization tends to be less common than specialization (Strong et al. 1984, Bernays and Graham 1988), implying that physiological tradeoffs in the ability to use multiple hosts dictate the evolution of diet breadth. However, even generalists may be comprised of a mosaic of differentially adapted genotypes exhibiting relative specialization on different hosts (Fox and Morrow 1981).

The extent to which tradeoffs in performance underlie host use patterns is unresolved. To date, there is little evidence to support the contention that negative genetic correlations define degree of specialization (Rausher 1984, Jaenike 1989, Fry 1990). To further confound the issue, Fry (1993) demonstrated that tradeoffs can exist even when genetic correlations based on full-sibling family means are zero or positive, and may not be present even when genetic correlations based on family means are negative. These spurious statistical results led Fry (1993) to conclude that research efforts to investigate correlated responses should focus on selection experiments. Unfortunately, the use of such experiments to resolve whether tradeoffs occur will likely not prove applicable in many cases. For many ecologically interesting insect species, selection experiments are so labor intensive as to be effectively impossible to carry out. Moreover, many species are difficult to maintain over multiple generations in a laboratory setting.

Given the problems associated not only with statistical procedures for analyzing correlated responses, but also with selection experiments, determining whether tradeoffs are important components of host breadth will necessitate the use of an array of techniques. Combining information obtained from a variety of sources should increase our interpretative abilities. When used in combination with statistical analyses, "norms of reaction" or "the profile of phenotypes across hosts produced by a given genotype..." (Via and Lande 1985), may be especially useful for investigating the genotypic structure of populations. Norms of reaction are pictoral representations of genotype by environment interactions and genetic correlations and permit direct observation of the response of a population of genotypes towards multiple host environments. In the absence of tradeoffs, the rank order of phenotypes on each host is maintained (Figure 12a). When tradeoffs are present, the rank order of phenotypes across hosts changes (Figure 12b). The degree of crossing among lines connecting family means on each host is an indication of the extent to which different genotypes are optimum on different hosts and the extent to which tradeoffs may be present.

Papilio glaucus L., the eastern tiger swallowtail butterfly, provides a unique opportunity for examining the role of differential performance and genotypic tradeoffs in defining patterns of host use. *P. glaucus* is an especially polyphagous tree-feeding insect, using hosts from at least seven plant families (Bossart and Scriber 1993). The ability of *P. glaucus* to feed on multiple hosts could result as a function of a single, especially robust genotype or conversely, as a function of differentially adapted genotypes (Fox and Morrow 1981). In this study I examine full-sibling performance of Ohio, Georgia and Florida *P. glaucus* populations on three hosts. Extensive variation among families within populations, coupled with interactions among families and host species, would be evidence that *P. glaucus* is comprised of a mosaic of relatively oligophagous genotypes. The degree to which physiological tradeoffs underlie this variation

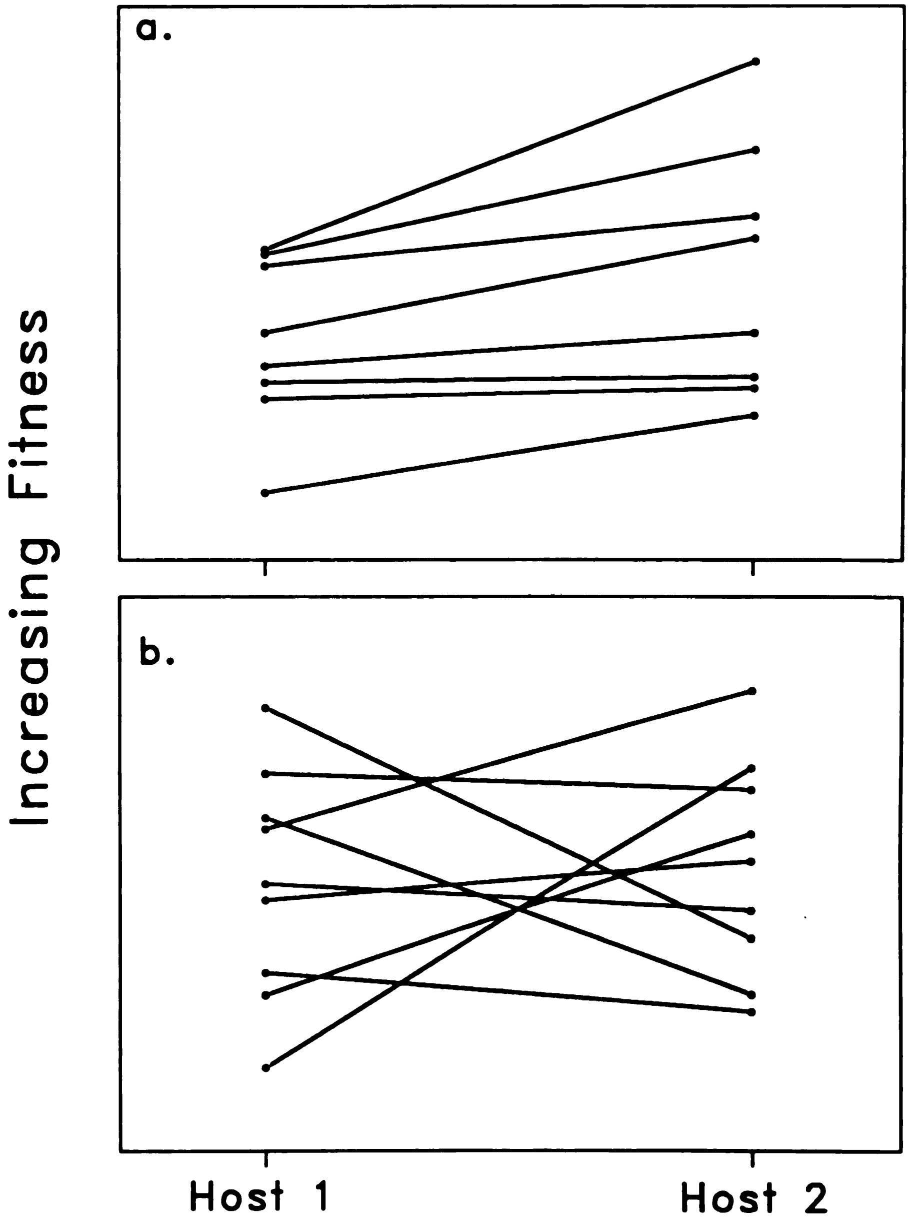

Figure 12. Reaction norms depicting two types of patterns that can underlie significant genotype x environment interactions: a) the rank order of phenotypes on each host is the same, tradeoffs are absent, b) the rank order of phenotypes is different on each host, tradeoffs may be present.

would be directly linked to the extent of change in rank order performance among *P. glaucus* families across different plant species.

