VARIATION IN THE NUTRITIONAL PHYSIOLOGY OF TREE-FEEDING SWALLOWTAIL CATERPILLARS

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INTRODUCTION

A key problem in addressing patterns of interaction between forest insects and their host trees is determining the level at which important ecological and evolutionary interactions occur. We commonly view plant-herbivore relations as herbivore species interacting with plant species, tacitly assuming that variation among members of either species is small and one can represent their interaction by the average characteristics of the insect and the tree species (Ehrlich and Raven 1964, Feeny 1976, Rhoades and Cates 1976, Scriber and Feeny 1979). This simplistic view is violated if insects within a species differ markedly in their ability to grow on a particular host, or if trees within a species differ substantially in quality as perceived by the insect. If variation within both insect and host species is large and pervasive, then insect-plant interactions may be best studied at the level of genotypes and populations (Edmunds and Alstad 1978, Fox and Morrow 1981, Thompson 1988a, Ng 1988, Karban 1989). Clearly intraspecific variation exists. For example, potato beetles, checkerspot butterflies, tortoise beetles, autumnal moths, and tiger swallowtails are each comprised of populations that differ in their ability to use key hosts (Hsiao 1978, Rausher 1982, 1984, Haukioja and Hanhimaki 1985, Scriber 1986a, Hare and Kennedy 1986). Likewise mountain birch, cottonwood, honeylocust, and a variety of other trees are comprised of populations that differ in their suitability for specific herbivores (Hanover 1980, Haukioja and Hanhimaki 1985, Pakash and Heather 1986, Herms et al. 1987). Even within populations, individual trees may be highly variable (Edmunds and Alstad 1978, Whitham and Slobodchikoff 1981, Smiley et al. 1985, Ayres et al. 1987, Whitham 1989). The emerging problem is to quantify variation within and between populations relative to variation at other levels.

Our studies have addressed variation in the nutritional physiology of tree-feeding swallowtail caterpillars (Papilionidae: Lepidoptera). A central objective has been to determine the level at which ecological and evolutionary divergence has occurred. For example, physiological adaptation of caterpillars to their host plants may occur at the level of genotypes and populations, at the level of species and species groups, or both. Genetic variation within and between populations implies a potential for adaptation to local conditions. Alternatively, substantial variation between closely related species, with little intraspecific variation, implies that adaptive shifts occur via reproductive isolation and are often associated with speciation events (Mayr 1963). In this case, it is appropriate to emphasize species attributes.

Our research has concentrated on traits of likely ecological importance, and especially on traits subject to geographically variable selective pressures. In addition to nutritional physiology and detoxification, these include mate choice and reproductive allocation (Burns 1966, Svard and Wiklund 1986, Lederhouse et al. 1989), cold hardiness and diapause biology (Shimado 1988, Hagen and Scriber

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1989, Kukal et al. in prep.), host choice and oviposition behavior (Grossmueller and Lederhouse 1985, 1987, Thompson 1988b, 1988c, Bossart and Scriber in prep.), and larval temperature responses (Scriber and Lederhouse 1983, Ritland and Scriber 1985). Our ability to draw inferences about the evolutionary history of these attributes is strengthened by the existence of a large number of *Papilio* taxa with differing degrees of relatedness (Hagen and Scriber 1990). Here we describe recently completed experiments testing the nature and extent of variation within two closely related species of *Papilio*, *P. glaucus*, and *P. canadensis*.

BACKGROUND

Nearctic Tree-feeding Swallowtails

Most of the common tree-feeding papilionids in North America belong to two sister clades within the genus *Papilio*: the *P. glaucus* and *P. troilus* species groups. Host use patterns in the *P. troilus* group are typical of the Papilionidae in that all its members (*P. troilus* L., *P. palamedes* Drury, and *P. pilumnus* Boisduval) are restricted to hosts from a single plant family (Lauraceae). In contrast, all six members of the *P. glaucus* group (*P. glaucus* L., *P. alexiares* Hoppfer, *P. canadensis* R & J, *P. eurymedon* Lucas, *P. rutulus* Lucas, and *P. multicaudatus* Kirby) feed on more than one family of hosts (Scriber 1973, 1984). Polyphagy appears to be a derived trait (synapomorphy) within the *P. glaucus* group (Scriber et al. 1991). Unlike the *P. troilus* group, neonate larvae of the *P. glaucus* group typically initiate feeding on virtually any foliage they encounter, toxic or not (Scriber et al. 1991). This behavioral difference may have been a critical precondition for the evolution of polyphagy in *P. glaucus* and its relatives because selection cannot screen for suitable physiologies unless the larvae feed (Feeny 1991). All members of the *P. glaucus* group share the ability to feed on black cherry (*Prunus serotina*, Rosaceae) and chokecherry (*Prunus virginiana*), but *P. glaucus* species differ in their growth performance on most host taxa other than *Prunus* (Scriber et al. 1991). *P. eurymedon* appears to be uniquely capable of developing on *Rhamnus* (Rhamnaceae). *P. canadensis*, *P. eurymedon*, and *P. rutulus* generally grow well on plants of the Salicaceae, yet few *P. glaucus* or *P. alexiares* survive beyond the first instar. Magnoliaceae are typically lethal to all *P. glaucus* taxa except *P. glaucus* and *P. alexiares*.

Papilio glaucus and *Papilio canadensis*

Best known among the tree-feeding swallowtails are two taxa formerly regarded as subspecies, but now believed to be separate species (Hagen et al. in prep.). The transition zone between *P. glaucus* (= "*glaucus*") and *P. canadensis* (= "*canadensis*," formerly *P. g. canadensis*) coincides with a complex ecotone that runs through central Wisconsin and Michigan and extends eastward through northern Pennsylvania, central New York, and southern New England (Scriber 1988). The ecotone has long been recognized as the transition between boreal coniferous forests and diverse deciduous southern forests (Curtis 1959, Braun 1974). This zone marks a climatic boundary between regions influenced by cool, dry air masses to the north and warmer, wetter air masses to the south; it approximates the 1,400 degree day isotherm (10° C base), which is the lower limit for bivoltine potential in *glaucus* (Scriber 1988). The ecotone also corresponds to the southern edge of Pleistocene continental glaciation and thus marks a discontinuity in soil types, topography, and history of occupancy by plants and animals.

