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ADAPTATION AND CONSTRAINT IN PAPILIO CANADENSIS:
GEOGRAPHIC VARIATION IN NUTRITIONAL PHYSIOLOGY
AND TEMPERATURE RESPONSES

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MATTHEW P. AYRES

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**ADAPTATION AND CONSTRAINT IN PAPILIO CANADENSIS:
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AND TEMPERATURE RESPONSES**

By

Matthew P. Ayres

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ABSTRACT

ADAPTATION AND CONSTRAINT IN *PAPILIO CANADENSIS*:
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AND TEMPERATURE RESPONSES

By

Matthew P. Ayres

Papilio canadensis, the Canadian tiger swallowtail butterfly, is a polyphagous herbivore common throughout the boreal forests of North America. Populations inhabiting Michigan feed on different tree species, under a different climatic regime, than those inhabiting interior Alaska. With respect to its host-use abilities and temperature responses, *P. canadensis* could be a mosaic of relatively specialized genotypes, each of which can only succeed in a subset of environments exploited by the species as a whole, or, it could be single cohesive genotype that is unusually robust. I evaluated these hypotheses by comparing the physiological ecology of *P. canadensis* populations from Alaska and Michigan on the host species normally encountered by each, and at the temperatures normally encountered by each.

The ability to consume, detoxify, assimilate, and convert chemically diverse plant tissue is highly conserved within *P. canadensis*. There was no differentiation of host-use abilities between Michigan and Alaskan populations, although there is little overlap between their host communities. Michigan caterpillars grew as well as Alaskan caterpillars on Alaskan hosts, and no better than Alaskan caterpillars on Michigan hosts. There was also no evidence of host specialists within

populations. Nonetheless, there were pronounced differences in host-use abilities between *P. canadensis* and its presumed progenitor (*P. glaucus*).

In contrast to host plant use, marked population divergence has occurred in response to climatic differences between Alaska and Michigan. At low temperatures (12°C), Alaskan larvae grew faster (fifth instar doubling time of 5.8 versus 9.1 d), and molted faster (fifth molt required 11.8 versus 17.8 d). Increased egg size and reduced adult size represent additional adaptations of Alaskan *P. canadensis* for short subarctic summers. In the Alaskan climate, Alaskan swallowtails have an estimated fitness of 3.00 relative to Michigan swallowtails. Changes in growth-temperature responses made the greatest apparent contribution to enhanced fitness, followed by increased egg size. Occasional extreme summers appear to be more important than average summers in shaping adaptive responses. Regional adaptation to climate allows *P. canadensis* to maintain a broader distribution than would otherwise be possible, but distribution limits are probably still constrained by summer temperatures.

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INTRODUCTION

Why do organisms occur where they do but not elsewhere? How do some species maintain very cosmopolitan distributions while others are highly endemic? Why do some populations respond to environmental change through adaptation while others go extinct? Is it possible to generalize about the types of ecological attributes that are likely or unlikely to respond to changes in selection pressure. I addressed these broad questions through a study of geographic differentiation in a pair of broadly distributed insect species that encounter very different environments across their range.

Two closely related species of the tiger swallowtail butterfly, *Papilio glaucus* and *Papilio canadensis* (Lepidoptera: Papilionidae), are abundant and conspicuous members of the herbivore fauna from southern Florida to interior Alaska. Their larvae are able to complete development on hosts representing at least 14 plant families (30-40 species; Scriber 1983, 1984) under summer temperature regimes that run the gamut from >3500 degree days to <700 degree days (10°C base). No single host species occurs throughout this north-south transect. Even within the species, populations encounter dramatically different environments from one end of their distribution to the other. How do these swallowtail populations succeed in completing their life cycle each year in such disparate environments? Under one scenario, each species has converged on a

single cohesive genotype (Mayr 1963) that is sufficiently robust to allow rather cosmopolitan success (super-genotype hypothesis). In the alternative view, species are composites of variable genotypes and differentiated populations (Dethier, 1954), any one of which is able to perform well in only a subset of conditions encountered by the species as a whole (genetic mosaic hypothesis). The genetic mosaic hypothesis suggests that local populations are continually being modified through natural selection to provide a match between the environment and the phenotype; i.e., the environment determines organism attributes. In contrast, support for the cohesive genotype scenario would indicate that species attributes are largely fixed, and suggest instead that an organism's attributes determine the environment where it can occur.

Using the experimental format of a reciprocal transplant, I evaluated these hypotheses by comparing the physiology ecology of *P. canadensis* populations from Alaska and Michigan on the host species normally encountered by each (Chapter 1), and at the temperatures normally encountered by each (Chapter 2). Interspecific comparisons with *P. glaucus* allowed a comparison of variation within and between species.

CHAPTER ONE

Conservation of polyphagous abilities in oligophagous populations of Papilio canadensis

Introduction

When Ehrlich and Raven (1964) formulated what was to become a dominant paradigm for the evolution of insect-host plant relations, they framed the model very explicitly in terms of species characteristics. Attributes such as detoxification abilities were viewed as constants within a species, with significant evolutionary advances being occasional, rapid, and most often associated with speciation events: "The evolution of secondary plant substances and the *stepwise* evolutionary responses to these by phytophagous organisms have clearly been the dominant factors in the evolution of butterflies and other phytophagous groups" (*my italics*). The same typological thinking underlies the influential papers written 22 years later by Feeny (1976) and Rhoades and Cates (1976). Although the idea that herbivore species may be genetically heterogeneous is old (e.g., Brues, 1924; Thorpe, 1930; and Dethier, 1954), the importance of this variability in understanding insect-host interactions has been commonly overlooked until recently. In 1978, Edmunds and Alstad emphasized that interactions between scale insects and ponderosa pine varied widely depending on characteristics of the particular insect genotype and the individual tree attacked. Since then, various

studies have reported variation between populations and/or genotypes in the ability to use particular hosts (Hsiao, 1978; Moran, 1981; Tavormina, 1982; Rausher, 1982, 1984; Blau and Feeny, 1983; Service, 1984; Via, 1984a; Haukioja and Hahnimäki, 1985; Weber, 1985; Bowers, 1986; Hare and Kennedy, 1986; Futuyma and Philippi, 1987; Horton et al., 1988; Nitao et al. 1991). Fox and Morrow (1981) and Scriber (1983, 1986) suggested that apparently polyphagous species may actually be mosaics of relatively oligophagous genotypes. If this hypothesis were substantiated, it would alter the scale on which we study and describe plant-herbivore interactions (Ng, 1988; Thompson, 1988). Recent reviews have addressed intraspecific variability in herbivores (Denno and McClure, 1983; Diehl and Bush, 1984; Futuyma and Peterson, 1985; Jaenike, 1990; Via, 1990), but its generality and its magnitude relative to variation between herbivore species remains poorly known.

Papilio canadensis R & J (formerly *P. glaucus canadensis*, Hagen et al., 1991) is a polyphagous tree-feeding insect (Lepidoptera: Papilionidae) common throughout the boreal and north-temperate forests of the Nearctic. Its hosts include species of *Populus* and *Salix* (Salicaceae), *Betula* and *Alnus* (Betulaceae), *Prunus* and *Amelanchier* (Rosaceae), *Fraxinus* (Oleaceae), *Tilia* (Tiliaceae), and probably more (Scriber and Ayres 1990). Its distribution is more extensive than most of the tree species on which it feeds, hence populations inhabiting the Great Lakes region of Wisconsin and Michigan necessarily feed on a different suite of host species than those inhabiting interior Alaska 4000 km distant.

With respect to its nutritional physiology, *P. canadensis* could be a mosaic of differentiated populations and variable genotypes that only collectively possess

the ability to use all of its reported host plants; alternatively, a single unusually robust genotype allows its polyphagous habits and broad distribution. I refer to these as the genetic mosaic and super-genotype hypotheses, respectively. They were tested by comparing the growth performance of *P. canadensis* populations from Alaska and Michigan on nine Alaskan host species and nine Michigan host species. If genetic variability is large and pervasive it should be evident within each population as interactions between insect families and host species.

Additionally, the genetic mosaic hypothesis predicts regional differentiation of the populations such that Alaskan populations should be generally better suited to Alaskan hosts and Michigan populations better suited to Michigan hosts (Scriber 1983, 1986). To provide context for evaluating such intraspecific variability, I included interspecific comparisons with *P. glaucus* L., the apparent closest relative of *P. canadensis* (Scriber et al., 1990; Hagen and Scriber, 1991).

Methods

Overview

In *Papilio*, host effects are often apparent as differences in neonate survival (Scriber et al., 1990; Hagen, 1990). My most extensive experiments compared first instar relative growth rates, which similarly reflect neonate growth performance, but provide a continuous rather than a discrete variable for analysis. Results of first instar trials allow a partitioning of variance due to (1) interspecific variation between sister species, (2) intraspecific variation at the level of populations and full-sib families, (3) effects of host species, and (4) interactions between insect genotypes and host species. One set of first instar growth trials

(*P. canadensis* from Alaska and Michigan tested on nine Alaskan host species) was extended to allow measurements of growth rate in the middle and final instars, pupal mass, larval duration, and survival by stadia. Fifth instar growth performance was further compared through measurements of consumption rate, apparent digestibility, growth efficiency, and nitrogen use efficiency.

Sampling of insects and hosts

During early June 1988, adult *P. canadensis* females were collected from sites in interior Alaska (vicinity of Fairbanks) and northern Michigan/Wisconsin (Gogebic and Iron counties in MI, and nearby Vilas and Iron Counties in WI). At the same time, adult *P. glaucus* females were collected from southern Michigan (Washtenaw, Ingham, and Cass Counties). All adults were transported to our laboratory in Michigan, fed a 20% honey solution daily, and induced to oviposit in clear plastic boxes (10 x 20 x 27 cm) with sprigs of appropriate foliage (100% humidity, 4 h dark at 24° alternating with 4 h bright light at 32°). Resulting neonate larvae were used for comparisons of *P. canadensis* and *P. glaucus* growth performance on six host species common in southern Michigan. A slightly later cohort of progeny from the same collection of adults were transported as eggs to Fairbanks, Alaska where they were used for comparisons of *P. canadensis* from Alaska and Michigan on nine Alaskan host species. The following year (June 1989), adult *P. canadensis* females were collected from the same sites in interior Alaska and northern Michigan and their progeny were tested on nine host species common in northern Michigan and the Great Lakes region. During July 1989, adult *P. glaucus* were collected from Gallia County

Ohio, and their progeny were tested on nine Alaskan host species. Each of the three *Papilio* populations was represented in my experiments by 15-20 full sib families (7-10 families in each year). Voucher specimens have been deposited with the Michigan State University Department of Entomology museum.

Alaskan hosts were represented by nine tree species in four genera and two families: *Betula resinifera*, *Alnus crispa*, and *Alnus tenuifolia* in the Betulaceae; and *Populus tremuloides*, *Populus balsamifera*, *Salix alaxensis*, *Salix bebbiana*, *Salix glauca*, and *Salix novae-angliae* in the Salicaceae (see appendix). Foliage was collected from or near the University of Alaska Fairbanks arboretum. Northern Michigan host species were represented by nine species in six genera and five families: *Fraxinus americana* (Oleaceae); *Prunus serotina* and *Prunus virginiana* (Rosaceae); *Tilia americana* (Tiliaceae); *Betula alleghaniensis*, *Betula papyrifera*, and *Alnus rugosa* (Betulaceae); and *Populus grandidentata* (Salicaceae). Foliage was collected from Dunn County, Wisconsin. Southern Michigan host species were represented by six species in five genera and five families: *Liriodendron tulipifera* (Magnoliaceae); *Ptelea trifoliata* (Rutaceae); *Tilia americana* (Tiliaceae); *Prunus serotina* and *Prunus virginiana* (Rosaceae); and *Fraxinus americana* (Oleaceae). Foliage was collected from Ingham County, Michigan. Each host species was represented in each first instar growth trial by foliage from five genets.

First instar growth performance

Recently eclosed larvae were weighed to ± 0.1 mg (W_0), then allocated individually to clear plastic cups (12 ounce) containing a leaf or shoot of one host

species. Plaster-of-paris bases in the cups were saturated with water, which provided high humidity and maintained leaf turgor throughout the trial. Larvae from each family were spread equitably across host species. On the third day (after about 60 h at 24°C, photoperiod of L:D 18:6), when larvae on the best hosts were nearly ready to initiate their first molt, larvae were reweighed (W_3). First instar relative growth rate was calculated as $RGR_1 = (\ln(W_3 \cdot 0.9) - \ln(W_0))/T$, where T = the elapsed time in days (Gordon, 1968). W_3 was reduced by 10% (= estimated mass of food in their gut, unpublished data) to make it comparable to W_0 (taken before the larvae had begun to feed). This adjustment affected no patterns or statistical results but corrected for what would otherwise be a bias in the growth rate. All population and species comparisons were based on concurrent measurements using foliage collected from the same trees at the same time, except for comparisons of *P. canadensis* and *P. glaucus* on Alaskan host species, where foliage was collected from the same sites but at different times (see appendix).