Materials and Methods

Larval sources

Larvae were generated from adult *P. glaucus* females collected from field populations in Lawrence Co., Ohio, Clarke Co., Georgia and Highlands Co., Florida. Butterflies were collected from several local sites in each region. Florida butterflies were collected once, during early April 1988. The Ohio and Georgia populations were sampled twice for comparative purposes. Butterflies were collected from Ohio in July 1988 and 1991 to provide information on larval performance across years; butterflies were collected from Georgia in August 1988 and April 1989 to provide information on larval performance across broods. Field collected butterflies were placed in individual glassine envelopes, then transported to Michigan State University. Females were fed a 20% honey solution upon arrival, then stored at 24°C for 24 h prior to being set up for oviposition. To induce oviposition, females were individually placed in (10 cm x 20 cm x 27 cm) clear plastic "shoeboxes" (Tristate Plastics) with sprigs of *L. tulipifera*, a preferred host of ovipositing *P. glaucus* females. Boxes were maintained under artificial illumination, alternating 4h:4h photo:scotophase. Ovipositing females were fed daily. Eggs were collected and stored at 24°C until eclosion.

Larval performance

Full-sibling larval performance was compared across three *P. glaucus* hosts: *Liriodendron tulipifera*, *Magnolia virginiana* and *Prunus serotina*. The frequency of use of these hosts in nature differs for different *P. glaucus* populations. *M. virginiana* is the only host common to Highlands Co., Florida, but occurs only rarely in Clarke Co., Georgia, and not at all in Lawrence Co., Ohio. *P. serotina* and *L. tulipifera* both occur commonly in Georgia and Ohio. *P. serotina* and *L. tulipifera* 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. *M. virginiana* foliage was collected daily from potted trees maintained on campus.

Eggs were checked for eclosion every 2-3 h from 7:30 am - 7 pm. Larvae eclosing between 7 pm - 7:30 am were excluded from the performance assays. Ten to 15 neonate larvae from 8 - 17 families were randomly allocated to each of the three host species. Larvae were reared individually to pupation in (150 cm x 25 cm) screened, plastic petri dishes containing sprigs of the appropriate host foliage. Foliage sprigs were placed in water-filled, rubber-capped plastic vials to maintain leaf freshness. Petri dishes were stacked randomly in growth chambers maintained at 24°C, 18h:6h photo:scotophase. Larvae were checked daily and fresh foliage 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 eclosion 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 after shedding their larval exoskeleton.

Larval survival was calculated as a family percentage. Relative growth rate (RGR) was calculated as,

$\text{RGR} = (W_P - W_I)/((W_P - W_I/2) \times D)$, where

(W_I) is initial larval mass,

(W_P) is pupal mass, and

(D) is larval duration

Statistical analyses

Larval duration, pupal mass and relative growth rate were analyzed using the general linear method, mixed-model analysis of variance (PROC GLM; SAS 1985). Family and family x host were designated as random effects. Expected mean squares and error terms used to test each effect followed Ayres and Thomas (1990). All population-collection date combinations were analyzed separately. Standard product-moment genetic correlations between family means were calculated for each host pair for each larval trait examined (Via 1984). Survival data was arcsine transformed, as recommended when percentage data spans a wide range.

Pupal mass and larval duration tend to be sexually dimorphic in *P. glaucus*. When 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 heavier and grew 1.2 d longer than males. Not all families were represented by both males and females, consequently, sex could not be included as a source of variation in the analysis of variance. To account for the potential

Table 4. Analysis of variance comparing larval duration for full-sibling *P. glaucus* families collected from different populations and broods and reared on *P. serotina*, *M. virginiana* or *L. tulipifera*.

Source of Variation	df	MS	F^a
A. Florida - 1988			
Host	2	36.11	7.25**
Family	7	6.16	2.28*
Host x Family	14	4.98	1.85*
Error	60	2.70	
B. Georgia August Brood - 1988			
Host	2	230.07	20.09***
Family	16	22.97	2.96***
Host x Family	32	11.45	1.48*
Error	175	7.75	
C. Georgia April Brood - 1989			
Host	2	246.30	18.08***
Family	11	10.26	1.25
Host x Family	21	13.62	1.67*
Error	181	8.18	
D. Ohio July Brood - 1988			
Host	2	180.11	19.51***
Family	9	4.06	0.54
Host x Family	18	9.23	1.22
Error	100	7.55	
E. Ohio July Brood - 1991			
Host	2	852.82	118.38***
Family	13	14.38	2.00*
Host x Family	23	7.17	0.99
Error	141	7.20	

* $P < .05$, ** $P < .01$, *** $P < .001$.

[a] The F test denominator for Host was $MS_{Host\ x\ Family}$. The F test denominator for Host x Family was MS_{error}.

Table 5. Analysis of variance comparing relative growth rate for full-sibling *P. glaucus* families collected from different populations and broods and reared on *P. serotina*, *M. virginiana* or *L. tulipifera*.

Source of Variation	df	MS	*F*[a]
A. Florida - 1988			
Host	2	3.0×10^{-4}	6.84**
Family	7	5.6×10^{-5}	2.50*
Host x Family	14	4.4×10^{-5}	1.99*
Error	83	2.2×10^{-5}	
B. Georgia August Brood - 1988			
Host	2	1.8×10^{-3}	28.77***
Family	16	1.4×10^{-4}	3.14***
Host x Family	32	6.4×10^{-5}	1.40
Error	175	4.6×10^{-5}	
C. Georgia April Brood - 1989			
Host	2	2.0×10^{-3}	30.27***
Family	11	8.7×10^{-5}	1.30
Host x Family	21	1.4×10^{-4}	2.02**
Error	179	6.7×10^{-5}	
D. Ohio July Brood - 1988			
Host	2	1.1×10^{-3}	22.31***
Family	9	2.8×10^{-5}	0.77
Host x Family	18	4.8×10^{-5}	1.32
E. Ohio July Brood - 1991			
Host	2	3.0×10^{-3}	127.29***
Family	13	5.1×10^{-5}	2.15**
Host x Family	23	2.4×10^{-5}	1.00
Error	141	2.4×10^{-5}	

* $P < .05$, ** $P < .01$, *** $P < .001$.

[a] The *f* test denominator for Host was $MS_{\text{Host x Family}}$. The *F* test denominator for Host x Family was MS_{error}.

Table 6. Analysis of variance comparing pupal mass for full-sibling *P. glaucus* families collected from different populations and broods and reared on *P. serotina*, *M. virginiana* or *L. tulipifera*.