P. glaucus and *P. canadensis* are distinguishable by wing morphology, allozymes, and mitochondrial DNA restriction sites (Luebke et al. 1987, Hagen 1990 and in prep.). They also differ in several characteristics of obvious ecological significance. Diapause is induced by short photoperiods in the multivoltine *glaucus*, while *canadensis* is necessarily univoltine (Rockey et al. 1987a, 1987b). The species also differ in the expression (y-linked trait in *glaucus*) and suppression (x-linked trait in

canadensis) of a mimetic dark morph in the females (Clarke and Sheppard 1962, Hagen and Scriber 1989). Host use differences between *glaucus* and *canadensis* are especially pronounced (Fig. 1). In laboratory trials, first instar *glaucus* larvae grew rapidly on tuliptree, *Liriodendron tulipifera*, foliage (Magnoliaceae), doubling their weight in less than a day, while comparable development in *canadensis* larvae required 3 to 4 days. *P. glaucus* larvae also grew somewhat faster on hoptree, *Ptelea* (Rutaceae). Yet on *Populus tremuloides* (quaking aspen) and *Betula papyrifera* (paper birch), *canadensis* larvae grew substantially faster than *glaucus* larvae. These patterns are consistent with those based on first instar survival, total development time, and pupal mass (Scriber 1983, Lindroth et al. 1986). The northernmost limits of tuliptree and hoptree, and the southernmost limits of aspen and birch, are coincident with the transition zone between the species. However, *glaucus* and *canadensis* range limits are not a simple consequence of these host distributions because other common hosts for each, e.g. cherries (*Prunus*) and ashes (*Fraxinus*), extend either side of the transition zone.

Laboratory handpairings of *glaucus* and *canadensis* adults produced F_1 hybrids that grew and survived on both aspen and tuliptree foliage (Scriber 1986b), suggesting the involvement of two discrete detoxification systems independently controlled by autosomal loci. The ability of *canadensis* larvae to use quaking aspen depends at least in part on the ability to detoxify phenolic glycosides (Lindroth et al. 1988, Scriber et al. 1989). Backcross studies indicated a detoxification threshold necessary for survival, beyond which performance on diets containing phenolic glycoside tremulacin increased with increasing proportions of *canadensis* genes.

Intraspecific Variation

Populations of *glaucus* and *canadensis* in different regions may encounter very different communities of host plants. This is true on a relatively fine scale (river valleys versus uplands) as well as on a coarser geographic scale (the swamp bayheads of Florida versus the Appalachian forests of southern Ohio, or the northern hardwoods of the Great Lakes region versus the boreal forests of interior Alaska). Populations inhabiting regions as distant and floristically disparate as Florida and Ohio, or Alaska and the Great Lakes, must be subject to selection favoring physiological traits that improve growth performance on local hosts. Indeed, larvae from southern Florida survived better on sweetbay, *Magnolia virginiana*, the only regionally abundant host, than larvae from other *glaucus* populations (Scriber 1986a). It has been suggested that Florida populations of *P. glaucus* represent a distinct race, *P. g. australis* Maynard, but electrophoretic comparisons indicate no barriers to gene flow, and we regard the Florida insects as southern populations of *P. glaucus*.

The remainder of this paper describes experiments designed to test the prevalence of regional specialization (Hsiao 1978, Scriber 1983, Scriber 1986a) and allow for quantitative comparisons of variation within and between populations with variation between species and species groups. One set of experiments compares *canadensis* populations from Alaska and the Great Lakes region. Other experiments compare *glaucus* populations from Florida, Georgia, and Ohio. Associated electrophoretic studies provide estimates of population divergence and substructuring independent of the growth performance studies and survey specific allozymes for linkage with host-use abilities.