Middle instar growth performance

On day 17 of the experiment comparing *P. canadensis* from Alaska and Michigan on Alaskan host species, all larvae were reweighed (W_{17}) and their instar was recorded. At this time, some larvae on the best hosts had just entered the fifth (final) instar. A relative growth rate encompassing these middle instars was calculated as $RGR_{mid} = (\ln(W_{17}) - \ln(W_3))/T$, where W_3 = mass at the end of the first instar trial, and T = time elapsed from W_3 to W_{17} .

Final instar growth performance

Shortly after larvae completed molting into the fifth and final instar (had resumed feeding and producing frass), they were weighed, given a previously weighed shoot of foliage, and allowed to feed and grow uninterrupted for the balance of the instar (ca. 3 d), when they were reweighed. A matched shoot of weighed foliage was placed in an adjacent cup and exposed to the same conditions as the foliage fed to the larva; this served as a control to estimate the initial dry mass of the experimental foliage. After the trial, the experimental foliage, the control foliage, and the frass produced during the 3 d trial were oven-dried and weighed. A subset of the control foliage and frass samples was analyzed for total nitrogen with standard micro-Kjeldahl techniques (sulfuric acid digestion followed by colorimetric analysis with a Technicon Auto-analyzer II; Herms, 1991). Non-experimental fifth instar larvae from Alaska and Michigan were used to estimate the dry mass of experimental larvae from their fresh mass ($DM = 0.125 \cdot FM$, $n = 20$; dry:wet ratio was unaffected by host species). These data allowed estimation of fifth instar relative growth rate (RGR_v), relative consumption rate (RCR), relative assimilation rate (RAR), apparent digestibility (AD), efficiency of conversion of digested matter (ECD), and nitrogen use efficiency (NUE). Calculations of RCR, RAR, and RGR follow formulae in Gordon (1968). $AD = RAR/RCR$, and $ECD = RGR/RAR$. NUE equalled the fraction of ingested nitrogen that did not appear in the frass (frass contains both undigested and excretory nitrogen): $NUE = (N_{\text{ingested}} - N_{\text{egested}})/N_{\text{ingested}}$. All indices of fifth instar growth performance are in units of dry mass.

Pupal mass, larval duration, and survival

Larvae were checked daily, and foliage replaced as needed (every 2-3 d) until larvae pupated or died. Larval duration was defined as the interval from initiation of feeding in neonate larvae (W_0), until one day before the conspicuous color change that signals prepupation. The diapausing pupae were moved to 12° for one month, then weighed and sexed. Larval mortality was recorded by day and instar.

Statistical analyses

All data were analyzed by ANOVA (GLM procedure, Type III sums of squares: SAS Institute, 1985) after evaluating assumptions of normality and homogeneity of variances. I tested for population differentiation in first instar growth rate with a mixed model design, where insect population (AK and MI), host community (AK and MI), and host species nested within host community were all treated as fixed effects. Insect families were nested within insect population and host community, and were treated as random effects. The expected mean squares and *F*-tests follow Scheffe's formulation of the mixed model ANOVA (Ayres and Thomas, 1990). I tested for interspecific patterns in first instar growth with an identical model except insect species was substituted for insect population and the host communities were from Alaska and southern Michigan. Both analyses excluded some families not represented on all hosts, which facilitated tests for family x host interactions, and did not alter the result of tests involving insect populations or insect species.

Larval mortality subsequent to the first instar trial reduced the number of

families that were represented on all hosts and reduced total degrees of freedom. Consequently, I used a simpler ANOVA model to analyze growth performance in the middle instars, growth performance in the final instar, pupal mass, and larval duration: insect population and host species were treated as fixed effects, and the analysis was run using family means (Hurlbert, 1984). All figures, including first instar growth rates, show means and confidence intervals (or SE's) calculated from family means.

Pupal mass and larval duration tend to be sexually dimorphic in *P. canadensis*, which could confound analyses because some treatments yielded unequal numbers of male and female pupae. Sex could not be readily included as a factor in the ANOVA model because some treatment combinations lacked male or female pupae. I evaluated sexual dimorphism by comparing mean male and female values for the 18 population-host combinations. The larval duration of females averaged 1.5 d (5%) longer than males ($t_{17} = 3.18$, $P < 0.001$), and the pupal mass of females averaged 39 mg (6%) greater than males ($t_{17} = 4.12$, $P < 0.001$). I developed the following linear regression equations:

$$\text{Days}_{\text{female}} = -1.00 + 1.09 \cdot \text{Days}_{\text{male}} \quad (P < 0.0001, \text{df} = 1, 17, r^2 = 0.90),$$

$$\text{Mass}_{\text{female}} = -141 + 1.26 \cdot \text{Mass}_{\text{male}} \quad (P < 0.0001, \text{df} = 1, 17, r^2 = 0.82),$$

which were used to adjust male measurements to that expected for females (Haukioja and Neuvonen, 1985; Ayres et al., 1987). Residuals from the regressions did not differ between populations or hosts ($P > 0.15$ for all four tests). Analyses of larval duration and pupal mass used these adjusted values. The effects of these adjustments were small (grand means changed by 2-3%) and



the ANOVA conclusions did not change when I reran the analyses using unadjusted values.

Results

First instar growth performance: intraspecific variation

Counter to the predictions of the genetic mosaic hypothesis, there was little evidence for enhanced performance of first instar *P. canadensis* larvae on regionally available hosts. Michigan larvae grew no faster than Alaska larvae when both populations were challenged with foliage from nine Michigan host species (Figure 1, upper; least square means $\pm$ SE equalled 0.410 ± 0.039 versus 0.418 ± 0.029 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ for Michigan and Alaskan larvae respectively, $P = 0.81$). Similarly, Alaskan larvae grew only slightly faster than Michigan larvae when both populations were challenged with foliage from nine Alaskan species (Figure 1, lower; least square means $\pm$ SE equalled 0.525 ± 0.029 versus 0.466 ± 0.019 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ for Alaska and Michigan respectively, $P = 0.06$). The interaction between insect population and host community was nonsignificant ($F_{1,20} = 0.64$, $P = 0.43$, Table 1). There was a weak overall tendency for Alaskan larvae to grow faster than Michigan larvae ($F_{1,20} = 3.34$, $P = 0.083$, Table 1).

The main effect of host species on first instar growth performance was large and significant ($F_{16,160} = 14.27$, $P = 0.0001$; Figure 1), and there was significant variation between insect families within populations ($F_{20,357} = 2.72$, $P < 0.0001$, Table 1). But, counter to the predictions of the genetic mosaic hypothesis, there was no evidence for within-population interactions between insect families and host species ($F_{160,357} = 0.74$, $P = 0.99$, Table 1). Some

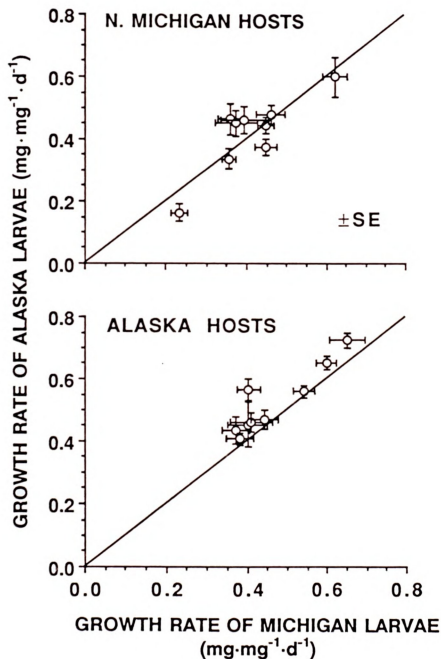


Figure 1: Growth rate of first instar *P. canadensis* from Alaska and Michigan compared on nine Michigan host species (upper) and nine Alaskan host species (lower). Points near the line of equality represent hosts on which the two insect populations performed equally well. Error bars show ± 1 SE. Host species are listed in appendix.

Table 1: ANOVA comparing the first instar growth rate of *P. canadensis* larvae from Alaska and Michigan on host species occurring in Alaska and Michigan.

SOURCE	DF	MS · 10 ⁴	F ^a	P
A. Insect population	1	2316	3.34	0.083
B. Host community	1	6654	9.59	0.0057
C. Insect pop. x Host community	1	445	0.64	0.43
D. Host species (community)	16	2682	14.27	0.0001
E. Insect pop. x Host species	16	162	0.86	0.50
F. Insect family (pop., comm.)	20	694	2.72	0.0001
G. Insect family x Host species	160	188	0.74	0.99
H. Error	357	255		

^a The *F* test denominator for sources A, B, and C was MS_{Family}.
 The *F* test denominator for sources D, and E was MS_{Family x Host species}.
 The *F* test denominator for sources F, and G was MS_{Error}.

families tended to have higher first instar growth rates than other families, but the pattern among families was unaffected by host species. This test for family x host interactions was very general in that it simultaneously considered nine host species from each of two regions. A strong interaction involving any pair of host species could have been obscured if the other host species contributed little to the family x host interaction. As a check of this possibility, I tested for family x host interactions using 72 different subsets of data that compared each possible pair of hosts from Alaska and from Michigan. None of the 72 F -tests was significant at $P < 0.05$ (degrees of freedom were 8-14 for the numerator and 37-47 for the denominator).

First instar growth performance: interspecific variation

In contrast to the absence of variation within *P. canadensis*, there were striking differences between *P. canadensis* and its sister species, *P. glaucus*. *P. glaucus* larvae grew significantly faster than *P. canadensis* when both species were challenged with six hosts from southern Michigan (Figure 2, upper; least square means $\pm$ SE equalled 0.486 ± 0.018 versus 0.355 ± 0.026 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ for *P. glaucus* and *P. canadensis* respectively, $P = 0.008$). The pattern was reversed in the reciprocal test, where *P. canadensis* larvae grew significantly faster than *P. glaucus* larvae when both species were challenged with nine Alaskan host species (Figure 2, lower; least square means $\pm$ SE equalled 0.525 ± 0.014 versus 0.326 ± 0.012 for *P. canadensis* and *P. glaucus* respectively, $P < 0.0001$). The interaction between insect species and host community was highly significant ($F_{1,19} = 71.49$, $P < 0.0001$, Table 2), and in the hypothesized direction (insect species grew fastest



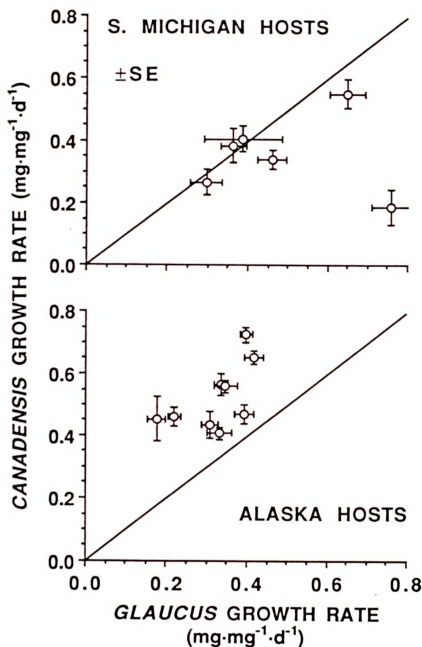


Figure 2: Growth rate of first instar *P. canadensis* and first instar *P. glaucus* compared on six host species from southern Michigan and nine from Alaska. Points near the line of equality represent hosts on which the two insect species performed equally well. Error bars show $\pm 1 \text{ SE}$. Host species are listed in appendix.

Table 2: ANOVA comparing the first instar growth rate of *P. canadensis* AK and *P. glaucus* MI on host species occurring in Alaska and southern Michigan.

SOURCE	DF	MS · 10 ⁴	F ^a	P
A. Insect species	1	726	4.02	0.060
B. Host community	1	25	0.14	0.71
C. Insect spp. x Host community	1	12926	71.49	0.0001
D. Host species (community)	13	2176	14.38	0.0001
E. Insect species x Host species	13	1061	7.01	0.0001
F. Insect family (A, B)	19	181	0.96	0.51
G. Insect family x Host species	131	151	0.80	0.92
H. Error	209	189		

^a The *F* test denominator for sources A, B, and C was MS_{Family}
 The *F* test denominator for sources D, and E was MS_{Family x Host species}
 The *F* test denominator for sources F, and G was MS_{Error}

on the hosts that they normally encounter). On southern tree species, the differences between *P. glaucus* and *P. canadensis* were most dramatic on *Liriodendron tulipifera* (Magnoliaceae), but *P. glaucus* also tended to grow faster on *Ptelea trifoliata* (Rutaceae) and *Prunus virginiana* (Rosaceae) (Figure 2, Appendix). On boreal tree species, the growth rates of *P. canadensis* larvae were generally elevated relative to *P. glaucus* larvae (Figure 2).