Source of Variation	df	MS	F[a]
A. Florida - 1988			
Host	2	0.435	5.51*
Family	7	0.096	2.67*
Host x Family	14	0.079	1.21
Error	60	0.036	
B. Georgia August Brood - 1988			
Host	2	0.339	11.69***
Family	16	0.106	4.69***
Host x Family	32	0.029	1.29
Error	175	0.227	
C. Georgia April Brood - 1989			
Host	2	0.195	10.87***
Family	11	0.098	5.43***
Host x Family	21	0.023	1.29
Error	179	0.018	
D. Ohio July Brood - 1988			
Host	2	0.294	8.65**
Family	9	0.114	6.46***
Host x Family	18	0.034	1.91*
Error	99	0.018	
E. Ohio July Brood - 1991			
Host	2	0.213	9.69***
Family	13	0.060	2.73**
Host x Family	23	0.015	0.70
Error	141	0.022	

* $P < .05$, ** $P < .01$, *** $P < .001$.

[a] The F test denominator for Host was $MS_{Host \times Family}$. The F test denominator for Host x Family was MS_{error}.

this variance attributable to families consists of both genetic and nongenetic maternal effects.

The most significant result with regard to differential adaptation and tradeoffs was that *P. glaucus* families from Florida and Georgia responded differently to the three host species. A significant host x family interaction effect was present for larval duration (Table 4) and relative growth rate (Table 5) in both the Florida and Georgia April broods. A significant interaction effect was also present for larval duration (but not relative growth rate, $p = .09$) in the Georgia August brood. Significant interactions among families and hosts were absent in both Ohio broods for these two traits. However, the Ohio brood collected in 1988 was the only brood to exhibit a significant interaction among families and host species for pupal mass (Table 6).

A significant host x family interaction can be present even if there is no change in the rank order of family means across hosts, i.e., the same families are optimum across species. However, tradeoffs in performance are implicated only when the host x family interaction reflects changes in rank order of family means. The norm of reaction diagrams for relative growth rate and larval duration indicate that *P. glaucus* families do not maintain the same level of performance across hosts (Figures 13-15) and rank order does change; only relative growth rates are depicted since this variable incorporates both larval duration and pupal mass. For example, the three Florida brood families growing at the slowest rate on *M. virginiana*, developed at the fastest rates on *L. tulipifera* (Figure 13). Similarly, many of the Georgia April brood families growing at the slowest rates on *M. virginiana*, developed at the fastest rates on *P. serotina* (Figure 14). Even

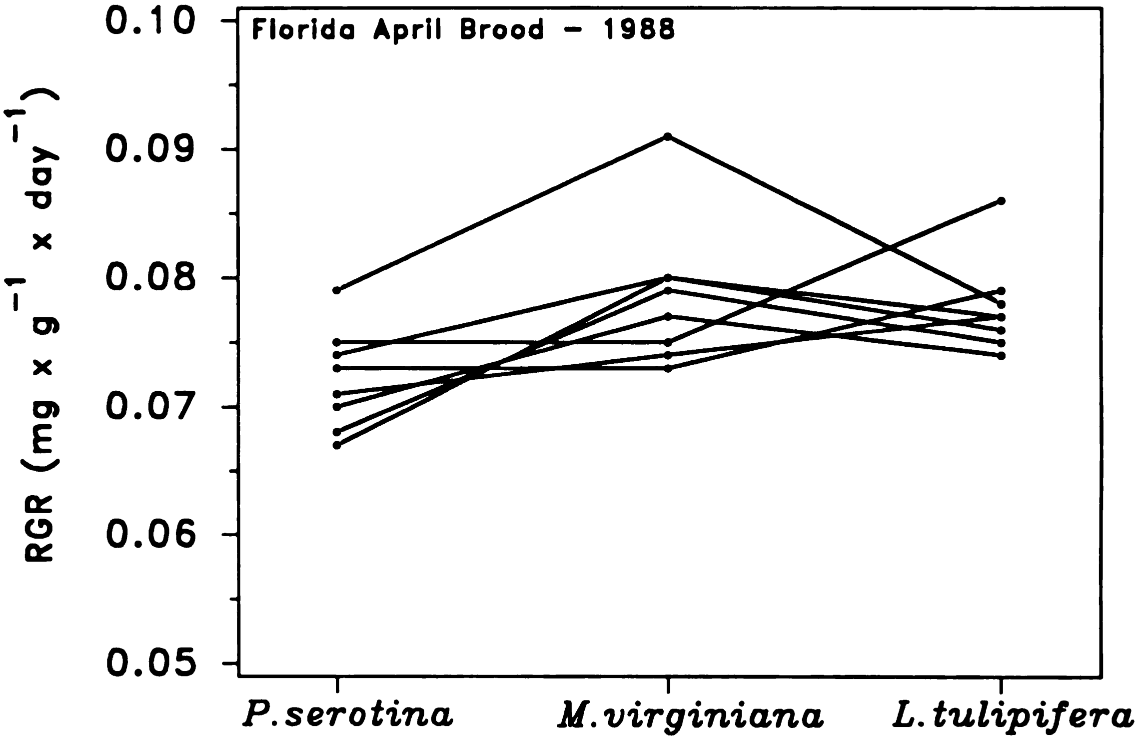

Figure 13. Reaction norms of relative growth rate for full-sibling, Florida *P. glaucus* larvae developing on *P. serotina*, *M. virginiana* or *L. tulipifera*.