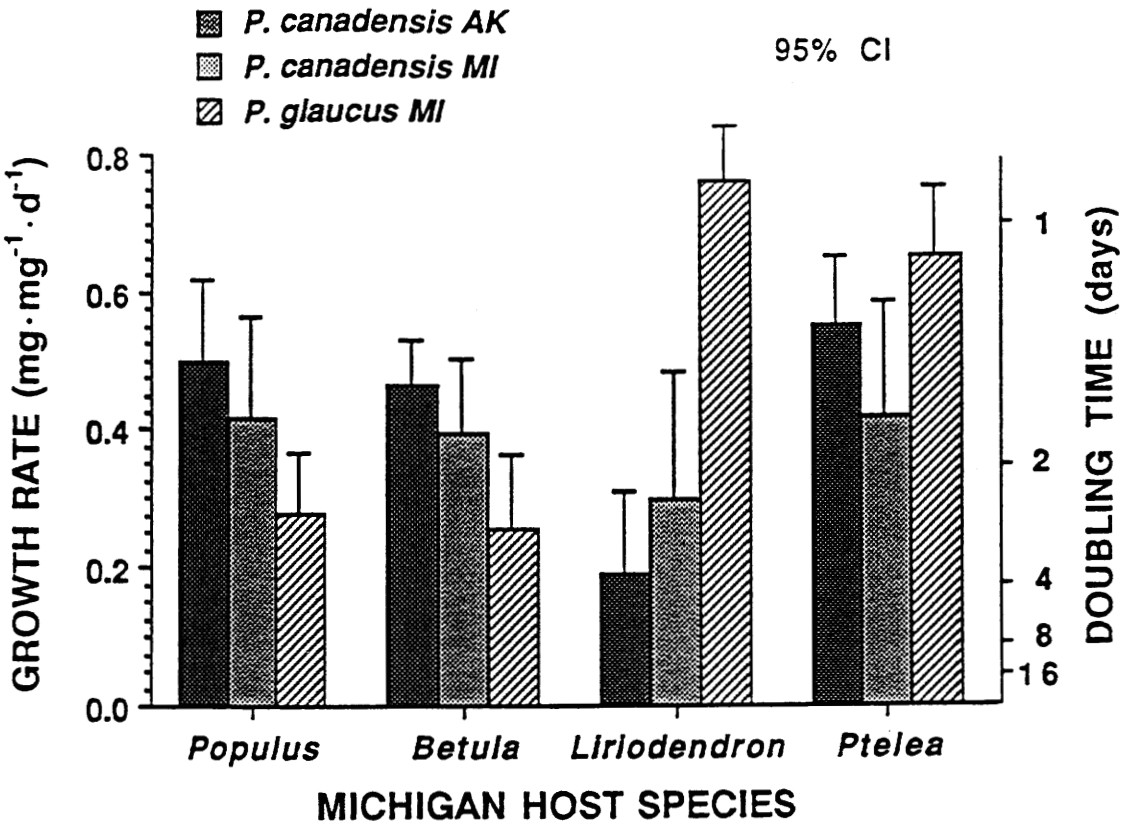

Figure 1. Relative growth rate of first instar larvae from three insect populations tested on four Michigan host species. Right hand axis shows the time required for larvae to double their weight at the corresponding growth rate. The insect populations are *P. canadensis* from Alaska, *P. canadensis* from northern Michigan, and *P. glaucus* from southern Michigan. The host species, from left to right, are *Populus tremuloides* (quaking aspen), *Betula papyrifera* (paper birch), *Liriodendron tulipifera* (tuliptree), and *Ptelea trifoliata* (hoptree). The experiment was conducted from 13 to 20 June 1988 using leaves collected from Ingham County, Michigan. Error bars indicate 95 percent confidence intervals.

METHODS

Butterfly Collection and Oviposition

Adult females were captured from populations in Florida, Georgia, Ohio, Michigan, Wisconsin, and Alaska, then transported on ice or shipped using overnight delivery to our laboratory for oviposition. Females were placed in clear plastic boxes (10 cm x 20 cm x 27 cm) with sprigs of appropriate foliage, held at 100 percent humidity under artificial illumination (4 hr dark alternating with 4 hr light), and fed a honey-water solution daily. Eggs were collected every other day, and resulting neonate larvae were used for the laboratory growth performance studies.

Electrophoresis

Butterflies were frozen at -80°C to preserve tissues for allozyme electrophoresis. Four polymorphic allozyme loci were examined using thin-layer cellulose acetate plates. Alleles were named according to their relative mobility, with the most common *P. glaucus* allele assigned the value of 100; negative numbers were assigned to cathodally migrating allozymes. Detailed techniques are described elsewhere (Hagen and Scriber 1989, Bossart in prep.)

Larval Performance of *P. canadensis*

We compared *P. canadensis* populations from interior Alaska (Fairbanks and vicinity) and the Great Lakes region (northern Wisconsin and the Upper Peninsula of Michigan), 4,000 km away. Some studies also included interspecific comparisons with *glaucus* larvae from southern Michigan and Ohio (500 to 1,000 km from the Michigan *canadensis* sites). We challenged the larvae with nine host species native to Alaska and nine host species native to the Great Lakes region. Of the Great Lakes hosts, none of the nine species and only two of the five families are sympatric with Alaska swallowtails. The Alaska tree species included the only two hosts regularly encountered by both populations (*Populus tremuloides* and *Populus balsamifera*) plus seven other species of *Salix, Betula,* and *Alnus*, which are encountered naturally only in Alaska. We had only limited knowledge of *canadensis* host use in Alaska, so we chose species likely to be hosts based on their distribution and taxonomy. In the first summer we documented natural use of four out of the nine species: *Populus tremuloides, Populus balsamifera, Alnus tenuifolia,* and *Salix novae-angliae* (Scriber and Ayres 1990).

Our standard measure of growth performance was first instar relative growth rate. The same protocol was used for experiments conducted in Fairbanks, Alaska, and East Lansing, Michigan. Each population was represented on each host by 17 to 20 larvae drawn from 5 to 8 full-sib families. Larvae from the two populations hatched at the same time and were tested concurrently on the same sample of leaves. Leaves were collected as the *canadensis* eggs began to hatch. Each host species was represented by leaves from five genetically distinct trees. Freshly hatched larvae were weighed, then distributed singly to clear plastic vials containing foliage from one of the experimental hosts. A moistened plaster-of-paris base in each vial provided high humidity and maintained leaf turgor throughout the trial. Nearly all larvae fed on the foliage offered them. After 2 days (24°C, photoperiod of L:D 18:6), the larvae were reweighed. A typical larva weighed 1.5 mg at hatch (T_i) and, on a good host, 6.2 mg at T_f. Final weights were reduced by 10 percent (= estimated weight of food in their gut) to make them comparable to initial weights (taken before the larvae had begun to feed). Relative growth rate was calculated as $(\ln(W_f) - \ln(W_i))/T$, where W_f equals the final weight, W_i equals the initial weight, and T equals the time in days (Gordon 1968, Ayres and MacLean 1987). Degrees of freedom for population comparisons were based on the number of families.

Larval Performance of *P. glaucus*

We compared *P. glaucus* populations from Lawrence County, Ohio, Clarke County, Georgia, and Highlands County, Florida, on three hosts: *Liriodendron tulipifera, Magnolia virginiana,* and *Prunus serotina*. These populations span 1,300 km, each separated from the nearest population by about 650 km. The three experimental hosts are all used naturally by *P. glaucus* and support generally high levels of growth performance. The frequency of use differs between populations. Florida *P. glaucus* are largely restricted to *Magnolia virginiana*, the only common host throughout much of peninsular Florida. In contrast, Georgia and Ohio populations rarely or never encounter *Magnolia virginiana.* A variety of host species occur in Ohio and Georgia, but the Ohio population probably encounters *Liriodendron* more frequently than other hosts and the Georgia population probably encounters *Prunus* most frequently.

In 1988 progeny from 6 Florida families, 8 Ohio families, and 14 Georgia families were reared individually in plastic petri dishes (24°C, photoperiod of L:D 18:6). Ten neonate larvae from each family were randomly allocated to each of the three hosts. *Liriodendron* and *Prunus* leaves were collected every other day from trees near East Lansing, Michigan. *Magnolia* leaves were collected from trees maintained in our greenhouses. Larvae were reared to adults. Larval duration, pupal mass, and sex were recorded. Similar experiments were conducted in 1989. Population comparisons may have been somewhat confounded by seasonal changes in the foliage because the natural phenology of the populations differed and we were unable to rear the larvae synchronously.