Growth performance during the middle instars

P. canadensis populations from Alaska and Michigan did not differ in their relative growth rate spanning days 3 to 17 (Figure 3, upper; population effect in Table 3). Most larvae were just completing the first instar on day 3. By day 17, larval development ranged from midway through the third instar (on poor hosts) to early in the fifth and final instar (on good hosts). Although relative growth rates were nearly identical between populations, Alaskan larvae had attained significantly greater mass by day 17 (Figure 3, lower; population effect in Table 3). This was attributable to larger egg size and greater neonate mass in Alaskan larvae. At hatch, Alaskan larvae were 1.4 times heavier than Michigan larvae (mean $\pm$ SE = 1.33 ± 0.03 versus 0.98 ± 0.03 mg; $F_{1,16} = 31.41$, $P < 0.0001$; based on 360 total larvae from 18 families). By day 17, typical larvae from both populations had increased their mass by 170 fold, but Alaskan larvae still averaged 1.5 times heavier than Michigan larvae (mean $\pm$ SE = 241 ± 9 versus 158 ± 9 mg). Host species had a large effect on growth performance during the middle instars (Figure 3, Table 3).

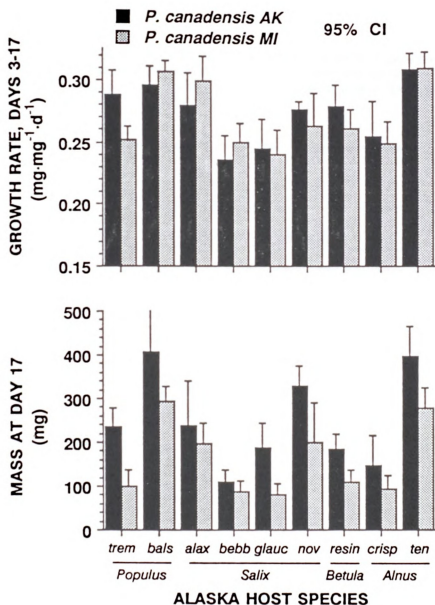


Figure 3: Growth performance of *P. canadensis* from Alaska and Michigan compared on nine Alaskan host species. Upper figure shows relative growth rate during the middle instars (day 3 to day 17). Lower figure shows larval mass on day 17. Error bars indicate 95% confidence intervals. Host species, from left to right, are *Populus tremuloides*, *Populus balsamifera*, *Salix alaxensis*, *Salix bebbiana*, *Salix glauca*, *Salix novae-angliae*, *Betula resinifera*, *Alnus crispa*, and *Alnus tenuifolia*.

Table 3: ANOVA results comparing the growth performance *P. canadensis* populations from Alaska and Michigan feeding on nine Alaskan host species. RGR_{mid} is the relative growth rate during the middle instars (day 3 - 17), and M_{17} is the larval mass on day 17.

Source	DF	RGR_{mid}		M_{17}	
		$MS \cdot 10^6$	F	$MS \cdot 10^{-1}$	F
Population	1	332	0.71	24014	49.15***
Host	8	8247	17.69***	12802	26.20***
Pop x Host	8	1076	2.30*	588	1.20
Error	107	468		488	

* $P < 0.05$; *** $P < 0.0001$

Growth performance during the final instar

The growth rates and efficiencies of final instar larvae were strongly affected by host species, but were nearly identical between *P. canadensis* populations (Figure 4, Table 4). Relative consumption rate ranged from about $1.4 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ on *Salix alaxensis*, *Salix bebbiana*, *Salix glauca*, and *Alnus crispa novae-angliae*, and *Alnus tenuifolia*. The apparent digestibility ranged from 0.23 on *Salix bebbiana* to 0.40 on *Populus tremuloides*. On the other hand, the efficiency with which assimilated matter was converted to new larval tissue ($\text{ECD} = \text{growth/assimilation}$) was relatively insensitive to host species, averaging about 0.40 on eight of nine hosts, but dropping to 0.30 on *Salix glauca*. Relative growth rate (the product of consumption rate, apparent digestibility, and ECD) ranged from about $0.30 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ on good hosts, to $0.13 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ on nutritionally inferior hosts. Thus, the time required for fifth instar larvae to double their mass varied from 2.3 d to 5.3 d depending on host, but was independent of the insect population.

Apparent digestibility and ECD are measures of efficiency based on dry mass. Nitrogen use efficiency is a physiological analog of apparent digestibility $\times$ ECD, and may be of equal or greater ecological relevance (Mattson, 1980). Nitrogen use efficiency differed markedly between hosts, but not between *P. canadensis* populations (Figure 5): $F_{8,35} = 7.10$, $P < 0.0001$, host effect; $F_{1,35} = 3.09$, $P = 0.09$, population effect.

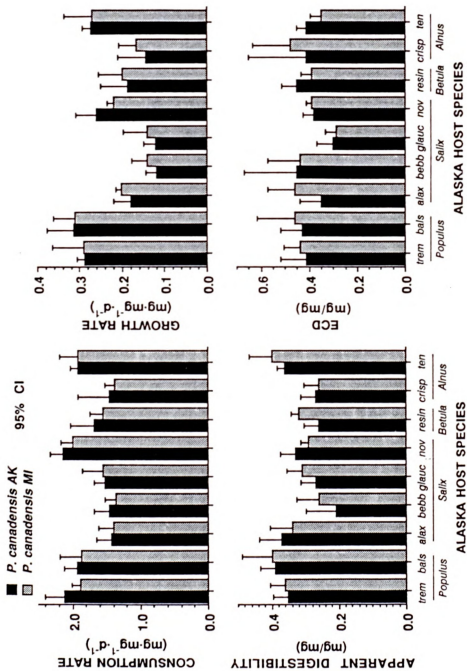


Figure 4: Growth performance of final instar *P. canadensis* from Alaska and Michigan compared on nine Alaskan host species. Growth rate (upper right) equals the product of consumption rate, apparent digestibility, and ECD. Error bars indicate 95% confidence intervals. Host species as in Fig. 3.

Table 4: ANOVA results comparing 5th instar growth-energetics of *P. canadensis* from Alaska and Michigan feeding on nine Alaskan host species.

Source	DF	Growth Rate		Consumption Rate		Apparent Digestibility		Assimilation Rate		ECD	
		MS ^a	F	MS ^a	F	MS ^a	F	MS ^a	F	MS ^a	F
Population	1	102	0.40	16478	2.82	473	1.56	6	0.00	361	0.28
Host	8	4981	19.69***	78363	13.39***	3127	10.33***	27895	13.85***	2490	1.91
Pop x Host	8	121	0.48	1758	0.30	404	1.34	1681	0.83	715	0.55
Error	80	253		5852		303		2013		1302	

^a MS = Mean Square · 10⁵

*** $P < 0.0001$

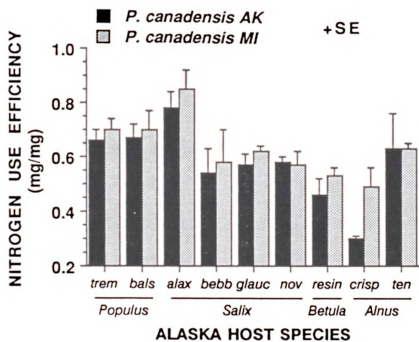


Figure 5: Nitrogen use efficiency of final instar *P. canadensis* from Alaska and Michigan compared on nine Alaskan host species. Error bars indicate 1 se. Host species as in Fig. 3.

Pupal mass, larval duration, and survival

By extending the duration of growth, Michigan larvae produced larger pupae than Alaskan larvae (Figures 6-7, Table 5), even though Alaskan larvae began their life larger, and tended to grow at the same relative growth rate. Female pupal mass ($\pm$ SE) averaged 688 ± 9 versus 750 ± 9 mg, and female larval duration averaged 28.0 ± 0.3 versus 33.0 ± 0.3 d, for Alaska and Michigan respectively. On all but the worst hosts, larvae growing for a longer time produced larger pupae; Michigan larvae tended to feed and grow for a longer time than Alaskan larvae, but the relationship between duration of growth and pupal mass did not differ between populations (Figure 7).

Pupal mass and larval duration were also strongly affected by host species (Figure 6, Table 5). On hosts where they grew slowly, larvae extended the duration of growth but still produced smaller pupae than on high quality hosts. There were interactions between host species and insect population in that population differences in pupal mass were least on low quality hosts, and population differences in larval duration were greatest on low quality hosts.

Larval survival was strongly affected by host species but was very similar between populations (Table 6). Survival to pupation ranged from 30% on *Salix glauca* to about 80% on *Populus balsamifera*, *Populus tremuloides*, *Betula resinifera*, and *Alnus tenuifolia*. Patterns of survival also differed; on *Alnus crispa*, virtually all of the mortality occurred during the first instar, while on *Salix novae-angliae*, there was some mortality at almost every stage. This suggests acute toxicity as opposed to chronic malnutrition. Survival was generally correlated with other measures of growth performance, but with some exceptions. *Betula resinifera* had



Table 5: ANOVA results comparing the pupal mass and larval period of *P. canadensis* from Alaska and Michigan reared on nine Alaskan host species.

Source	DF	Female Pupal Mass		Female Larval Period	
		MS · 10 ⁻¹	F	MS · 10 ²	F
Population	1	10570	22.54 ^{***}	69531	122.59 ^{***}
Host	8	10286	21.94 ^{***}	27979	49.33 ^{***}
Pop x Host	8	12670	2.70 ^{**}	1255	2.21 [*]
Error	98	469		567	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

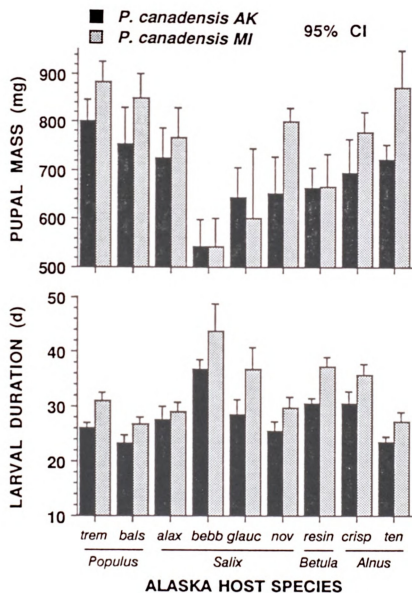


Figure 6: Pupal mass and larval duration of *P. canadensis* from Alaska and Michigan reared on nine Alaskan host species. Error bars indicate 95% confidence intervals. Host species as in Fig. 3.

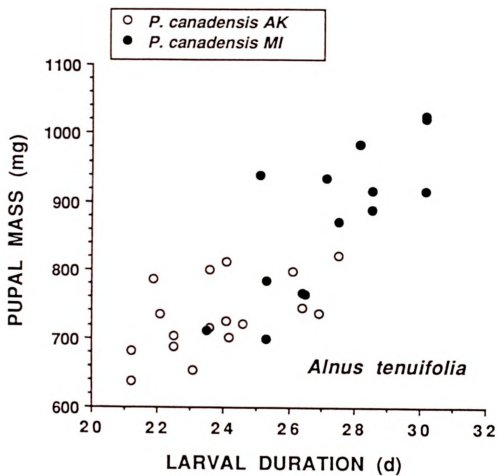


Figure 7: Pupal mass as a function of larval duration in *P. canadensis* from Alaska and Michigan reared on *Alnus tenuifolia*. Each point represents one pupa.

Table 6: Cumulative survival of *P. canadensis* larvae from Alaska and Michigan reared on nine Alaskan host species. Each host began with 19 or 20 neonate larvae from each population. Pupal survival only includes the first half of winter diapause.

Tree species	Insect Population	Percent of initial insects surviving to the stadium indicated					Pupae
		1	2	3	4	5	
<i>Populus tremuloides</i>	AK	80	80	80	80	80	80
	MI	80	75	75	75	75	75
<i>Populus balsamifera</i>	AK	90	85	85	85	75	70
	MI	95	90	90	90	90	90
<i>Salix alaxensis</i>	AK	79	79	79	74	68	68
	MI	80	75	75	70	60	60
<i>Salix bebbiana</i>	AK	75	75	75	70	50	30
	MI	70	65	65	60	55	45
<i>Salix glauca</i>	AK	55	45	45	45	40	40
	MI	55	50	50	35	20	20
<i>Salix novae-angliae</i>	AK	90	70	65	65	60	55
	MI	90	68	63	63	63	58
<i>Betula resinifera</i>	AK	95	84	84	84	79	79
	MI	85	85	85	85	85	80
<i>Alnus crispa</i>	AK	58	58	58	58	58	53
	MI	70	70	65	65	65	65
<i>Alnus tenuifolia</i>	AK	95	95	85	85	85	85
	MI	90	85	85	80	75	70

very high survival even though larval growth rates and pupal mass were below average. Conversely, *Salix novae-angliae* allowed generally high growth rates and produced large pupae, even though survival was less than 60%.