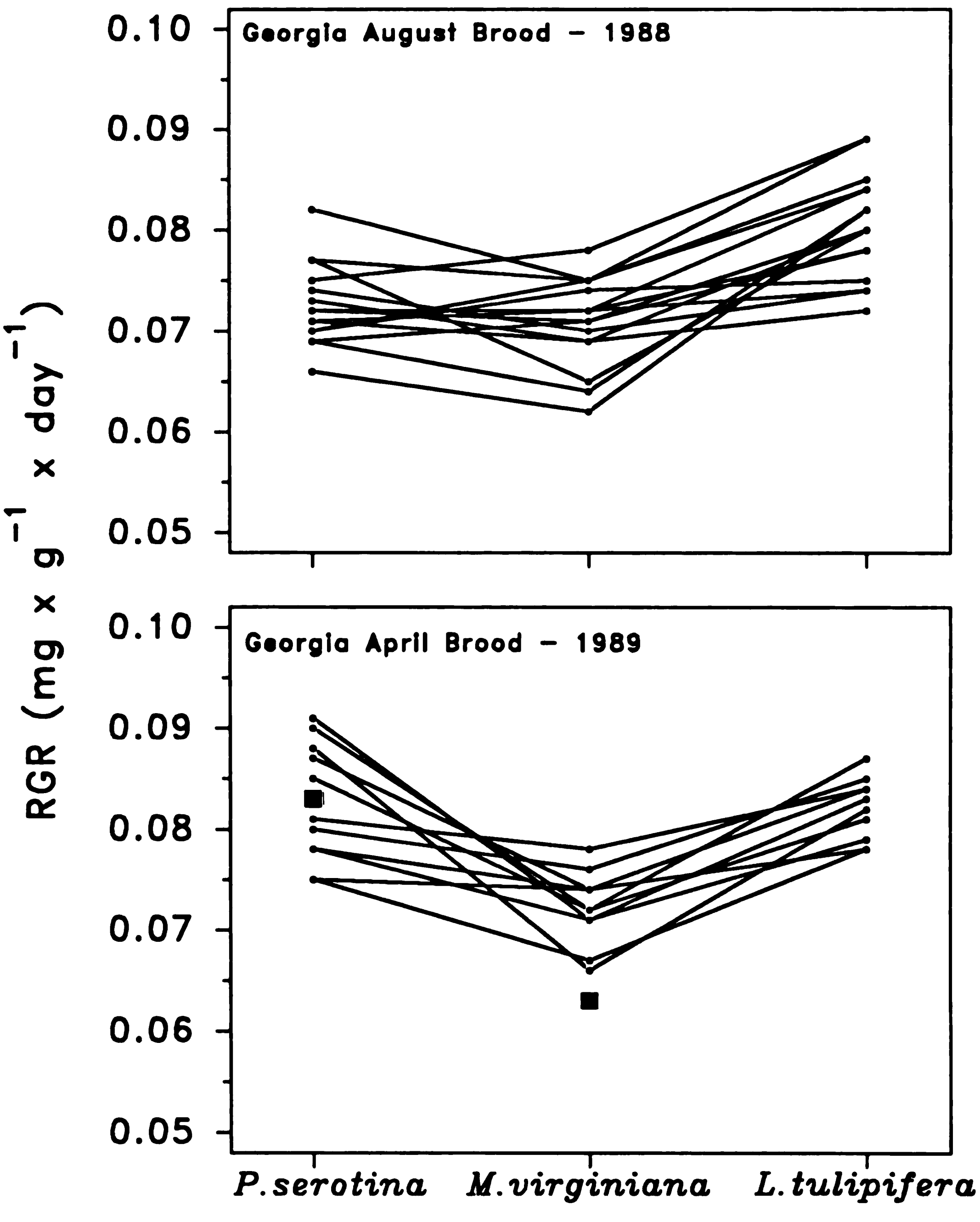

Figure 14. Reaction norms of relative growth rate for full-sibling, Georgia *P. glaucus* larvae developing on *P. serotina*, *M. virginiana* or *L. tulipifera*. The use of the symbol indicates a family not represented on *L. tulipifera*.

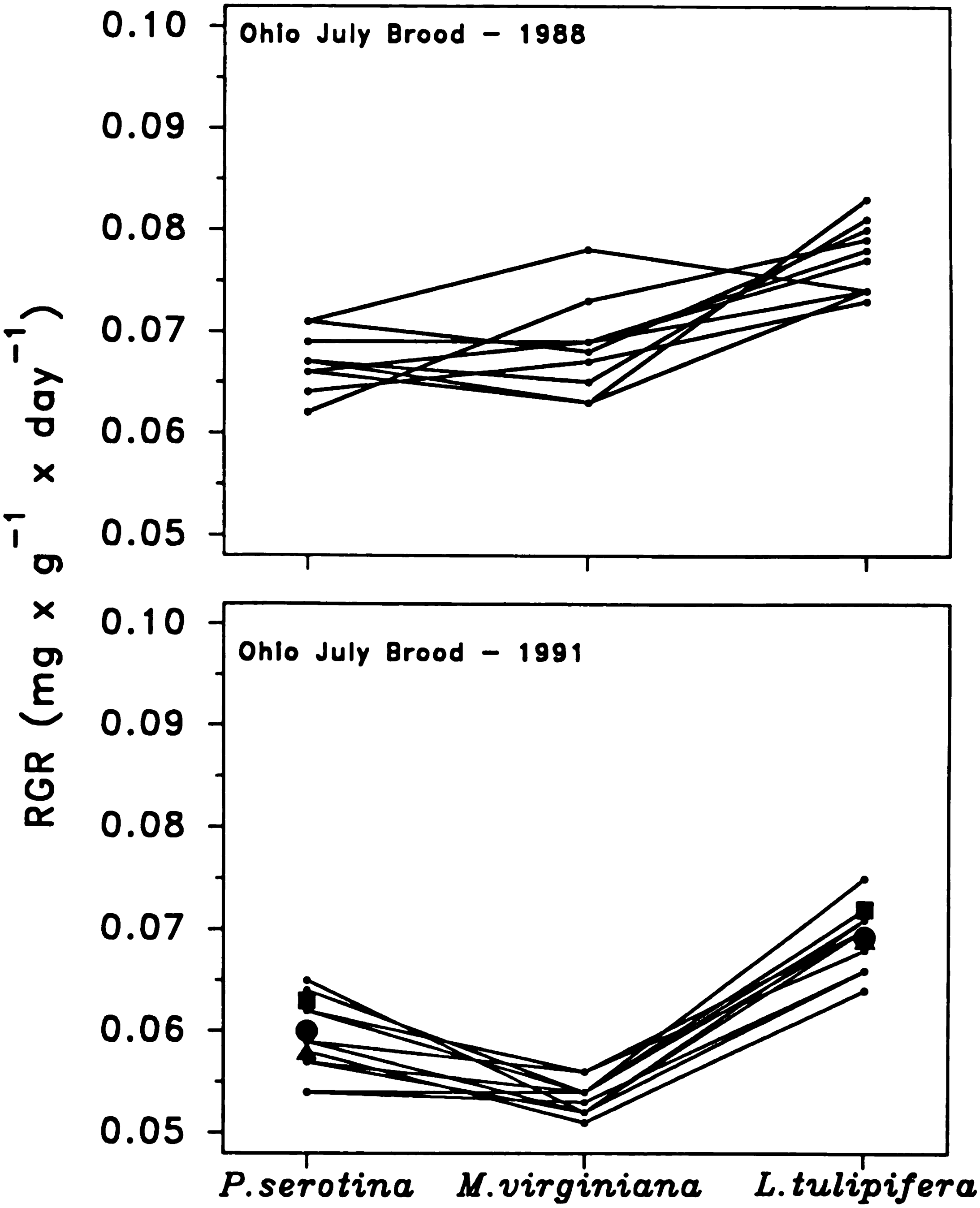

Figure 15. Reaction norms of relative growth rate for full-sibling, Ohio *P. glaucus* larvae developing on *P. serotina*, *M. virginiana* or *L. tulipifera*. The use of the symbols indicates families not represented on *M. virginiana*.

when host x family effects were not significant and the majority of families maintained the same level of performance across hosts, families with low rankings on one host and higher rankings on other hosts were present, as was the case in the Georgia August brood (Figure 14) and Ohio July brood (Figure 15).