RESULTS AND DISCUSSION

Variation within *P. canadensis*

Relative growth rates on nine Wisconsin tree species ranged from 0.61 $mg \cdot mg^{-1} \cdot d^{-1}$ on *Betula nigra* to 0.20 $mg \cdot mg^{-1} \cdot d^{-1}$ on *Betula allegheniensis* (Fig. 2). These rates correspond to doubling times of 1.1 versus 3.5 days, respectively, indicating a broad spectrum of host quality. Overall, the Alaska and Great Lakes insect populations did not differ: least square means ± 1 SE equalled 0.425 ± 0.029 versus 0.414 ± 0.039 $mg \cdot mg^{-1} \cdot d^{-1}$ for Alaska and Wisconsin respectively ($P = 0.81$). The contrast between populations was not significant for any of the nine hosts treated separately ($P > 0.05$). These results counter predictions of the regional specialization hypothesis. A match between the physiological attributes of Wisconsin caterpillars and the nutritive characteristics of Great Lakes hosts might have arisen through the acquisition of new abilities in the Wisconsin population (e.g. a detoxification and digestive system suitable for basswood) or the loss of ancestral and unneeded abilities in the Alaska population. There is no evidence that either of these processes has occurred for any of the host taxa examined.

The reciprocal comparison between *canadensis* larvae from Alaska and Wisconsin on nine Alaska host species revealed a similar pattern (Fig. 3). There was a weak overall tendency for Alaska *canadensis* to grow faster than Wisconsin *canadensis* (least square means ± 1 SE equalled 0.525 ± 0.029 versus 0.458 ± 0.019 $mg \cdot mg^{-1} \cdot d^{-1}$ for Alaska and Wisconsin respectively, $P = 0.06$). But Alaska larvae also tended to grow slightly, though not significantly, faster on Wisconsin hosts. The contrast between *canadensis* populations was significant for only one of nine Alaska hosts (*Salix glauca*, $t = 3.44$, df = 13, $P < 0.01$). We do not interpret these modest effects as evidence of local adaptation to host characteristics. The most general test for the hypothesized pattern of local specialization to regional hosts was the ANOVA interaction between *canadensis* populations (Alaska and Wisconsin) and host community (nine Alaska host species and nine Wisconsin host species), which was insignificant ($P = 0.43$, $F = 0.64$, df = 1, 20). The test for interactions between host species and families within populations was also insignificant ($P = 0.99$, $F = 0.74$, df = 160, 357). Yet these were robust tests involving 696 larvae and 18 host species; given the observed variance, we had a high probability of detecting any ecologically meaningful differences. Far from being a mosaic of differentiated populations and variable genotypes, the host-use abilities of *P. canadensis* appear to be remarkably constant across a broad geographic range.

In contrast to the absence of variation within or between *canadensis* populations, there was a marked difference between *canadensis* and *glaucus*. First instar growth rates of *P. glaucus* from Ohio were 34 percent lower overall when tested on the same Alaska host species (Fig. 3). On all nine hosts considered separately, the mean growth rate of *glaucus* larvae was lower than both *canadensis* populations, and on six of the nine hosts, the contrast between species was significant ($P < 0.05$). Only on *Salix alaxensis*, *Salix bebbiana*, and *Betula resinifera* did *glaucus* larvae grow at rates approaching that of *canadensis* larvae. The two swallowtail species seem fundamentally different in their ability to feed on a broad array of boreal host plants.