Discussion

Evaluation of hypotheses

There was no evidence for population differences in the ability of *P. canadensis* larvae to ingest, detoxify, assimilate, or convert the leaf tissue of various tree species that they encounter throughout their broad geographic distribution. Populations from Alaska were no better able to use Alaskan hosts, and no worse able to use Michigan hosts, than populations from Michigan (absence of population x host community interaction, Table 1, Figure 1). Similarly, there was no evidence of host specialists among families within populations (absence of family x host species interaction, Table 1). Instead, all genotypes appeared equally capable of using all hosts, thereby refuting the genetic mosaic hypothesis both at the inter- and intra-population level. Among 500+ species in the Papilionidae, *P. canadensis* is extraordinary in its ability to exploit so many taxonomically and phytochemically diverse hosts (Scriber, 1984). The explanation for these polyphagous abilities lies not within the population structure of the species, but within the genome of any individual within the species (super-genotype hypothesis).

Apparently, future evolutionary change in the nutritional physiology of *P. canadensis* will be constrained by the appearance of novel variation. Thus I would not expect *P. canadensis* to respond rapidly to climate-induced changes in

the plant community, nor would I expect that other *P. canadensis* populations, in other regions of the boreal forest with other communities of hosts, differ in their host-use abilities. The detection of variation is partly a function of sampling intensity, but my conclusions seem robust. Population comparisons were based on nearly 600 larvae representing over 30 families. Given the observed variance, I was likely to detect population differences in growth rate of >15% during the first instar, >3% during the middle instars, and >10% during the final instar. Furthermore, the same experimental design readily detected interspecific differences between *P. glaucus* and *P. canadensis* (Table 2, Figure 2). Populations of *P. canadensis* at the northern and southern edges of the boreal forest have been associated with different tree communities for most of the Holocene (Webb et al., 1983). If there were meaningful intraspecific variation in the nutritional physiology of *P. canadensis*, even if it were undetectable within populations, I would have expected measurable divergence between these distant populations.

Although host-use abilities appear to be invariant, there is substantial variation in other attributes of *P. canadensis*. Alaskan females lay eggs that are 1.5 times larger than those produced by Michigan females of the same size (which benefits larval development, Figure 3, Table 2), but only produce 60% as many (Lederhouse and Ayres, unpub.). Alaskan caterpillars initiate pupation at a smaller size than their Michigan counterparts; hence both larval duration and pupal mass are less in the Alaskan population (Figures 6-7, Table 5). Alaskan caterpillars are capable of more rapid growth at low temperatures (e.g., doubling time of 6 d versus 9 d for 5th instar larvae at 12°C), and require less time to molt at low temperatures (6 d versus 8 d for the penultimate molt at 12°, and 12 d

versus 18 d for the final molt) (Chapter 2). Each of these differences is an apparent response to the selective pressures imposed by short cool Alaskan summers. *P. canadensis* populations have diverged in their temperature responses and life history strategies yet have remained surprisingly invariant in their nutritional physiology.

Explanation for invariance of nutritional physiology

Endler (1977) considered clinal differentiation as a balance between the homogenizing effects of gene flow and the diversifying effects of selection gradients. The biology of *P. canadensis* seems to match Endler's gradient selection model in that suitable habitat is more or less continuous from Michigan to Alaska (ca. 4000 km), and the dispersal capabilities of individual butterflies are small with respect to the species distribution. In Endler's terms, the lack of differentiation in *P. canadensis* could be due to high gene flow or shallow selection gradients.

We lack detailed information on gene flow in *P. canadensis*, but based on adult longevity in the laboratory (Lederhouse et al., 1990), behavioral studies of the closely related *P. glaucus* (Lederhouse, 1982; Berger, 1986), and my own field observations, it appears that most mating and oviposition takes place within 20 km of the site of larval development, and I doubt that much occurs beyond 60 km. The vagility of *P. canadensis* is certainly greater than such insect herbivores as *Euphydryas* butterflies (Ehrlich et al., 1975), *Alsophila* moths (Futuyma et al., 1984), and *Nuculaspis* scales (Alstad and Corbin, 1990), but probably less than others such as *Danaus* butterflies (Eanes and Koehn, 1978; Malcolm and Brower,



1989) and *Schistocerca* locusts (Chapman, 1990). In any case, it is obvious that gene flow is not so extensive to preclude differentiation, because these populations have diverged markedly in other traits (Chapter 2).

Weak selection gradients also seem an unlikely explanation for the lack of divergence. Host use patterns differ between the populations (Scriber and Ayres, 1990, unpublished), and it is clear that the plant species differ in the secondary chemistry of their foliage (Palo, 1984). Leaves of *Prunus serotina* contain the cyanogenic glycoside prunasin (Horsley and Meinwold, 1981; Reilly et al., 1986). *Alnus crispa* contains the stilbene pinosylvin; *A. crispa* and *A. tenuifolia* contain the triterpene α -amyrin, and *A. rugosa* contains what may be the shikimate glycoside oregonin (Bryant, 1981; Clausen et al., 1986; Ayres and Reichardt, unpublished). *Fraxinus* contains an assortment of phenolic acids and flavonoids (Kowalczyk and Olechnowicz, 1988). *Tilia* contains the triterpene β -amyrin, plus a number of flavonoids (Hickok and Anway, 1972; Guelz et al., 1988). *Populus tremuloides* and *P. grandidentata* contain the phenolic glycosides tremulacin and salicortin (Lindroth et al., 1987); *P. balsamifera* buds and leaves contain a complex mixture of compounds including cineol and benzyl alcohol (Reichardt et al., 1990, pers. comm.). *Betula papyrifera* and *B. alleghaniensis* differ in their qualitative composition of flavonoids (Pawlowska, 1983). *Salix novae-angliae* contains high concentrations of the flavonoid kaempferol-3-arabinsyl-7-rhamnoside (T. P. Clausen, pers. comm.). *Salix alaxensis*, *S. bebbiana*, *S. glauca*, *S. novae-angliae*, *Betula alleghaniensis*, *B. papyrifera*, and *B. resinifera*, all produce high concentrations of condensed tannins that are structurally distinguishable (percent procyanidin, stereochemistry of C₄ relative to C₃, and the number of

flavonoid subunits per molecule) (Ayres et al., in prep.) Even under favorable laboratory conditions, larval mortality of *P. canadensis* was substantial on most hosts (Table 6), and larval growth rates (neonate to prepupa) were only half that of specialist relatives in the *Papilio troilus* group (Nitao et al., 1991; Lederhouse et al, 1992). Natural selection should favor genotypes that survive better and grow faster, and it seems that the optimum physiology should differ across this diverse spectrum of hosts.

Implications for herbivore evolution

I suggest that the nutritional physiology of *P. canadensis*, *Epirrita autumnata* (Ayres et al., 1987), and perhaps other insect herbivores, has been canalized such that mutations and recombination seldom lead to phenotypic variation. Thus natural selection may have little on which to operate. (Endler's single locus, two allele, model assumes the pre-existence of variation.) Under this canalization scenario, substantive evolutionary change is predicted to involve the occasional breakdown and successful rearrangement of gene interaction systems (Wright, 1977, pp. 443-473), which would be unlikely except in small populations, and may be commonly associated with speciation events. This model reconciles the apparent stasis of nutritional physiology in *P. canadensis* with the marked discontinuity between *P. canadensis* and its putative ancestor *P. glaucus* (compare Figures 1 and 2, Tables 1 and 2). This is not a new idea. It is consistent with models of stepwise evolution (Ehrlich and Raven, 1964) and punctuated equilibria (Eldredge and Gould, 1972), and is linked with concepts of "metastable equilibria" (Haldane, 1931), "coadapted genes" (Dobzhansky, 1955), and "the unity

of the genotype" (Mayr, 1963, pp. 263-298).

This view of herbivore evolution emphasizes the importance of epistasis. I propose that larval growth performance is more complex than the additive effects of multiple gene products. The role of gene interactions in detoxification physiology could be tested by experimentally inactivating enzyme systems singly and in combination and measuring the response of the insect (e.g., Hedin et al., 1988; Lindroth, 1989a); statistical interactions will indicate epistasis. If nutritional physiology has been canalized, the composite process of growth should be buffered from genetic variation in the underlying components. Thus, genetic variation in relative growth rate is predicted to be less than variation in mixed function oxidase activity, esterase activity, proteinase activity, permeability of the peritrophic membrane, pH of the gut lumen, rate of gut passage, or the number and type of carrier proteins in the gut wall. Homeostatic properties relevant to insect growth may arise at many levels, including gene duplication (8 esterase isozymes have been electrophoretically resolved in *P. canadensis*, K. S. Johnson, unpublished data), Mendelian dominance (Sved and Mayo, 1970), regulation of gene expression (Lindroth, 1989b), kinetics of biochemical pathways (Kacser and Burns, 1981), and neuroendocrine control (Bernays and Simpson, 1982).

Broad patterns of nutritional adaptation in herbivorous insects may not be a simple extension of the microevolutionary processes occurring within most populations; i.e., intraspecific patterns may be poor predictors of interspecific patterns. Intraspecific variation provided no hint of the extensive divergence between *P. canadensis* and *P. glaucus*, nor of the strong negative correlations (apparent tradeoffs) between their growth performance on different hosts.

Studies in the *Papilio troilus* species group have similarly indicated that intraspecific variation is of a completely different scale and pattern than variation between closely related species (Nitao et al., 1991; Lederhouse et al., 1992). Negative genetic correlations in insect growth performance on different hosts are predicted by prevalent explanations for the evolution of specialization (Levins and MacArthur, 1969; Futuyma and Moreno, 1988), but recent reviews of intraspecific studies have noted that such negative correlations are surprisingly rare (Jaenike, 1990; Via, 1990). This is less surprising if intraspecific variation is so low as to be of modest ecological importance (e.g. Via, 1984b; Bowers, 1986; James et al., 1988; Nitao et al., 1991). Fair tests of correlation require meaningful variation, even if it requires the complications of interspecies comparisons. Available data may even overestimate the importance of intraspecific tradeoffs in host-use abilities, because most published studies involve systems where there were *a priori* expectations of low gene flow and high intraspecific variation.

Traits that are canalized may still evolve in predictable patterns according to general principles, but describing the patterns and testing the principles will require the tools of comparative biology in addition to quantitative genetics (Ehrlich and Raven, 1964; Stearns, 1980; Cheverud et al., 1985; Huey, 1987; Pagel and Harvey, 1988; Wanntorp et al., 1990). Further studies that compare variation within and between closely related species will test my suggestion that nutritional physiology is typically canalized and evolves during uncommon rearrangements of the genome.

CHAPTER TWO

Local adaptation to regional climates in Papilio canadensis

Introduction

Why do insect herbivores occur where they do, but not elsewhere?

Herbivore distributions are commonly less extensive than that of their host plants (MacLean 1983, McClure 1989), implying that they are not a simple function of host plant distributions. Interspecific competition may occur among insect herbivores (Fritz et al. 1986, Karban 1986), but competitive exclusion is probably rare because food resources are not usually limiting (Lawton and Strong 1981, Fritz and Price 1990). Here I explore the role of abiotic constraints, specifically summer temperatures, in determining the geographic range of the northern tiger swallowtail, *Papilio canadensis* R & J (Lepidoptera: Papilionidae).

P. canadensis is a polyphagous tree-feeding insect that occurs throughout much of the boreal forests of North America, from New England and upstate New York, west and north through the Great Lakes states and Canada to interior Alaska (Scriber 1988). Preliminary information suggested an important role of temperature in the ecology of *P. canadensis*. Larval growth rates are strongly temperature sensitive in swallowtails (Scriber and Lederhouse 1983, Ritland and Scriber 1985), as in most insects (Scriber and Slansky 1981, Taylor 1981). Also,

the northern limit of *Papilio glaucus* L., a southern parapatric sister species (Hagen et al. 1991), closely corresponds to the 1400 degree day isotherm (10°C base), which approximates the thermal sum required for completion of two generations (Scriber 1988). *P. canadensis* is obligatorily univoltine while *P. glaucus* is typically multivoltine (Hagen and Scriber 1989); if the biology of these two taxa were otherwise identical, *P. canadensis* would require about 700 degree days to complete its development.