Of the traits examined, family survival was the most labile (Figures 16-18). The Florida brood exhibited the least variation; variation was lower not only on a given host, but also across hosts. The Ohio 1991 brood was the only brood to exhibit an obvious host effect, with survival on *L. tulipifera* being 40 and 50% higher on average than survival on *P. serotina* and *M. virginiana*, respectively. However, survival of the Ohio 1991 brood on *P. serotina* and *M. virginiana* was much lower than for any of the other broods.

There were no major differences in host use patterns between the 1988 and 1991 Ohio broods. Although the Ohio 1988 brood grew 20% faster overall than the Ohio 1991 brood, the majority of families expressed the same general pattern across hosts (Figure 15). It's noteworthy, however, that two of the Ohio 1988 families were unique in their ability to grow at a fast rate on *M. virginiana*. A significant difference did occur between the late season, 1988 Georgia brood and the early season, 1989 Georgia brood. Despite virtually identical development for both Georgia broods on *M. virginiana* and *L. tulipifera*, development of the Georgia April brood was 15% faster than the August brood on *P. serotina* (25.4 d vs. 30.1 d; Figure 14).

Pupal mass (Table 7) and survival (Table 8) were the only traits for which significant family-mean genetic correlations were present between host pairs. All significant correlations were positive. The particular correlated host

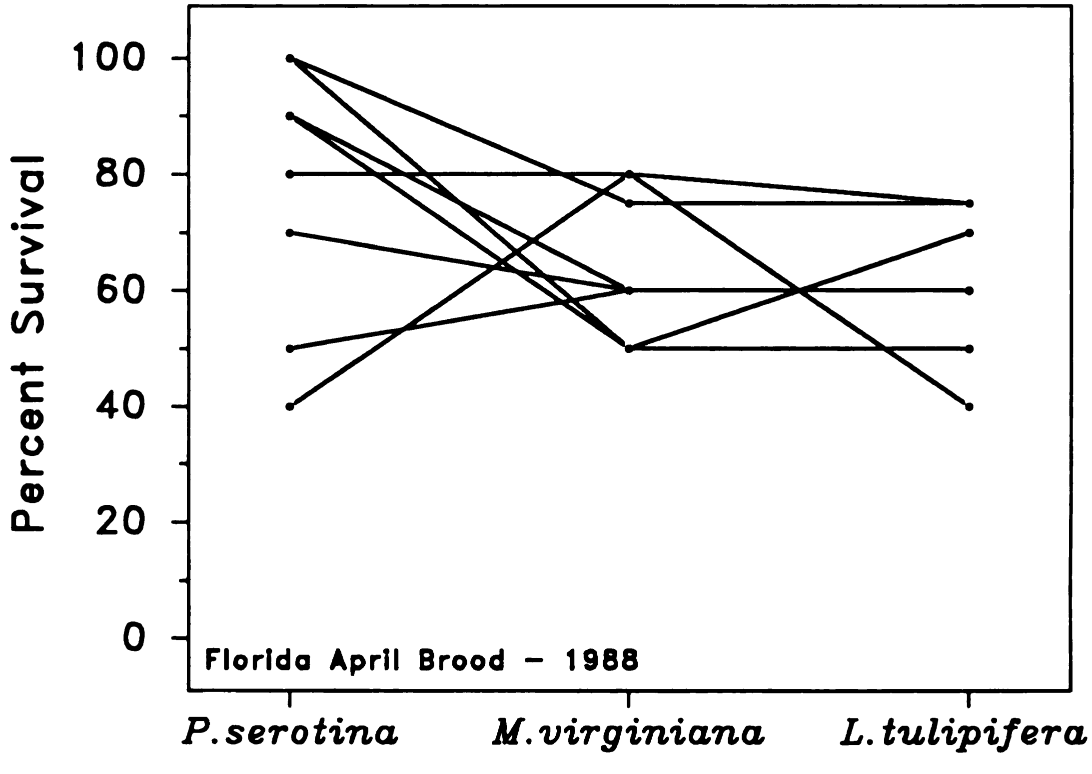

Figure 16. Reaction norms of percent survival of full-sibling, Florida *P. glaucus* larvae developing on *P. serotina*, *M. virginiana* or *L. tulipifera*.