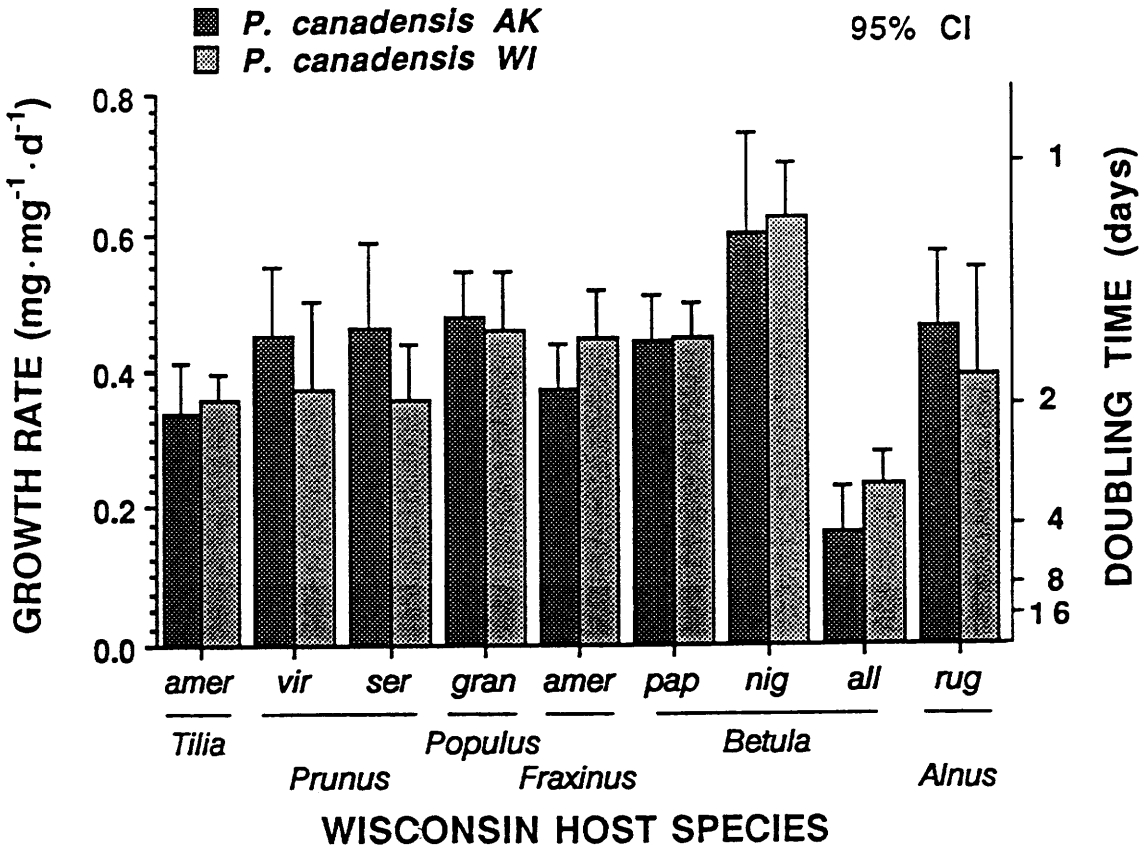

Figure 2. Relative growth rate of first instar *P. canadensis* from Alaska and Wisconsin on nine Wisconsin host species. The host species, from left to right, are *Tilia americana* (basswood), *Prunus virginiana* (chokecherry), *Prunus serotina* (black cherry), *Populus grandidentata* (bigtooth aspen), *Fraxinus americana* (white ash), *Betula papyrifera* (paper birch), *Betula nigra* (river birch), *Betula allegheniensis* (yellow birch), and *Alnus rugosa* (speckled alder). The experiment was conducted from 5 to 8 July 1989 using leaves from Dunn County, Wisconsin. Error bars indicate 95 percent confidence intervals.

First instar growth rates are just one measure of growth performance, but we have found them to be a powerful and generally reliable index. As part of a larger study (Ayres and Scriber in prep.), the 360 *canadensis* larvae initiated on Alaska hosts (Fig. 3) were reared to pupation and additional measurements collected. These data support our conclusion based on neonate performance that the populations do not differ in their nutritional physiology. Relative growth rates measured over the middle instars (day 3 to 17) did not differ between populations, nor did fifth instar growth rates, consumption rates, or conversion efficiencies. Hosts that produced high first instar growth rates tended to have higher overall survival and yield larger pupae in less time than hosts that produced low first instar growth rates. Larger pupae produce larger male and female adults that produce larger spermatophores and more eggs respectively (Lederhouse et al. in prep.).

The apparent absence of variation in detoxification systems and nutritional physiology is in contrast to other traits that do vary between the populations. Alaska females produced eggs 150 percent larger than their Great Lakes counterparts, but were able to produce only 63 percent as many

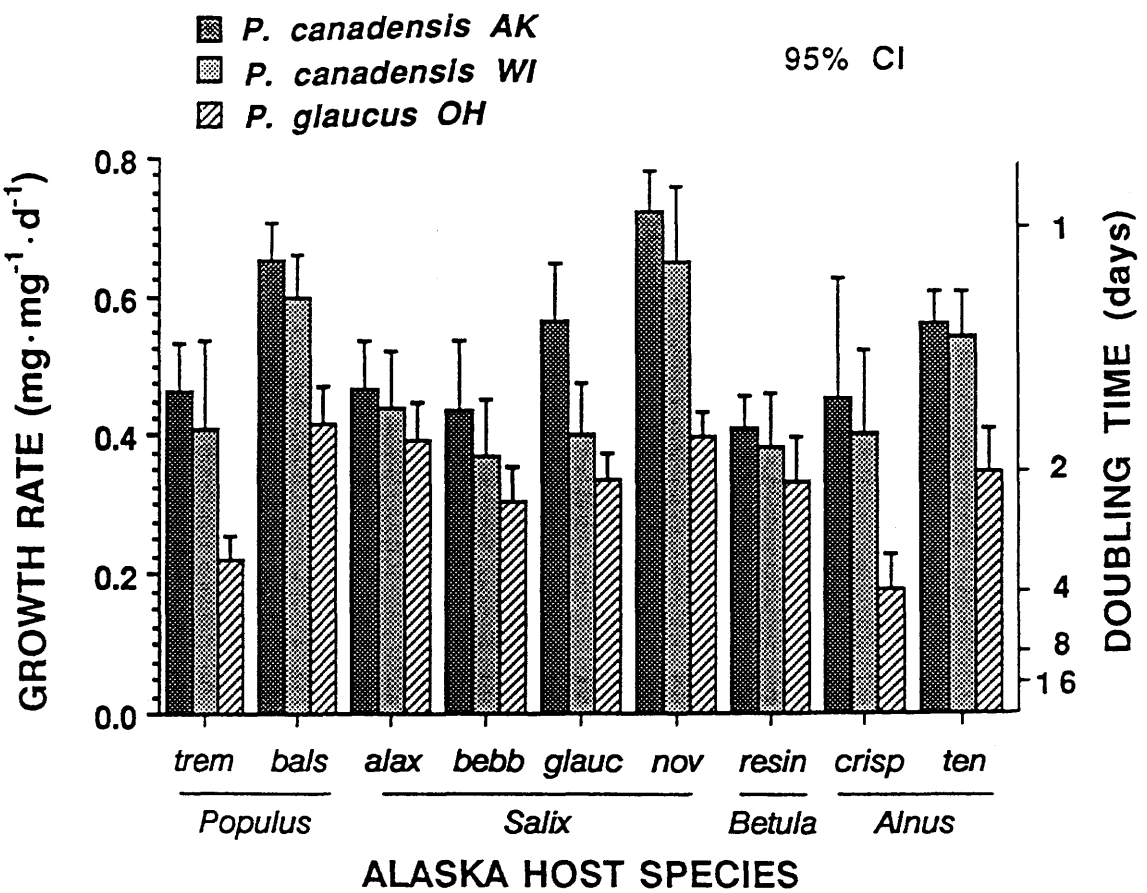

Figure 3. Relative growth rate of first instar larvae from three insect populations tested on nine Alaska host species. The insect populations are *P. canadensis* from Alaska, *P. canadensis* from Wisconsin, and *P. glaucus* from Ohio. The host species, from left to right, are *Populus tremuloides* (quaking aspen), *Populus balsamifera* (balsam poplar), *Salix alaxensis* (feltleaf willow), *Salix bebbiana* (bebb willow), *Salix glauca*, *Salix novae-angliae*, *Betula resinifera* (Alaska paper birch), *Alnus crispa* (green alder), and *Alnus tenuifolia* (thinleaf alder). The *canadensis* measurements were made from 26 to 29 June 1988 and the *glaucus* measurements from 19 to 22 August 1989; all leaves were collected from the same sites near Fairbanks, AK. Error bars indicate 95 percent confidence intervals.