As a first step in assessing the importance of summer temperatures for *P. canadensis*, I analyzed the climates encountered by populations near the southern and northern limits of their distribution. The thermal sums accumulated at Chatham, Michigan (86° 50' W, 46° 25' N) ranged from 760 to 1254 degree days over 50 years, with an average of 985 degree days (Figure 8). In contrast, the warmest summer in Fairbanks, Alaska (147° 30' W, 64° 50' N) accumulated fewer degree days than the coldest year in Chatham: ranging from 453 to 747 degree days over 48 years, with an average of 583 degree days (Figure 8). If we take 700 degree days as the minimum for completion of one generation, the upper peninsula of Michigan always accumulates sufficient degree days for completion of one generation, but never two, while only 5 years out of 48 would have allowed even a single generation in interior Alaska. In fact, *P. canadensis* butterflies were nectaring outside my Fairbanks laboratory as I completed these analyses.

Summers in interior Alaska are relatively cool as well as short. The average daily mean temperature during the period of *P. canadensis* larval development was 14.4°C compared to 18.8° in northern Michigan (Figure 9). In

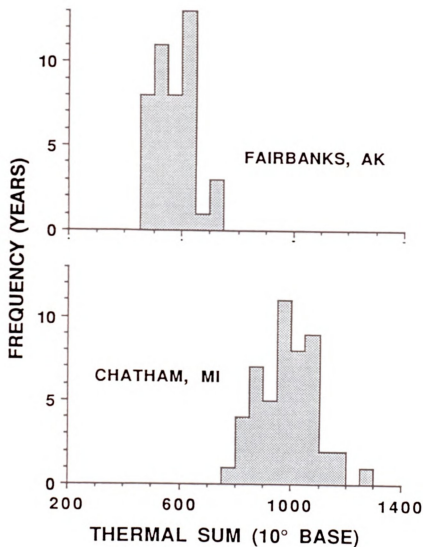


Figure 8: Frequency distribution of thermal sums accumulated above a 10°C base in Fairbanks, Alaska (48 years) and Chatham, Michigan (50 years). Michigan weather records included 1931-1980; Alaska records included 1936-1980 (excluding 1969) and 1987-1990. Annual accumulations were begun on 1 April and terminated near the time of leaf fall (15 September in Alaska and 1 October in Michigan). Thermal sums were calculated from daily maxima and minima using a sine function recommended by Watanabe (1978).

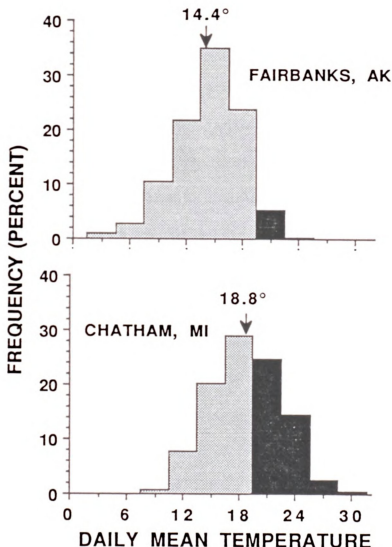


Figure 9: Distribution of daily mean temperatures encountered by *P. canadensis* larvae in Fairbanks, Alaska (48 years) and Chatham, Michigan (50 years) (weather records as in Fig. 1). The period of *P. canadensis* larval development was defined as 350-700 degree days in Michigan and 250-550 degree days in Alaska (truncated at 15 September when necessary). The percentage of days in each temperature class was calculated separately for each season, and the figure shows the average percent across 48 or 50 years of records (otherwise the distribution would be weighted towards long cold seasons).

Alaska, the daily mean temperature was 19.5° or less on most days (80%), while in Michigan it was 21° or warmer on nearly half the days (43%).

This climatic comparison led me to turn the original question around and ask how *P. canadensis* maintains as broad a distribution as it does. I suggest two alternative hypotheses: (1) *P. canadensis* is a composite of regionally adapted populations that collectively maintain a broader distribution than would otherwise be possible, or (2) *P. canadensis* is a single cohesive genotype that is surprisingly robust in its tolerance of climate. The scenario of regional adaption (H_1) suggests that populations are continually being modified through natural selection to conform to the local environment; i.e., the environment determines the genotype and therefore climatic limits to distribution and abundance may be nonexistent or transient. This contrasts with the notion of a cohesive genotype (H_2), which emphasizes genetic constraints, and argues instead that the genotype determines the environment where the organism occurs.

These hypotheses were evaluated by comparing the developmental physiology of *P. canadensis* from northern Michigan and interior Alaska across a range of temperatures in the laboratory and in the field.

Methods

Overview

P. canadensis populations were compared with respect to fourth instar growth rates (RGR_4), fifth instar growth rates (RGR_5), the duration of the fourth molt ($Molt_4$, fourth instar to fifth instar), the duration of the fifth molt ($Molt_5$, fifth instar to pupa), consumption rate (RCR), assimilation rate (RAR), average

daily metabolic rate (ADMR), apparent digestibility (AD), the efficiency of conversion of digested matter (ECD), nitrogen use efficiency (NUE), pupal mass, and survival to imagos. Studies were conducted during two seasons and included three host species: the temperature response of Alaskan and Michigan larvae were compared on *Populus tremuloides* Michx. (quaking aspen) in 1988 and 1989, and on *Populus balsamifera* L. (balsam poplar) in 1988 (both hosts are used naturally by both populations); 1988 experiments also included the temperature response of Alaskan larvae feeding on *Betula resinifera* Britton (Alaska paper birch). These experiments were conducted under controlled laboratory temperatures using freshly detached leaves. In addition, I compared the ability of the two populations to elevate larval body temperatures through basking behavior under field conditions, and compared the growth rates of larvae feeding naturally in the field with growth rates of larvae at an equivalent experimental temperature, feeding on detached leaves in the laboratory.

Population sampling

Female butterflies were collected during early June 1988 and 1989 from sites in interior Alaska (vicinity of Fairbanks) and northern Michigan/Wisconsin (Gogebic and Iron counties in MI; Vilas, Taylor, and Chippewa counties in WI), then transported to the laboratory, fed a 20% honey solution daily, and allowed to oviposit on sprigs of quaking aspen foliage (*Populus tremuloides*). Resulting larvae from the two populations were reared simultaneously and fed the same foliage (same trees, same leaf samples) until early in the fourth instar when growth trials began. Larvae destined for experiments with different host species

were reared on those hosts from the time of egg hatch. Thus, maternal parents, eggs, and larvae of both populations were handled with the same protocol, in the same laboratory, at the same time. This should have precluded any spurious differences between populations due to differing environments.

Experiments during 1988 included 9 Alaskan families (total of 108 larvae) and 6 Michigan families (96 larvae); 1989 experiments included 10 Alaskan families (76 larvae) and 10 Michigan families (55 larvae). Larvae from each family were allocated evenly across temperatures and hosts, but it was not possible to maintain equal sample sizes of each family in each treatment, and mortality resulted in some families being unrepresented in some treatments.

Larval growth during the fourth and fifth instars

Early fourth instar larvae (recently molted but feeding, ca. 100 mg fresh mass) were weighed (W_4), offered previously weighed leaves, and allocated to one of four experimental temperatures (12, 18, 24, or 30°C; photoperiod of L:D 18:6). Larvae were individually confined to clear plastic vials (12 ounce cups) with water-saturated plaster-of-paris bases that provided a high humidity atmosphere and maintained leaf turgor near natural levels. Paired control leaves (one sample per larva) were placed in adjacent vials, and exposed to the same conditions as the foliage fed to the larvae; the ratio of dry mass to wet mass of control leaves allowed me to estimate the initial dry mass of the foliage fed to the larvae. When larvae began to approach the fifth molt (ca. 380 mg; after 1.5 d at 30°, 4-5 d at 12°), they were reweighed (W_5), given fresh foliage, and returned to the same temperature. The unconsumed leaf tissue and the accumulated frass

were dried and weighed. The dry mass of experimental larvae was estimated from their fresh mass as $DM = 0.125 \cdot FM$. (The dry:wet ratio of *P. canadensis* larvae did not differ between populations and was unaffected by host plant.; $N = 20$)

Fourth instar relative growth rates were calculated as:

$$RGR_4 = [\ln(W_{4f}) - \ln(W_{4i})] / T_{4i-4f}$$

where $\ln$ = natural logarithm, and T_{4i-4f} = the elapsed time in days. Relative consumption rate (RCR) and relative assimilation (RAR) were calculated following formulae in Gordon (1968), which are similar to those of Waldbauer (1968) (produced values within $\pm 5\%$), but better describe a system of exponential growth. Apparent digestibility (AD) = RAR/RCR . The efficiency of conversion of digested matter (ECD) = RGR/RAR . Average daily metabolic rate (ADMR) was calculated as $RAR - RGR$. All are expressed in units of dry mass.

When larvae had completed the fourth molt and begun feeding in the fifth instar (ca. 400 mg), they were reweighed (W_{5i}), and again offered fresh, weighed foliage. Fifth instar trials lasted the majority of the fifth instar (1.5 to 5 d depending on temperature), and produced similarly derived estimates of all parameters measured in the fourth instar. In addition, a subset of the control leaves and frass samples was analyzed for total nitrogen content with standard micro-Kjeldahl techniques (sulfuric acid digestion followed by colorimetric analysis with a Technicon Auto-analyzer II; Herms, 1991). Nitrogen use efficiencies (NUE) were calculated as the fraction of ingested nitrogen that did not appear in the frass: $NUE = (N_{\text{ingested}} - N_{\text{egested}})/N_{\text{ingested}}$.

In 1989, larvae were returned to their experimental temperatures after the fifth instar growth measurements, fed fresh foliage, and monitored daily (12 and 18°) or twice daily (24 and 30°) until they completed the fifth molt and entered the diapausing pupal stage. Four weeks after pupation, pupae were sexed, weighed, and stored at 2°C in darkness through the winter. The following spring they were placed outdoors (Fairbanks) in screen emergence traps until the adults emerged or the pupae died.

The duration of molts

Because the process of molting between instars is not entirely discrete from that of growth within instars, its contribution to total development time is difficult to measure by simple counting the number of days or hours that an animal appears to be molting (Ayres and MacLean 1987). I estimated the duration of the fourth molt by calculating, based on fourth and fifth instar growth rates, how long it would have taken a larva to grow from its early fourth instar mass to its late fifth instar mass in the absence of molt ($T_4 + T_5$), and subtracting this from how long it actually took (T_{4i-5f}). The difference was defined to be the duration of the fourth molt (Molt_4). Calculations follow Ayres and MacLean (1987).

$$\text{Molt}_4 = T_{4i-5f} - (T_4 + T_5).$$

$$T_4 = [\ln(M_{4max}) - \ln(M_{4i})]/\text{RGR}_4.$$

$$T_5 = [\ln(M_{5f}) - \ln(M_{5min})]/\text{RGR}_5.$$

$$M_{5min} = M_{4max} - EXUV - RESP.$$



The mass of fifth instar larvae when they have first filled their gut after ecdysis (W_{5min}) equals the maximum mass of fourth instar larvae (M_{4max}) minus exuvial losses ($EXUV$) and respiratory expenses during molt ($RESP$). For *P. canadensis*, $EXUV$ was estimated as 2.5 mg and $RESP$ as $M_{4max} \cdot 0.05$. $EXUV$ and $RESP$ are difficult to estimate precisely, but halving or doubling them has little effect on $Molt_4$ (Ayres and MacLean 1987).

For simplicity I refer to the "duration of molt", but because of temporal overlap between the processes of growth and molt, $Molt_4$ is somewhat less than the time between the hormonally mediated initiation and completion of molt. $Molt_4$ is also somewhat longer than the time when a molting larva is obviously immobile and nonfeeding, because growth slows in preparation for apolysis, and only gradually resumes after ecdysis.

The duration of the fifth and final molt ($Molt_5$) was calculated similar to $Molt_4$ except was only based on growth rate in the preceding (fifth) instar because growth ceases after pupation.

$$Molt_5 = T_{5i-pupa} - [\ln(DM_{pupa}) - \ln(DM_{5i})]/RGR_5.$$

The dry mass of pupae (DM_{pupa}) were estimated from pupal fresh masses as $-18.4 + 0.243 \cdot FM_{pupa}$ for males and $-56.9 + 0.316 \cdot FM_{pupa}$ for females (Ayres, unpublished data). The dry mass of early fifth instar larvae (DM_{5i}) was calculated as before ($FM \cdot 0.125$) with a correction for indigestible gut contents (-15% fresh mass). As an alternative to this estimate of $Molt_5$, I also measured the time elapsed from when late fifth instar larvae cleared their guts and the completion of ecdysis.