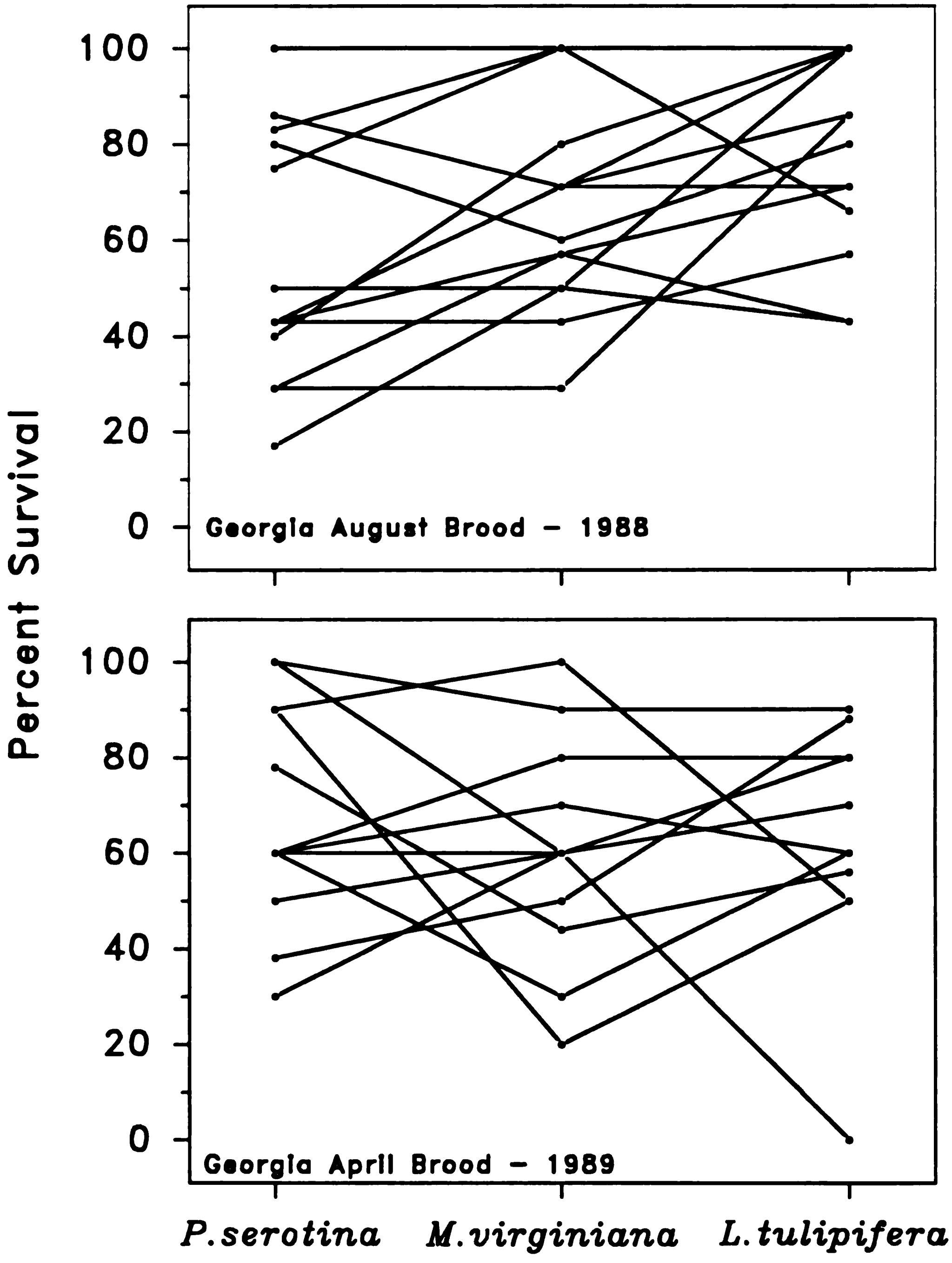

Figure 17. Reaction norms of percent survival of full-sibling, Georgia *P. glaucus* larvae developing on *P. serotina*, *M. virginiana* or *L. tulipifera*.

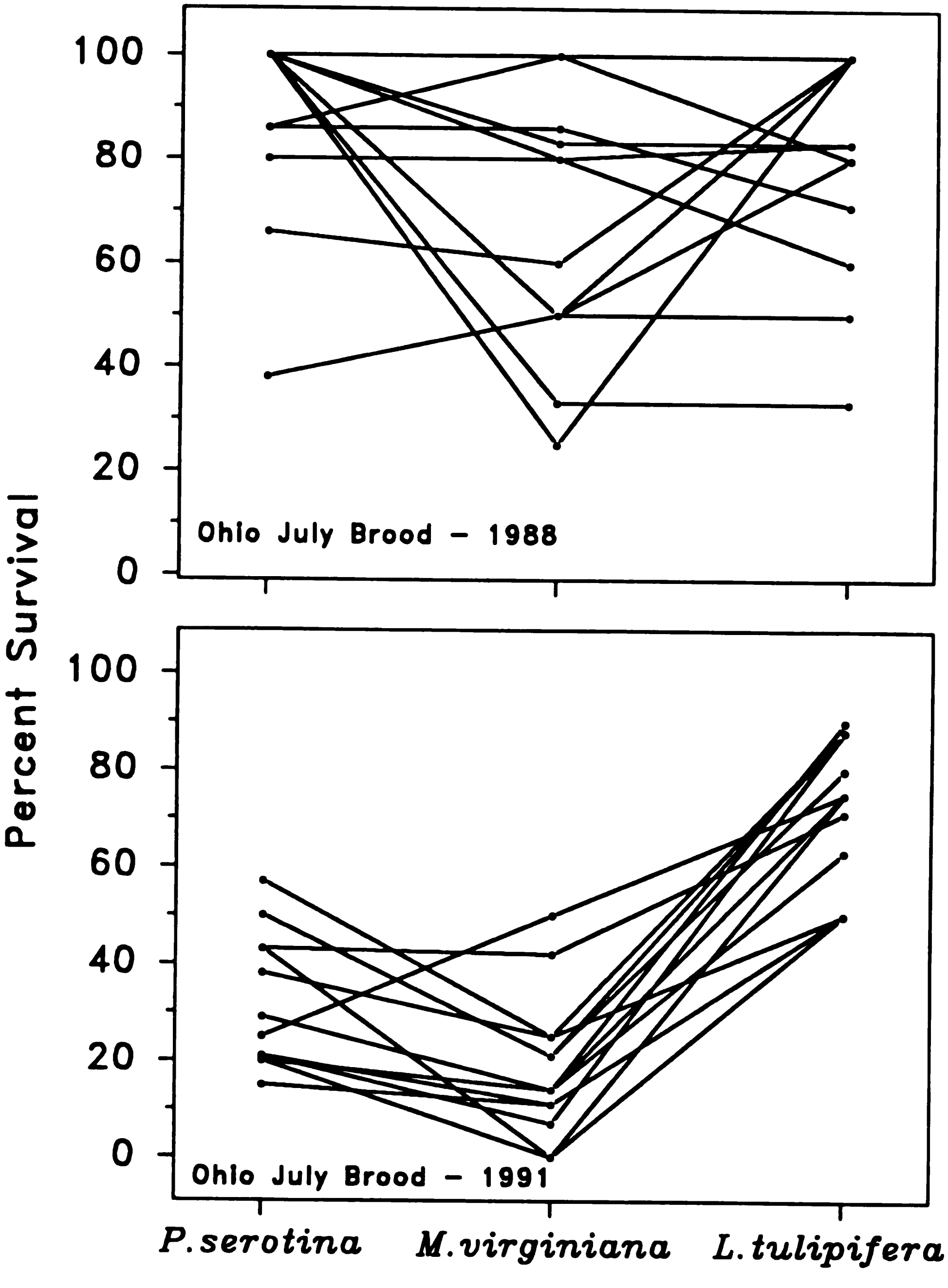

Figure 18. Reaction norms of percent survival of full-sibling, Ohio *P. glaucus* larvae developing on *P. serotina*, *M. virginiana* or *L. tulipifera*.

Table 7. Correlation matrix for *P. glaucus* populations and broods. Product-moment correlations are of larval host use traits expressed by full-siblings reared in different host environments. PSday, MVday and LTday = larval duration on *P. serotina*, *M. virginiana* and *L. tulipifera*, respectively; PSwgt, MVwgt and LTwgt = pupal mass on *P. serotina*, *M. virginiana* and *L. tulipifera*, respectively; PSrgr, MVrgr and LTrgr = relative growth rate on *P. serotina*, *M. virginiana* and *L. tulipifera*, respectively.

	PSday	MVday	LTday		PSwgt	MVwgt	LTwgt		PSrgr	MVrgr	LTrgr
A. Florida April Brood - 1988 (n=8)											
PSday	1.00	0.38	0.58	PSwgt	1.00	0.85**	-0.16	PSrgr	1.00	0.56	0.43
MVday		1.00	-0.15	MVwgt		1.00	0.05	MVrgr		1.00	-0.19
B. Georgia August Brood - 1988 (n=16)											
PSday	1.00	0.40	0.07	PSwgt	1.00	0.64**	0.51*	PSrgr	1.00	0.37	0.13
MVday		1.00	0.32	MVwgt		1.00	0.58**	MVrgr		1.00	0.13
C. Georgia April Brood - 1989 (n=12)											
PSday	1.00	-0.07	-0.21	PSwgt	1.00	0.14	0.86***	PSrgr	1.00	-0.18	-0.10
MVday		1.00	0.46	MVwgt		1.00	0.32	MVrgr		1.00	0.43
D. Ohio August Brood - 1988 (n=11)											
PSday	1.00	-0.11	0.25	PSwgt	1.00	0.20	0.14	PSrgr	1.00	0.19	0.08
MVday		1.00	-0.29	MVwgt		1.00	0.63*	MVrgr		1.00	-0.25
E. Ohio August Brood - 1991 (n=14)											
PSday	1.00	0.15	0.46	PSwgt	1.00	0.60	-0.15	PSrgr	1.00	0.15	0.47
MVday		1.00	0.01	MVwgt		1.00	0.48	MVrgr		1.00	0.39

* $P < .05$, ** $P < .01$, *** $P < .001$.