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as Wisconsin females of equal size (Lederhouse et al. in prep.). These larger eggs conferred an important developmental advantage. At 17 days after hatch, the Alaska *canadensis* larvae were still 1.5 times larger, even after a 180-fold increase in weight (relative growth rates were virtually identical) (Ayres and Scriber in prep.). Further, Great Lakes larvae tended to grow for a longer period and consequently produced larger pupae, even though they hatched smaller and grew at the same rate. The populations further differed in their larval temperature responses. In particular, Alaska larvae were capable of more rapid growth and development at the relatively low temperatures of 12° and 18°C (Ayres in prep.).

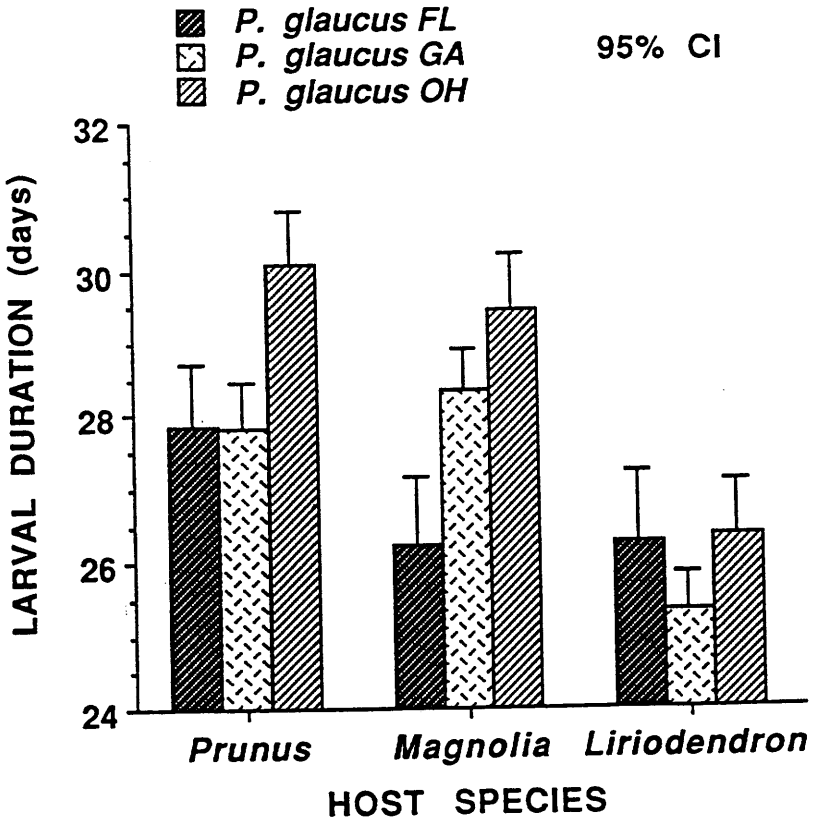

Figure 4. Larval duration of *P. glaucus* populations from Florida, Georgia, and Ohio tested on *Prunus serotina* (black cherry), *Magnolia virginiana* (sweetbay), and *Liriodendron tulipifera* (tuliptree). Measurements were made during May, August, and September 1988 for Florida, Ohio, and Georgia populations respectively. Error bars indicate 95 percent confidence intervals.

Variation within *P. glaucus*

Unlike *P. canadensis*, *P. glaucus* appears to harbor substantial genetic variation in nutritional physiology. Patterns in development time, especially significant interactions between insect populations and host species, support the hypothesis of regional specialization (Fig. 4, Table 1). Overall, the development time of Ohio *glaucus* tended to be longer than that of Florida *glaucus* (population effect, Table 1), but this difference was most pronounced on *Magnolia* (the prevalent Florida host) and nonexistent on *Liriodendron* (the prevalent Ohio host, population x host interaction, Table 1). Likewise, Georgia populations required a longer development time on *Magnolia* than Florida populations, even though development times were identical on *Prunus* (a prevalent Georgia host, Fig. 4). Significant variation among families within populations suggests an even finer level of ecological specialization, although the variation within populations was far smaller than that between populations (F = 14.95 versus 1.83 for main effects of population and family respectively, and F = 4.15 versus 1.39 for host x population interactions and host x family interactions respectively, Table 1).

Table 1. Mixed Model ANOVA (families nested within population) of larval duration for three *P. glaucus* populations tested on three host species. Data are shown in Fig. 4.

Source of variation	df	MS	Error term[a]	*F*	*P*
Population	2	134.41	Fam(Pop)	14.95	< 0.001 ***
Host	2	308.66	H x F(Pop)	45.32	< 0.001 ***
Family(Pop)	25	8.99	Error	1.83	< 0.05 *
Host x Pop	4	28.23	H x F(Pop)	4.15	< 0.01 **
Host x F(Pop)	54	6.81	Error	1.39	< 0.05 *
Error	315	4.91			

[a] Mean square used as denominator in *F* test (Ayres and Thomas 1990)

Further evidence for local adaptation lies in the relationship between larval duration (development time) and pupal mass. On *Magnolia*, Florida families with long development times tended to produce larger pupae (Fig. 5); this result is to be expected if all families grow at similar rates (are uniformly well adapted), but vary in the timing of pupation. In contrast, the negative correlation between larval duration and pupal mass of Ohio families feeding on *Magnolia* suggests differential detoxification abilities among families. For example, larvae from relatively maladapted families may partially have compensated for their low growth rates by extending the duration of growth, but the prolonged development time was inadequate to produce pupae of equal mass. On *Liriodendron*, Ohio families with relatively long development times tended to produce larger pupae, as expected for a population well adapted to *Liriodendron* (r = 0.31, P = 0.16). There was no relation between development time and pupal mass for Florida families on *Liriodendron* (r = -0.06, P = 0.86), indicating a mix of the two patterns.