Basking success

During August 1989, I compared the ability of *P. canadensis* larvae from Alaska and Michigan to elevate their body temperatures with solar radiation. Fifth instar larvae from Alaska and Michigan ($n = 18$ and 11 , respectively) were placed outdoors on quaking aspen trees where they were allowed to move, feed, and bask naturally. Temperatures were recorded to the nearest 0.1°C using a Sensortek BAT-12 telethermometer with a blunt tip, fine gauge copper/constantan sensor (Sensortek model MT-4, needle diameter 0.33 mm , time constant 0.025 sec). Caterpillar temperatures were measured non-invasively by sliding the sensor tip into a natural fold dorso-lateral to the first pair of legs. With each caterpillar temperature, I also measured air and leaf surface temperatures, as well as the temperature of a standardized "black body" exposed to the same insolation as the caterpillar. My black body was a black, caterpillar-sized piece of open cell foam rubber ($30 \times 10 \times 10\text{ mm}$), which I suspended next to each caterpillar until its temperature stabilized under the ambient radiation ($1\text{--}4\text{ minutes}$). Black body temperatures provided a measure of basking potential. All temperatures were recorded with the sensor tip shaded.

I measured the relative growth rates of larvae in the field (for comparison with laboratory growth rates) by weighing the Alaskan *P. canadensis* prior to being placed outdoors, and 3 d later. The ambient daily mean temperature encountered by field larvae was measured with a shaded maximum-minimum thermometer. A matched set of *P. canadensis* larvae was weighed one day later and grown for 3 d in the laboratory at a constant temperature set each day to the

mean temperature encountered the previous day by the field caterpillars.

Laboratory larvae were fed leaves collected from the same trees.

Statistical analyses

Statistical comparisons of *P. canadensis* populations from Alaska and Michigan were based on the number of families representing each population and the variance among families. Except as noted, figures and tables show population means and standard errors calculated from family means. RGR_4 , RGR_5 , and $Molt_4$, were analyzed separately at each temperature (due to extreme heteroscedasticity among temperatures) with an ANOVA model that included population (AK versus MI) and host (*Populus tremuloides* in 1988, *Populus balsamifera* in 1988, and *Populus tremuloides* in 1989) as fixed effects, and families nested within population as a random effect. $Molt_5$ (only measured in 1989) was analyzed with an identical ANOVA model except that temperature was substituted for host. Nitrogen use efficiency (incomplete factorial) was analyzed with two two-way ANOVAs, one comparing two populations across four temperatures on a single host (main effects = population and temperature), and one comparing a single population (AK) across three hosts and four temperatures (main effects = host and temperature). Pupal mass was analyzed with a three-way ANOVA that included temperature, population, and sex. Family was not explicitly included in analyses of nitrogen use efficiency and pupal mass because of limited sample sizes and because variance among families contributed only trivially to the other models. $Molt_5$ was square root transformed to correct for heteroscedasticity (figure shows non-transformed data); all other variables

satisfied assumptions of normality and equal variance. ANOVAs were calculated using the SAS General Linear Models procedure (SAS 1985). The effects of population and temperature on survival were tested with a two-way contingency analysis (CATMOD procedure, linear model, SAS 1985).

Results

Larval growth rates

As expected, temperature had an enormous effect on larval growth rate, with higher temperatures generally resulting in higher growth rates (Figure 10). In addition there were substantial differences between populations, and interactions among the effects of temperature, population, and host (Table 7). At low temperatures (12°C), Alaskan *P. canadensis* had consistently higher fourth and fifth instar growth rates than Michigan *P. canadensis* (Figure 10): least square means $\pm$ SE = 0.152 ± 0.008 versus 0.114 ± 0.007 mg · mg⁻¹ · d⁻¹ for fourth instars and 0.119 ± 0.008 versus 0.076 ± 0.010 mg · mg⁻¹ · d⁻¹ for fifth instars ($F_{1,23} = 14.10$ and $F_{1,19} = 11.69$, Table 7). Thus Alaskan fifth instars at 12° were doubling their mass in 5.8 d versus 9.1 d for their Michigan counterparts (doubling time = $\ln(2)/\text{RGR}$). At 18°, Alaskan growth rates were higher in six of six comparisons (Figure 10), significantly so for fifth instars (Table 7). At high temperatures (24 and 30°), population comparisons differed depending upon the host (Figure 10, Population x Host interactions in Table 7). In 1988, growth rates of Alaskan fourth instars feeding on *Populus tremuloides* declined from 24 to 30°C, while the growth rate of Michigan fourth instars increased across the full range of experimental temperatures (Figure 10, upper left); consequently, Michigan larvae

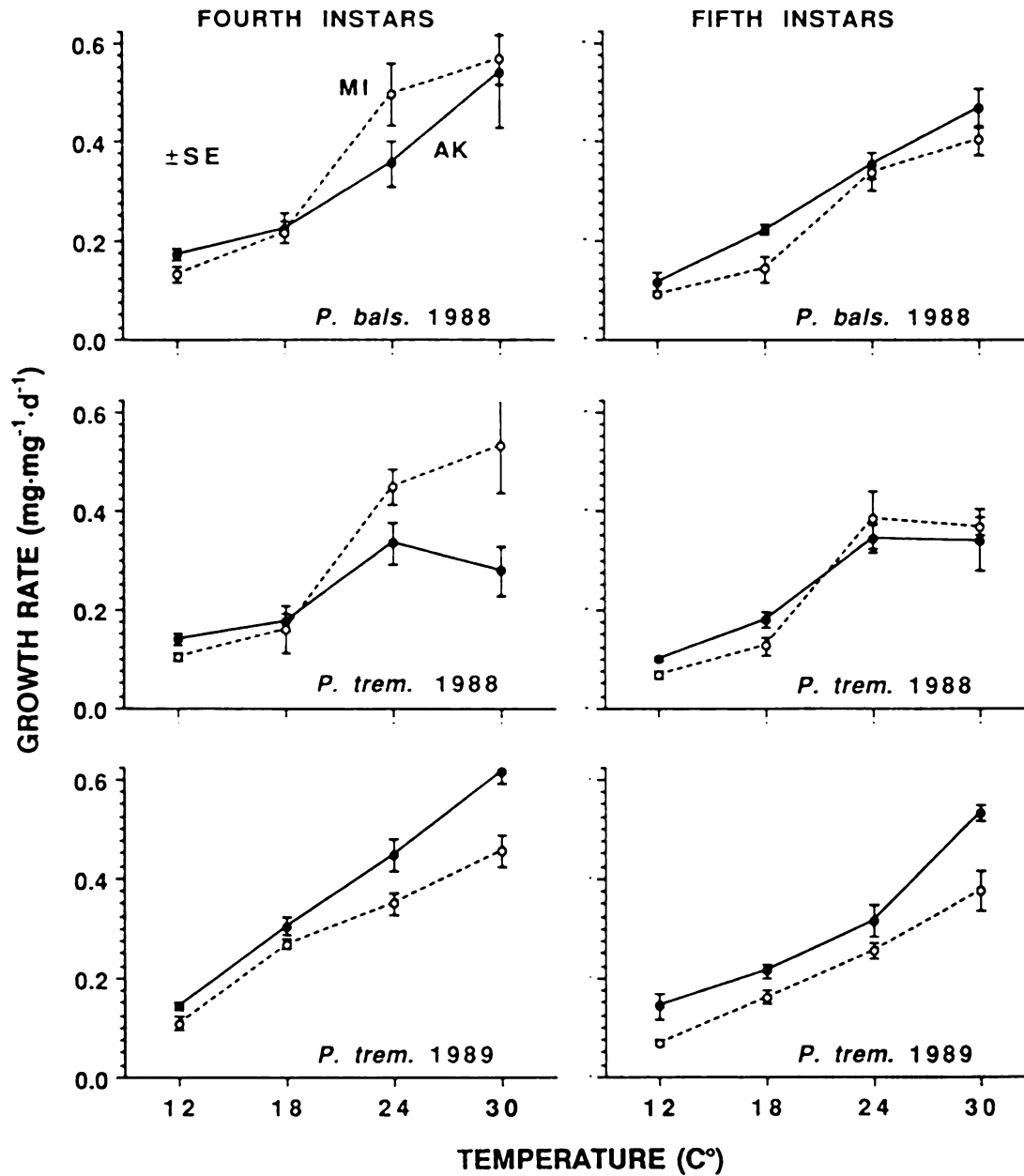


Figure 10: Relative growth rates of *P. canadensis* larvae from Alaska (solid lines) and Michigan (dashed lines) as a function of temperature. Experiments included fourth and fifth instars reared on *Populus balsamifera* and *P. tremuloides* in 1988 and *P. tremuloides* in 1989. Some standard errors are obscured by the data point. See Table 7 for corresponding ANOVAs.

Table 7: Summary of ANOVAs comparing the relative growth rates of fourth (RGR₄) and fifth (RGR₅) instar *P. canadensis* from Alaska and Michigan on three hosts at four temperatures. Corresponds to data in Fig. 10.

Source	df ^b	F statistics ^a							
		12°		18°		24°		30°	
		RGR ₄	RGR ₅	RGR ₄	RGR ₅	RGR ₄	RGR ₅	RGR ₄	RGR ₅
Population	1	14.10***	11.69**	1.44	20.58***	2.10	0.26	0.42	4.67*
Host	2	2.40	2.89	7.18*	1.02	0.26	4.43*	5.79**	5.43**
Pop. x Host	2	0.02	3.00	0.10	0.11	6.10**	1.50	13.08***	4.83*
Family(Pop.)	17-28	0.76	2.14	0.56	0.37	0.91	1.37	2.58*	1.68

^a. The F test denominator for Population was MS_{Family(Pop.)}; other terms were tested over MS_{error}.

^b. Error degrees of freedom equalled 10-45.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

were growing significantly faster than Alaskan larvae at 30° (mean $\pm$ SE = 0.533 ± 0.043 versus 0.279 ± 0.054 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$, $P = 0.0009$). Of the remaining five comparisons at 30°, the populations grew at similar rates in two comparisons, and Alaskan larvae tended to grow faster in three comparisons (significantly so in both 1989 comparisons, $P < 0.001$; Figure 10).

Dry mass and nitrogen budgets

Population comparisons of dry mass consumption rates and conversion efficiencies were summarized through analysis of two most disparate trials: fourth instars on *Populus tremuloides* in 1988, and fifth instars on *Populus tremuloides* in 1989 (upper left and lower right in Figure 10). At 12° in 1988 (Figure 11), the high growth rates of Alaskan larvae relative to Michigan larvae was attributable to 44% higher consumption rates ($\text{RCR} \pm \text{SE} = 0.85 \pm 0.07$ versus 0.59 ± 0.03 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$, $P < 0.05$); neither apparent digestibility (AD) nor the efficiency of conversion of digested matter (ECD) differed between populations at 12° (note that $\text{RGR} = \text{RCR} \cdot \text{AD} \cdot \text{ECD}$). At 30°, the reduced growth of Alaskan larvae relative to Michigan larvae was due to elevated respiratory expenses leading to a 42% reduction in the efficiency with which digested matter was converted into new larval tissue ($\text{ECD} \pm \text{SE} = 0.28 \pm 0.05$ versus 0.48 ± 0.06 mg/mg for AK and MI respectively, $P < 0.05$); in this trial, neither consumption rate nor apparent digestibility differed between populations at 30° (Figure 11).