Table 8. Correlation matrix of full-sibling larval survival across 3 host species for *P. glaucus* populations and broods. PSsrv, MVsrv and LTsrv = survival on *P. serotina*, *M. virginiana* and *L. tulipifera*, respectively.

	PSsrv	MVsrv	LTsrv
A. Florida April Brood - 1988 (n=8)			
PSsrv	1.00	0.40	-0.31
MVsrv		1.00	0.11
B. Georgia August Brood - 1988 (n=16)			
PSsrv	1.00	0.21	0.81***
MVsrv		1.00	0.36
C. Georgia April Brood - 1989 (n=11)			
PSsrv	1.00	0.04	0.30
MVsrv		1.00	0.13
D. Ohio July Brood - 1988 (n=11)			
PSsrv	1.00	0.05	-0.05
MVsrv		1.00	0.08
E. Ohio July Brood - 1991 (n=14)			
PSsrv	1.00	0.51*	0.25
MVsrv		1.00	0.08

* $P < .05$, *** $P < .001$.

combinations were brood dependent. The exception was the Georgia August brood, where pupal mass was significantly correlated for all host combinations, i.e., families with the heaviest mass on one host tended to attain the heaviest mass on the other hosts (Table 7). Many of the nonsignificant host/trait correlations were fairly large, suggesting that at least some of the same genes were controlling development. Twice as many of the nonsignificant correlations were positive, as negative.

Discussion

The significant host x family interactions and change in rank order of performance across hosts provides evidence that *P. glaucus* is comprised of a mosaic of differentially adapted genotypes, exhibiting differing abilities to use different plant species. The presence of genetically based variation in diet breadth establishes that *P. glaucus* is a composite generalist rather than a true generalist (Fox and Morrow 1981), i.e., polyphagy in *P. glaucus* is a function of the genotypic structure of the species as a whole, not of one individual, especially robust genotype. However, there is also a homeostatic component of polyphagy in *P. glaucus*. A number of families in both Georgia broods, but particularly the Georgia August brood, exhibited comparable relative growth rates across all three hosts. That these genotypes were observed only in the Georgia population is especially noteworthy since this population is the only population in which all three hosts occur sympatrically. The Florida population encounters only *M. virginiana* and the Ohio population, only *P. serotina* and *L. tulipifera* (Bossart, this volume; Scriber 1986). Such developmental stability is predicted as an end result of differential selection in a heterogeneous, stable environment when negative genetic correlations are absent and gene flow is balanced (Via and Lande 1985). However, equilibrium conditions are likely extremely rare in natural populations and since all the homeostatic genotypes exhibited lower growth rates than any of the other families on *L. tulipifera*, and lower than most other families on the other two hosts, it is unlikely that their frequency in the population will increase unless they display an advantage on hosts not examined.

Differences in host use patterns among Georgia broods likely reflect phenological variation in the quality of *P. serotina* as a food resource. A higher nutritive value of early season *P. serotina* foliage would explain the faster relative growth rates of early brood larvae. Activity levels of ß-glucosidase in *P. glaucus* larvae developing on *P. serotina* are known to be seasonally variable (Lindroth 1988), presumably reflecting seasonal change in plant glycosides. Plants, in general, express seasonal changes in both the quality and quantity of nutrients and allelochemicals (Mattson 1980, Scriber and Slansky 1981). That different *P. glaucus* populations and broods exhibit variable host use patterns argues the importance of analyzing intrapopulation variation when addressing evolutionary questions. The variation among *P. glaucus* broods and populations illustrates that interpretations based on population means will not adequately describe the evolutionary potential of separate populations (Via 1990). Moreover, since the sign and magnitude of genetic correlations are dependent upon, not only environmental effects, but also developmental stage, and specific traits examined (Stearns et al. 1991), it may not be valid to extrapolate from population to population or year to year.

The degree of correlation between performance on different host species within a given population or brood reflects the extent to which host use traits are governed by the same alleles. Significant correlation indicates that traits are linked or controlled by pleiotropy, while low correlation indicates that different genes underly each trait (Falconer 1981). Most genetic correlations calculated for *P. glaucus* were positive and nonsignificant, suggesting that adaptation to different host species is generally unconstrained by other hosts. Few negative correlations were observed and those that were, were weak and nonsignificant. It is interesting, though, that when negative correlations were detected, they tended to be associated with populations and hosts that might be expected to show evidence of tradeoffs if such existed. In both the Florida and Ohio 1988 populations, larval duration and relative growth rate on *L. tulipifera* tended to negatively covary with these same two traits on *M. virginiana*. If tradeoffs between use of these two hosts are present, then selection for enhanced use of locally occurring hosts (*M. virginiana* in Florida and *L. tulipifera* in Ohio) would decrease use of the host not present (*L. tulipifera* in Florida and *M. virginiana* in Ohio).

The reaction norms suggest that tradeoffs in host use may be present, as indicated by the crossing among family lines on different hosts. In all *P. glaucus* broods, there were genotypes that expressed optimum performance on one host, while expressing suboptimum performance on a second host. Tradeoffs were also implied by the absence of an optimal genotype across all hosts; this is despite ample opportunity for the evolution of optimization. At least 20,000 generations have elapsed since glacial retreat, yet a predominance of genotypes exhibiting high fitness on all hosts is absent; *P. glaucus* populations continue to be comprised of a number of presumably nonadapted genotypes.

The evidence for genetic tradeoffs in the response of *P. glaucus* larvae towards different host species is indirect, but comes from multiple sources: the presence of significant family x host interactions of the crossing type, the presence of weak, negative genetic correlations between traits that would be expected to show such correlations if tradeoffs exist, and the lack of optimization across hosts despite ample evolutionary opportunity. Even such minimal evidence necessitates that the potential impact of tradeoffs on evolutionary trajectories be considered (Via 1990). Tradeoffs are implicit in theories pertaining to the evolution of specialization in polyphagous insects and can maintain genetically-based variation in patterns of host use (e.g., Rose 1983). Differentiation already occurs between Florida and Ohio *P. glaucus* for host use traits (Bossart, this volume, Scriber 1986). The presence of a tradeoff in the use of *M. virginiana* and *L. tulipifera* could effect continued divergence between these two populations until such time that they become completely, independently evolving entities. At the very least, such tradeoffs could impose significant evolutionary constraints on host adaptation, especially initially. Tradeoffs in host use can impede not only the rate, but direction of evolution (Lande 1984, Via 1984, Arnold 1987).