Visual inspection of reaction norms further supported a physiological tradeoff (genotype-environment interaction) in the ability of full-sib families to use *Liriodendron* and *Magnolia* (Fig. 6). Within both Ohio and Florida populations, families with rapid development on *Magnolia* took relatively longer on *Liriodendron*. The tradeoff between performance on *Liriodendron* and *Magnolia* appears linked to the presence of particular glucosephosphate isomerase (GPI) alleles (Table 2). Within each population, homozygous $GPI_{100/100}$ genotypes tended to be fast growers on *Liriodendron* and heterozygous $GPI_{100/-104}$ genotypes tended to be fast growers on *Magnolia* (Bossart in prep.). Moreover, the frequency of the GPI $_{-104}$ allele tended to be higher in Florida, where *Magnolia* is the prevalent host (Table 3). We are currently unable to distinguish between genetic linkage and physiological causality as the basis for this relationship. No fixed differences in allozymes have been found between the populations, and the suggested clinal patterns were not statistically significant.

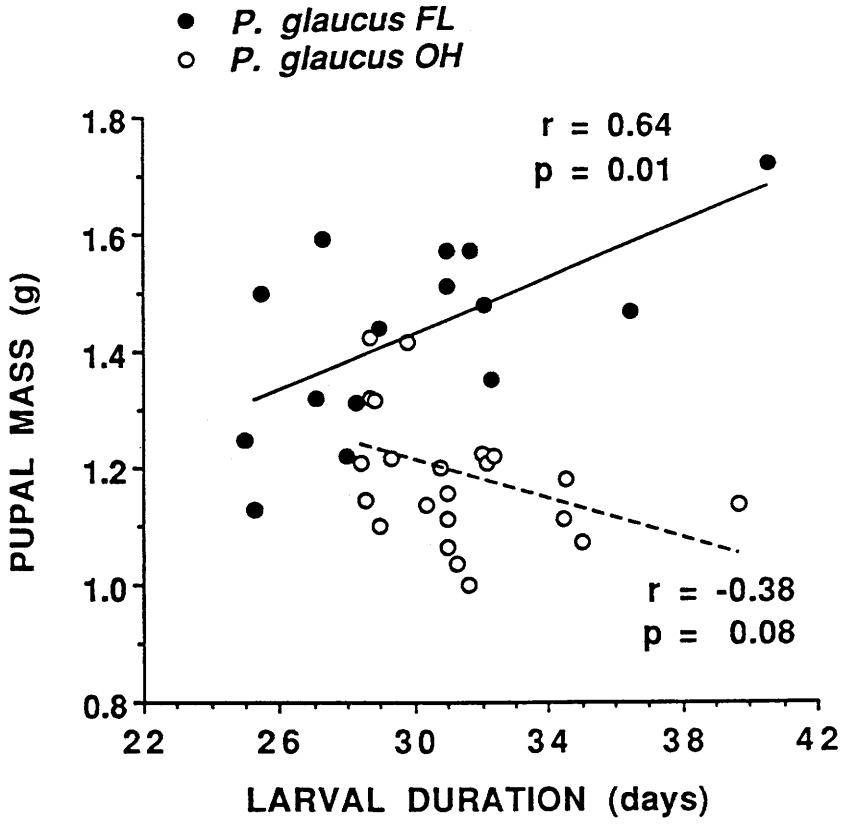

Figure 5. Relationship between larval duration and pupal mass of *P. glaucus* from Florida and Ohio reared on *Magnolia virginiana*. Each point represents the mean of 3 to 30 full-siblings. Figure includes data from 1988 and 1989.

Table 2. Larval duration of different glucosephosphate isomerase (GPI) genotypes from Florida and Ohio tested on *Magnolia virginana* and *Liriodendron tulipifera*. Values are mean ± SE (*N*). The interaction between host and genotype was significant at $P = 0.0008$ ($F = 12.15$, df = 1, 76).

	Liriodendron		*Magnolia*	
	$GPI_{100/100}$	$GPI_{100/-104}$	$GPI_{100/100}$	$GPI_{100/-104}$
Florida	22.36 ± 0.45 (11)	26.75 ± 0.75 (8)	26.06 ± 0.45 (18)	24.86 ± 0.67 (7)
Ohio	23.88 ± 0.44 (16)	27.55 ± 1.14 (11)	29.64 ± 0.95 (11)	28.50 ± 0.50 (2)