In trials where the growth rate of Alaskan larvae remained higher than Michigan larvae across all temperatures (e.g., fifth instars in 1989), it was due to generally elevated consumption rates (average of 40% higher in Alaskan larvae,

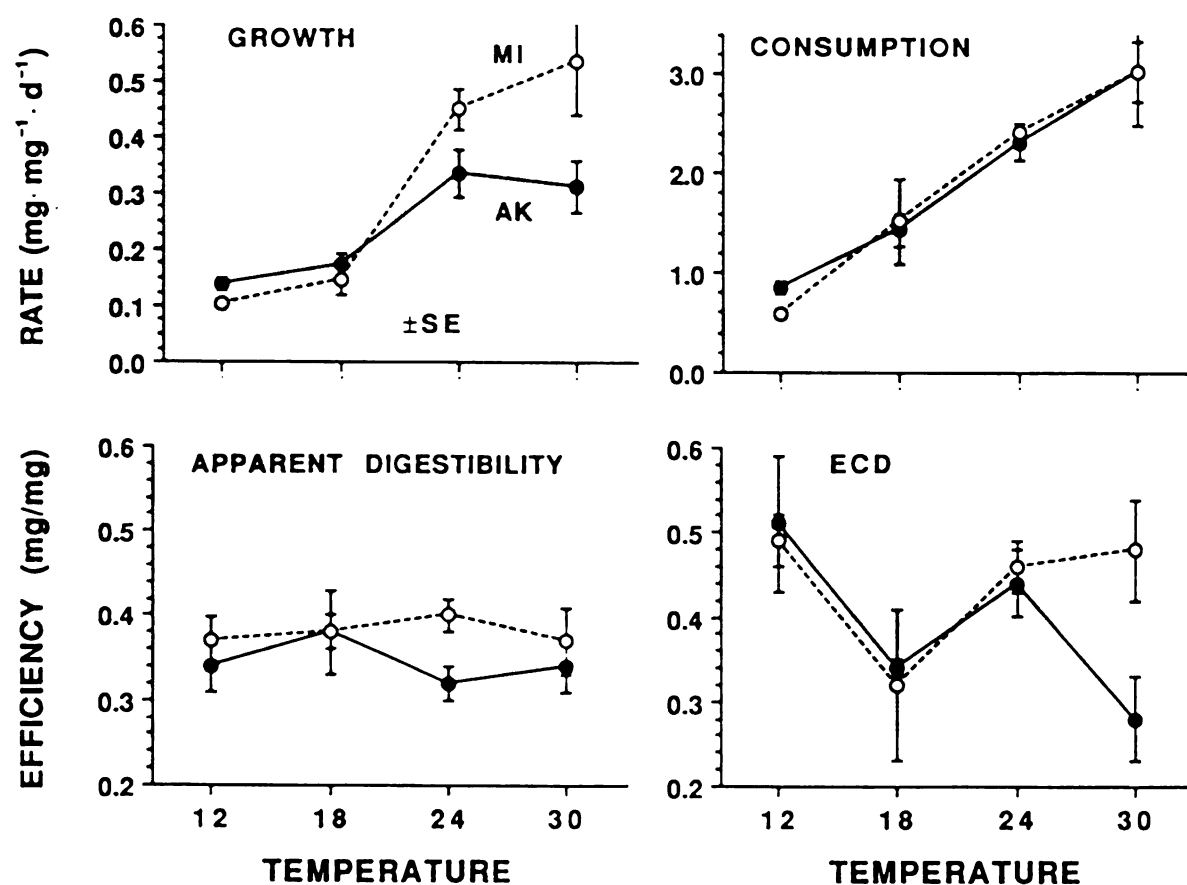


Figure 11: Consumption rate, apparent digestibility, and growth efficiency (ECD, growth/assimilation) of *P. canadensis* larvae from Alaska (solid lines) and Michigan (dashed lines) as a function of temperature. Corresponding growth rates appear in middle left of Fig. 10 (fourth instars feeding on *Populus tremuloides* in 1988). Some standard errors are obscured by the data point.

$P < 0.001$ at each temperature, Figure 12). Digestive efficiency (AD) did not differ between populations so assimilation rates also averaged 40% higher in Alaskan larvae (Figure 12). Although respiratory expenses (= average daily metabolic rate, = assimilation rate - growth rate) averaged 35% higher in Alaskan larvae, the higher assimilation rate was more than enough to compensate, and larvae maintained higher growth rates at all temperatures (Figure 12). Because population differences in metabolic rate balanced population differences in assimilation rate, there were no population differences in growth efficiency (= ECD = growth/assimilation) (Figure 12).

A powerful interaction between temperature and host quality was revealed in comparing the temperature responses of Alaskan *P. canadensis* on three different host species (Figure 13). On *Populus balsamifera*, fifth instar growth rate increased linearly with temperature from 12 to 30° ($Q_{10} = 2.16$). On *Populus tremuloides*, where consumption rates were indistinguishable from *P. balsamifera*, growth rate increased from 12 to 24°, then remained constant from 24 to 30°. The higher growth rates on *P. balsamifera* compared to *P. tremuloides* at 18 and 30°, were due to lower metabolic rates ($\text{ADMR} \pm \text{SE}$ at 30° = 0.57 ± 0.08 versus $0.91 \pm 0.03 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$). Apparent digestibility, and therefore assimilation rate, was equal or lower on *P. balsamifera* compared to *P. tremuloides*, so ECD, (= $(\text{RAR} - \text{ADMR})/\text{RAR}$), was higher (Figure 13). On *Betula resinifera*, growth rate was wholly insensitive to temperature ($Q_{10} = 1.16$ from 12 to 30°), even though consumption rate increased steadily with temperature ($Q_{10} = 1.68$) (Figure 13). Consumption did not, however, increase as steeply with temperature as on the other hosts (compare to RCR $Q_{10} = 2.21$ on *Populus balsamifera*). The apparent

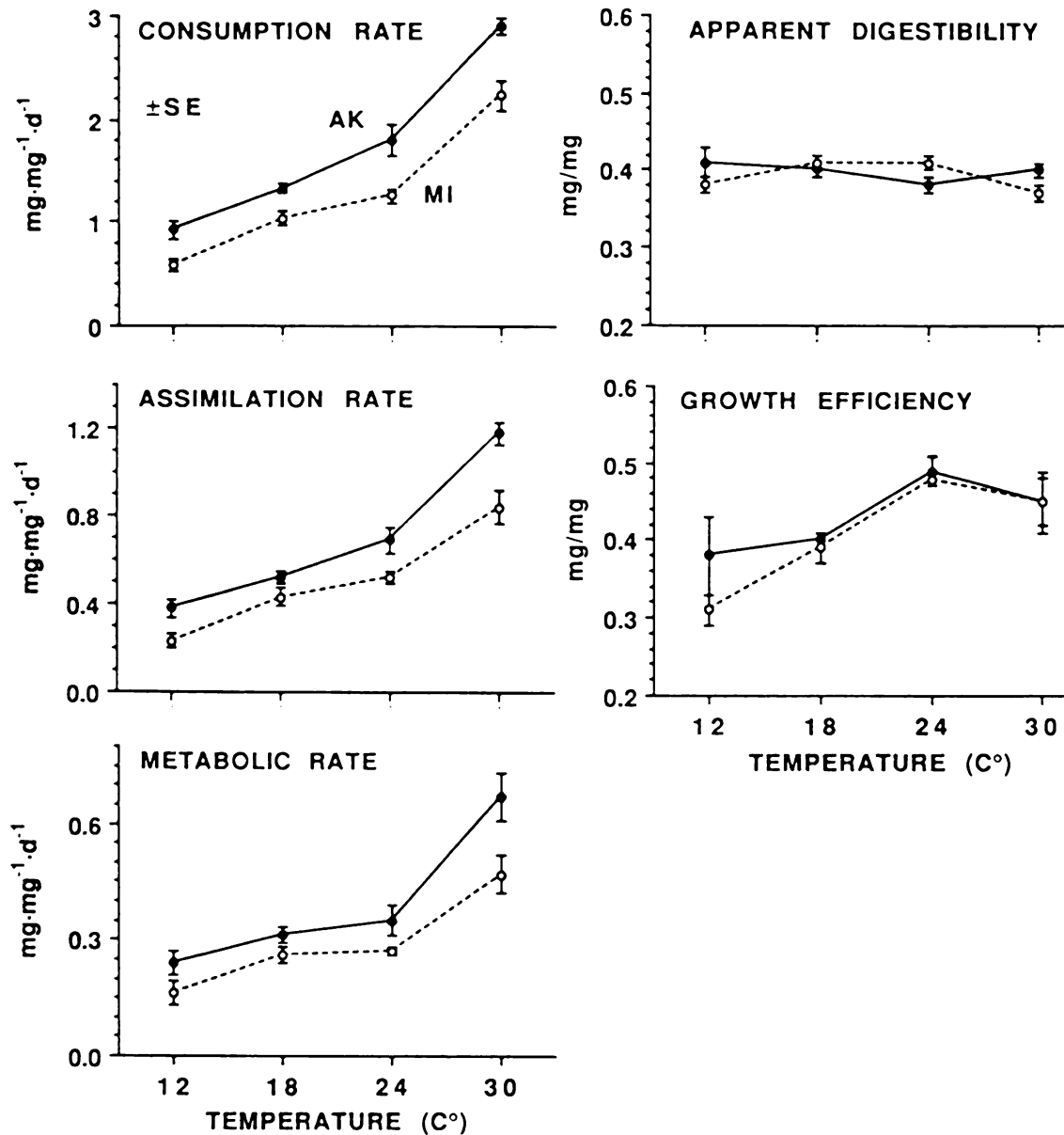


Figure 12: Consumption rate, assimilation rate, metabolic rate (ADMR), apparent digestibility, and growth efficiency (ECD, growth/assimilation) of *P. canadensis* larvae from Alaska (solid lines) and Michigan (dashed lines) as a function of temperature. Corresponding growth rates appear in lower right of Fig. 10 (fifth instars feeding on *Populus tremuloides* in 1989). Some standard errors are obscured by the data point.

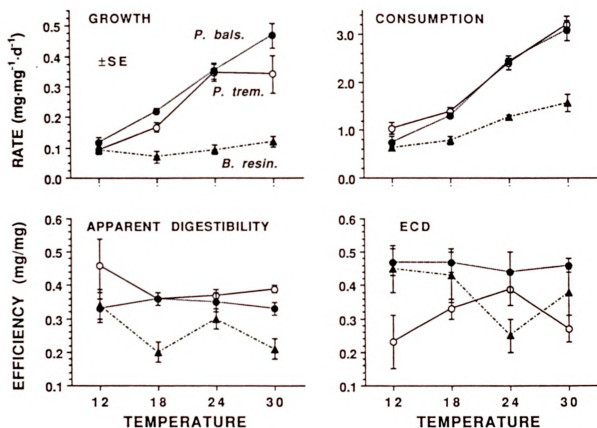


Figure 13: Growth rate, consumption rate, apparent digestibility, and growth efficiency (ECD, growth/assimilation) of *P. canadensis* fifth instars feeding on three hosts as a function of temperature. Hosts were *Populus balsamifera* (dotted lines), *P. tremuloides* (solid lines), and *Betula resinifera* (dashed lines). Some standard errors are obscured by the data point.

digestibility of *Betula* was low (least square mean $\pm$ SE = 0.26 ± 0.02) and relatively insensitive to temperature (Figure 13). Consequently, assimilation rate on *Betula* was low (RAR $\pm$ SE at 30° = $0.34 \pm 0.06 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$), and the arithmetic difference between assimilation rate and metabolic rate (= growth rate), did not change from 12 to 30°C even with a 2.5 fold increase in consumption rate.

There were no apparent differences in the nitrogen use efficiency of *P. canadensis* populations from Alaska and Michigan (Table 8; NUE least square means $\pm$ SE = 0.67 ± 0.02 versus $0.69 \pm 0.02 \text{ mg/mg}$ for Alaska and Michigan respectively; $F_{1,28} = 1.34$, $P = 0.26$). As with dry mass conversion efficiencies (AD and ECD), nitrogen use efficiency was relatively insensitive to temperature (Table 8; $F_{3,44} = 1.94$, $P = 0.14$). However, there were large effects of host species (Table 8; $F_{2,44} = 66.71$, $P < 0.0001$). Nitrogen use efficiency may have been somewhat less on *Populus balsamifera* than on *P. tremuloides* (least square means $\pm$ SE = 0.61 ± 0.02 versus 0.67 ± 0.02 , $P = 0.063$), but it was much less on *Betula* (0.36 ± 0.02) than on either *Populus* species ($P < 0.0001$). Leaf nitrogen content (% dry mass) was lower in *Betula* foliage (mean $\pm$ SE = $2.10 \pm 0.13\%$) than in *P. tremuloides* ($2.67 \pm 0.11\%$) or *P. balsamifera* ($2.44 \pm 0.10\%$), and larval dry mass consumption rates were also lower (Figure 13). Consequently, nitrogen consumption rates (NCR) at 30° were 2.3 times higher on *P. balsamifera* than on *Betula* (75.4 versus $33.0 \text{ mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$), and because of the reduced nitrogen use efficiencies on *Betula*, nitrogen accumulation rate (= NCR $\cdot$ NUE) at 30° was 4.1 times higher than on *Betula* (47.5 versus $11.6 \text{ mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$).

Table 8: Nitrogen use efficiency (NUE) of fifth instar *P. canadensis* from Alaska and Michigan feeding at four temperatures on three hosts (*Populus tremuloides*, *Populus balsamifera*, and *Betula resinifera*). $N = 4-5$ larvae (3-5 families) in each treatment combination (SE in parentheses).