The lack of convincing evidence of genetic tradeoffs from natural populations to date is not surprising. Negative correlations should only be apparent in populations currently in a state of evolutionary flux. If negative correlations were the driving force underlying specialization on a particular host, alleles permitting use of a second host would have been eliminated from the population and hence, would not be detected. Moreover, given that selective forces acting on populations are numerous and variable over time, tradeoffs in feeding ability on different plant species would likely be concealed unless they were particularly strong. The sample sizes needed to detect such correlations would need to be large (Stearns et al. 1991). When coupled with the statistical and logistical difficulties associated with testing and detecting genetic tradeoffs, it is little wonder that their presence has gone virtually undetected in natural populations. The approach used herein was to combine information obtained from analyses of variance, product-moment correlations and reaction norms. Unfortunately, despite providing useful information, none of these techniques permit unequivocal conclusions to be drawn (Fry 1993, Rausher 1983). While laboratory selection experiments permit valid conclusions, they are impractical for most ecologically interesting species, and may not be representative of natural populations since natural populations never encounter laboratory conditions. Given the difficulties associated with demonstrating tradeoffs in host use, clear evidence for such tradeoffs in herbivore populations will likely continue to be elusive.

GENERAL CONCLUSIONS

With regard to the traits studied in this project, host adaptation in *P. glaucus* is a function of both female host preference and larval physiology. That the host environment encountered by *P. glaucus* is heterogeneous is evidenced by the differential performance of populations and genotypes within populations on different host species. Presumably, these differences in host use are due to differences in the physical and chemical attributes of the plants. *L. tulipifera* appears to be the most optimal host species. Not only does this host tend to elicit the highest response in ovipositing females, it also tends to support the fastest growing larvae which develop into the heaviest pupae. Much of the extensive phenotypic variation that exists in *P. glaucus* for host preference and larval tolerance (Ayres et al. 1991; Bossart, this volume) is genetically based. The presence of adaptively significant variation both within and among geographic populations provides ample opportunity for local selective factors to shape host-use patterns. No one host is coincident with the range of this butterfly and separate populations have differentiated with respect to their ability to recognize and develop on locally abundant hosts. Florida *P. glaucus* oviposit more willingly, and develop at a faster rate, on *M. virginiana*, the prevalent Florida host, than Ohio or Georgia *P. glaucus*. Presumably, this response is a result of the selective pressures imposed by this host.

The presence of significant interactions between host species and families establishes that polyphagy in *P. glaucus* is not a function of a single, particularly robust genotype, but of a collection of differentially adapted genotypes. Not all genotypes responded equally well in all host environments; optimum genotypes on one host were often suboptimumal on another. Evidence from indirect sources suggests that physiological tradeoffs in the ability to use *M. virginiana* and *L. tulipifera*, in part, underlie this differential performance. Definitive evidence, however, will likely remain elusive. The use of selection experiments to address the existence of tradeoffs in *P. glaucus* was unproductive in this study and will probably be unproductive in ensuing studies since generations subsequent to the F2 are extremely difficult to obtain.

The geographic differentiation observed for larval tolerance and host preference contrasted with the lack of any such pattern for electrophoretically detectable, presumably neutral variation. In the absence of knowledge regarding differentiation in host use traits, the electrophoretic results indicate virtual panmixia in *P. glaucus*. That *P. glaucus* is a single, panmictic population, however, is extremely unlikely. Isolation by distance should preclude completely random mating. Mating would also be nonrandom if restrictions to gene flow prove to be present, as suggested by other studies (R. C. Lederhouse, unpubl.; J. L. Bossart, unpubl.). A more likely explanation for the absence of differentiation among populations in neutral variation is that the electrophoretic analysis of isozymes was probably not sufficiently sensitive to detect genetic differentation within *P. glaucus*, differentiation that might be revealed by other, more sensitive molecular techniques. This interpretation is supported by results of other studies which demonstrate that an analysis of nuclear or mitochondrial DNA may uncover differentiation even when an electrophoretic analysis suggests that genetic differentiation does not occur (Zink 1991, Karl and Avise 1992).

Regardless of whether other molecular techniques reveal latitudinal variation in *P. glaucus*, the electrophoretic analysis suggests that gene flow is sufficiently common throughout the range of this butterfly so as to act as a homogenizing influence. In a heterogeneous environment, gene flow would counteract differential selection on different hosts thereby drastically slowing the rate of optimization across hosts. However, even in a heterogeneous environment, optimization would be expected to evolve eventually such that genotypes with high fitness on all hosts would predominate, given stable environmental conditions and equal gene flow among hosts. The observation that optimization has not evolved in *P. glaucus* in over 20,000 generations since glaciation, implies that either tradeoffs are present or conditions are not at equilibrium, or both. Both of these factors probably define host use patterns in *P. glaucus*. Though weak and indirect, evidence of tradeoffs in host use is present. Moreover, host suitability likely changes both temporally and spatially. Optimum genotypes are probably dynamic, reflecting a stochastic, constantly changing host environment. Equilibirium conditions, in general, are probably very rare in natural populations. I predict that differential selection on ecologically important variation in *P. glaucus* will continue to be countered by gene flow, thereby maintaining genetic variation for host-use traits. In the absence of more substantial barriers to gene flow, the eastern tiger swallowtail will continue to be comprised of a mosaic of genotypes exhibiting differing abilities to use different host species.

APPENDIX

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1993-1

Title of thesis or dissertation (or other research projects):

Differential selection and adaptation in different host environments: genotypic and phenotypic variation in host use traits in the tiger swallowtail butterfly, *Papilio glaucus* L.

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Janice L. Bossart

Date 7 June 1993

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original:	Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies:	Included as Appendix 1 in copies of thesis or dissertation. Museum(s) files. Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio glaucus L.	FL: Highlands Co. April 1989 J. L. Bossart					7	5		MSU
Papilio glaucus L.	GA: Clarke Co. Lab reared 1989 J. L. Bossart					6			MSU
Papilio glaucus L.	GA: Clarke Co. August 1988 J. Maudsley						5		MSU
Papilio glaucus L.	OH: Lawrence Co. Lab reared 1989 J. L. Bossart					7			MSU
Papilio glaucus L.	OH: Lawrence Co. May 1991 M. P. Ayres						4		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

J. L. Bossart

Date 7 June 1993

Voucher No. 1993-1

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

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