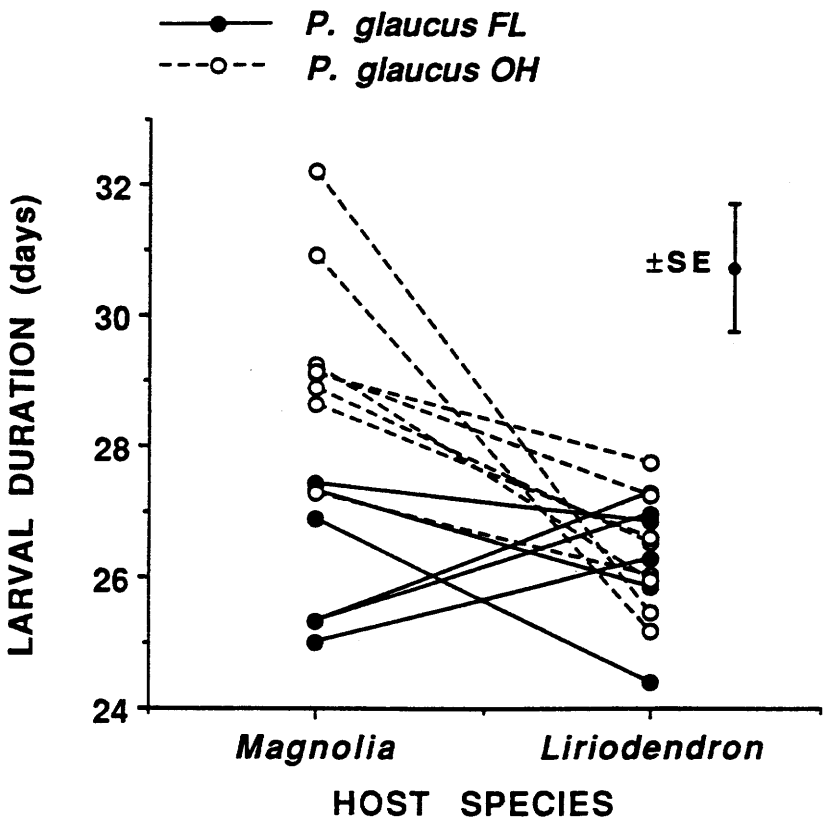

Figure 6. Larval duration of *P. glaucus* families from Florida and Ohio tested on *Liriodendron tulipifera* and *Magnolia virginiana*. Each point represents the mean of 6 to 10 full-siblings. Lines show family reaction norms. Standard error associated with family means is indicated ($SE^2 = MS_{Error}/N = 4.91/5$).

CONCLUSIONS

P. canadensis appears to be a recently evolved taxon derived from a *glaucus*-like ancestor (Scriber et al. 1991, Hagen and Scriber 1990). Our results suggest that the detoxification systems and nutritional physiology of ancestral *canadensis* became greatly modified in ways that allowed them to exploit a diversity of northern hosts. In the process, some ancestral abilities were lost (e.g. tuliptree detoxification) and others were retained (e.g. hoptree detoxification). The absence of detectable differentiation between widely separated *canadensis* populations encountering very different hosts suggests that the nutritional physiology of *canadensis* has remained largely unchanged since its divergence from a *glaucus* ancestor. The absence of detectable variation within *canadensis* populations implies that future changes in nutritional physiology will be limited by the appearance of new mutations. Its implied history of rapid evolutionary change followed by stasis is consistent with the stepwise mechanism of herbivore adaptation described by Ehrlich and Raven (1964). We note, however, that direct reciprocal evolutionary responses by the hosts are unlikely because of the low levels of damage inflicted by these herbivores. In striking contrast, *P. glaucus* appears to be a vast complex of differentially adapted genotypes and populations, which suggests that its nutritional physiology is being shaped by local selective pressures such as result from varying regional availability of hosts. The polyphagous habits of *P. glaucus* are enhanced by its variability within and between populations, which is not so for *P. canadensis*, all genotypes of which appear equally capable of using any host species within its range.

Table 3. Allele frequencies (%) at four polymorphic loci in *P. glaucus* populations from Ohio (OH), Georgia (GA), and Florida (FL). Number of alleles per sample = 48 to 84.

Locus	Allele	Population OH	GA	FL
PGM	91	3	1	0
	94	4	1	4
	97	12	29	16
	100	35	30	36
	103	38	27	28
	106	8	11	16
	109	0	1	0
IDH-2	100	83	92	71
	102	17	8	25
	103	0	0	4
MPI	90	3	0	0
	92	0	3	0
	94	0	11	13
	96	16	14	11
	98	22	29	20
	100	47	32	51
	102	12	11	5
GPI	-105	3	0	0
	-104	7	12	21
	100	79	78	75
	104	1	0	0
	105	7	10	4

We suggest several possible explanations for the markedly different levels of intraspecific variation within *glaucus* and *canadensis*. 1) The two species differ in population structure such that gene flow is more restricted in *glaucus* than *canadensis*. 2) *P. canadensis* originated as a very small population, perhaps as a result of Pleistocene isolation in some periglacial refugium (Scriber 1988), and low variation is a result of the associated genetic bottleneck. 3) *P. canadensis* is of such recent evolutionary origin that populations have not yet differentiated, but will in the future. 4) There is less variation in *canadensis* host use; perhaps *Populus tremuloides* is the prevalent host for all *canadensis* populations, and other host-use records are rare "oviposition mistakes" that contribute only insignificantly to selective pressures shaping the nutritional physiology of *canadensis*. 5) The boreal forest is characterized by low phytochemical diversity relative to that of temperate forests, the most ubiquitous boreal hosts being restricted to four genera in two families: *Populus*, *Salix*, *Betula*, and *Alnus*. Consequently, natural selection has favored the development of a single detoxification system that is optimal for most hosts, i.e. *canadensis* detoxification systems have been canalized such that mutations and recombination seldom lead to phenotypic variation.

Hypothesis 1 can be tested by comparing the divergence in allozyme frequencies between populations within species. If it holds true, the fixation index (F_{ST}) of *glaucus* populations should be greater than that of *canadensis* populations separated by the same geographic distance. The available data are limited, but they indicate extensive gene flow within both species (Hagen 1990); the estimated genetic distance between *P. glaucus* from Ohio and Florida was no greater than that between *P. canadensis* from Alaska and Michigan: Nei's genetic identities equalled 0.99 in both cases (Hagen and Scriber 1990). Hypotheses 2 and 3 cannot account for divergence between *canadensis* populations in traits other than nutritional physiology such as egg size, adult size, and temperature responses. Hypothesis 2 would be supported by lower levels of allozyme heterozygosity in *canadensis* than in *glaucus* (Bonnell and Selander 1974). Hypothesis 3 predicts that other recently derived swallowtail taxa (as estimated from allozyme and mtDNA sequence divergence) would be similarly invariant in their nutritional physiology. Hypothesis 4 questions our view of *P. canadensis* as a polyphagous herbivore and requires that suitable hosts go generally unused. It can be tested through quantification of oviposition patterns in the field. We presently favor hypothesis 5, which predicts that the various secondary metabolites present in boreal hosts are detoxified by *canadensis* using only one or a few biochemical systems. It further predicts that other polyphagous herbivores of the boreal forest will similarly exhibit low variation in nutritional physiology, and will be consistently less variable than related species of polyphagous herbivores from temperate and tropical forests (Mattson et al. 1988, Mattson et al. in this volume). An important correlate is that physiological preadaptation to alternative hosts will be more common in boreal forests than elsewhere. That is, boreal herbivores challenged with sympatric nonhost plant species should be more likely to successfully detoxify that foliage than temperate or tropical herbivores challenged with nonhost species from their environment.

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