Host	Nitrogen Use Efficiency							
	12°		18°		24°		30°	
	AK	MI	AK	MI	AK	MI	AK	MI
<i>P. tre.</i>	0.71 (0.04)	0.78 (0.03)	0.59 (0.05)	0.67 (0.07)	0.67 (0.02)	0.71 (0.03)	0.70 (0.03)	0.64 (0.04)
<i>P. bal.</i>	0.59 (0.03)		0.61 (0.04)		0.62 (0.04)		0.63 (0.04)	
<i>B. res.</i>	0.33 (0.04)		0.31 (0.05)		0.44 (0.04)		0.35 (0.04)	



Molting physiology

The fourth molt (Molt₄) required 14-50% more time for Michigan larvae than for Alaskan larvae (Figure 14). Population differences were significant at 12° (least square means $\pm$ SE = 8.13 ± 0.39 versus 6.58 ± 0.38 d for Michigan and Alaska respectively), at 18° (3.12 ± 0.28 versus 2.08 ± 0.25 d), and at 30° (1.18 ± 0.09 versus 0.86 ± 0.11 d) (Table 9). Molt duration was highly sensitive to temperature (required 7.2 times longer at 12° than at 30°, Figure 14), but was relatively insensitive to host (no significant host effects or population x host interactions in Table 9).

The fifth molt (Molt₅, Figure 15) required 50% longer for Michigan larvae than for Alaskan larvae at 12° ($P < 0.0025$), 42% longer at 18° ($P = 0.02$), 32% longer at 24° ($P < 0.0025$), and actually required slightly less time at 30° ($P = 0.18$) (population effect and temperature x population interaction in Table 10). The duration of the fifth molt was very temperature sensitive between 12 and 18° (Q_{10} of Molt₅ = 5.38 and 5.92 for Alaska and Michigan respectively), temperature insensitive from 18 to 24° (Q_{10} = 1.02 and 1.21), and moderately temperature sensitive from 24 to 30° (Q_{10} = 1.70 and 2.92). The fifth molt required a surprisingly long time (up to 18 d for Michigan larvae at 12°), but this did not appear to be an artifact of the way it was estimated. The interval from when late fifth instars cleared their gut to when they completed ecdysis to enter the pupal stage averaged 55% as long as Molt₅ (range of 41 to 69% in eight treatments). This interval must be an underestimate of the contribution of the fifth molt to total development time. In other experiments (at 24°), repeated weighings of

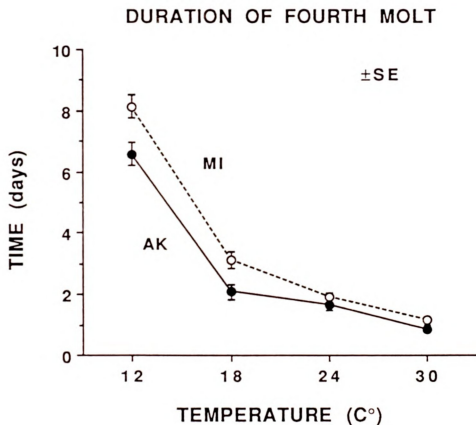


Figure 14: The duration of the fourth molt (Molt₄, fourth instar to fifth instar) in *P. canadensis* from Alaska (solid line) and Michigan (dashed line) as a function of temperature. Experiments included larvae reared on *Populus balsamifera* and *P. tremuloides* in 1988 and *P. tremuloides* in 1989. Some standard errors are obscured by the data point. See Table 9 for corresponding ANOVAs.



Table 9: Summary of ANOVAs comparing the duration of the fourth molt (Molt₄) in *P. canadensis* from Alaska and Michigan on three hosts at four temperatures. Corresponds to data in Fig. 14.

Source	df ^b	F statistics ^a			
		12°	18°	24°	30°
Population	1	7.53*	7.81**	1.23	4.62*
Host	2	0.18	1.55	0.70	2.51
Pop. x Host	2	2.03	0.12	0.25	1.46
Family(Pop.)	19-25	0.35	0.53	0.96	1.51

^a The *F* test denominator for Population was MS_{Family(Pop.)}; other terms were tested over MS_{error}.

^b Error degrees of freedom equalled 10-37.

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.0001

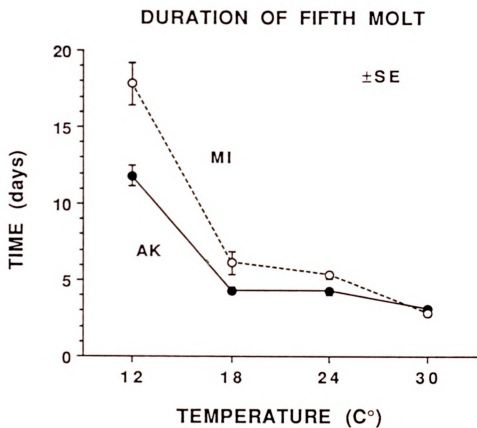


Figure 15: The duration of the fifth molt (Molt₅, fifth instar to pupa) in *P. canadensis* from Alaska (solid line) and Michigan (dashed line) as a function of temperature. Larvae were reared on *P. tremuloides* in 1989. Some standard errors are obscured by the data point. See Table 10 for corresponding ANOVA.

Table 10: ANOVA comparing the duration of the fifth molt in *P. canadensis* larvae from Alaska and Michigan at four temperatures (square root transformed data). Corresponds to data in Fig. 15.

SOURCE	DF	MS · 10 ³	F ^a
Temperature	3	11565	189.15***
Population	1	1313	20.60***
Temp. x Pop.	3	502	8.21***
Family(Pop.)	17	64	1.04
Error	64	61	

^a. The *F* test denominator for Population was MS_{Family(Pop.)}; all others were tested over MS_{error}.

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.0001

larvae throughout the fifth instar indicated nearly exponential growth for the first several days, then 1-2 days longer when larvae continued to feed but grew very little (unpublished data). My estimates of Molt₅ (Figure 15) attribute the dramatically slowed growth in this stage to the early physiological demands of molt and preparations for metamorphosis to the pupal stage.

Pupal mass

Michigan caterpillars produced significantly larger pupae than Alaskan caterpillars (Figure 16; $F_{1,88} = 11.89$, Table 11). There were also strong main effects of temperature and sex on pupal mass (Table 11). Pupal mass averaged 25% less at 12° than at 18°, and female pupae averaged 9% larger than male pupae (Figure 9). Population differences were less pronounced in males and at low temperatures.

Survival

Populations did not differ in their survival across temperatures or at any one temperature (Table 12; chi-square for population effects = 0.12, $P = 0.73$, df = 1; chi-square for population x temperature interaction = 2.28, df = 3, $P = 0.52$). However, survival was influenced by temperature (chi-square = 39.77, df = 3, $P = 0.0001$). Survival to the reproductive adult stage was only about 25% among animals reared through the final two instars at 12°C, primarily due to low pupal survival (Table 12). Overall survival was highest at 18° (average of 91%), and intermediate at 24 and 30° (average of 60%).

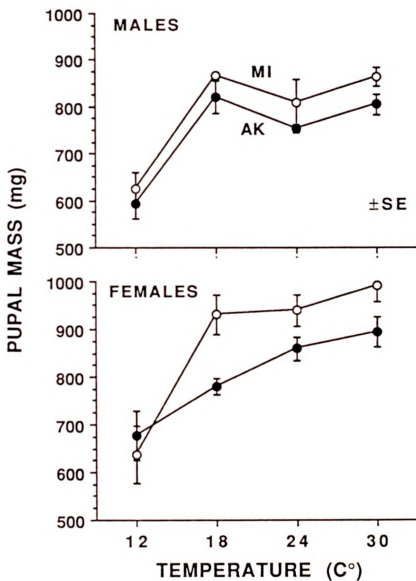


Figure 16: Pupal mass of male and female *P. canadensis* from Alaska (solid line) and Michigan (dashed line) as a function of temperature. Larvae were reared on *P. tremuloides* in 1989. See Table 11 for corresponding ANOVA.

Table 11: ANOVA comparing the pupal mass of male and female *P. canadensis* from Alaska and Michigan reared at four temperatures. Corresponds to data in Fig. 16.

Source	df	MS · 10 ⁻¹	F ^a
Temperature	3	19372	36.12***
Population	1	6375	11.89***
Sex	1	9213	17.18***
Temp. x Pop.	3	666	1.24
Temp. x Sex	3	1140	2.13
Pop. x Sex	1	250	0.47
Temp. x Pop. x Sex	3	458	0.85
Error	88	536	0.85

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

Table 12: Survival of *P. canadensis* from Alaska and Michigan reared at four temperatures in 1989. Larval survival is the percentage of early fourth instar larvae beginning the experiment that successfully pupated. Pupal survival is the percentage of pupae that survived 9 mo of diapause and emerged as adults the following spring. Cumulative survival is the product of larval and pupal survival.

	12°			18°			24°			30°		
	AK	MI		AK	MI		AK	MI		AK	MI	
Initial number	13	9		15	10		21	14		27	22	
Larval survival	77%	67%		93%	90%		81%	86%		81%	95%	
Pupal survival	33%	40%		100%	100%		83%	63%		63%	75%	
Total survival	25%	27%		93%	90%		63%	54%		51%	71%	

Development time

To develop from early fourth instars to pupae, *P. canadensis* in 1989 required 9.5 to 58 d depending on temperature and genotype (Figure 17). Michigan larvae required 4.9 times longer at 12° than at 30° (mean $\pm$ SE = 57.5 $\pm$ 2.4 versus 11.7 $\pm$ 0.6 d). Alaskan larvae were somewhat less temperature sensitive (required 4.0 times longer at 12° compared to 30°: 37.7 $\pm$ 2.6 versus 9.5 $\pm$ 0.3 d), and required 19-34% less time overall (larger percentage differences at low temperatures) (Figure 17). The temperature sensitivity of development time would have been even more pronounced if fifth instars at 12° had grown as large as at warmer temperatures (Figure 9). The fourth and fifth molts together accounted for 35 to 51% of development time (Figure 17). In both populations, molt made up the largest fraction of development time at 12°. The fourth instar required less time for Alaskan larvae than Michigan larvae partly because of higher relative growth rates (Figure 10) and partly because they began the instar at a slightly larger size ($W_{4i} \pm$ SD = 112 $\pm$ 54 versus 86 $\pm$ 42 mg; P = 0.006) (our estimates of time in the fourth instar assume that larvae began the instar at 90% of W_{4i}). The mass of larvae at the start of the fifth instar did not differ between populations ($W_{5i} \pm$ SD = 449 $\pm$ 151 versus 492 $\pm$ 170 mg for Alaska and Michigan respectively; P = 0.21), and only modestly (if at all) across temperatures ($F_{3,94}$ = 2.54, P = 0.061, maximum difference among 4 temperatures = 14%).

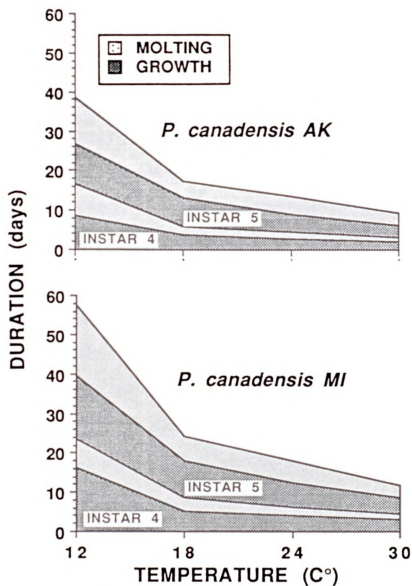


Figure 17: Development time from early fourth instars to pupae of *P. canadensis* from Alaska (upper) and Michigan (lower) as a function of temperature. Durations of the fourth instar, the fourth molt, the fifth instar, and the fifth molt, are indicated.

Basking success

I found only rare circumstances that allowed *P. canadensis* larvae in Alaska to elevate their body temperature much above ambient. Larvae were deliberately placed in high radiation microsites (maximally exposed south-facing branches) within a high-radiation site (a south-facing forest edge on a south-facing slope), yet in 145 caterpillar observations on 8 sunny days (7-12 and 18-20 August 1989, I never found a larva more than 4.5° above ambient air temperature (mean $\pm$ SD = $0.9 \pm 1.3^{\circ}\text{C}$). Generally the basking potential (measured by black body temperatures) was not very high (maximum of 11.0 °, mean $\pm$ SD = $2.7 \pm 2.5^{\circ}$) because of breezes and/or shading from nearby leaves. Larvae seldom moved. Most larvae remained within 20 cm of where I placed them throughout the experiment (3-5 d). I did not observe larvae moving to high radiation microsites even though such sites were often only cm away. On 22-23 August, in an attempt to maximize basking potential, I placed larvae individually on freshly cut branches (ca. 50 cm high) positioned on a lawn in full sunlight but protected from the breezes. Under these conditions, larvae were able to elevate body temperatures as much as 10-14°C above air temperatures. Across a range of environmental conditions, the basking success of larvae was about 50% of basking potential as measured by black body temperatures (Figure 18). There was no evidence for population differences in basking success (Figure 18). The body temperatures of Alaskan larvae did not differ from that of Michigan larvae when exposed to the same field conditions (Figure 19).

Growth rates of larvae in the field and laboratory were indistinguishable when fed the same foliage and subjected to the same mean daily air temperature

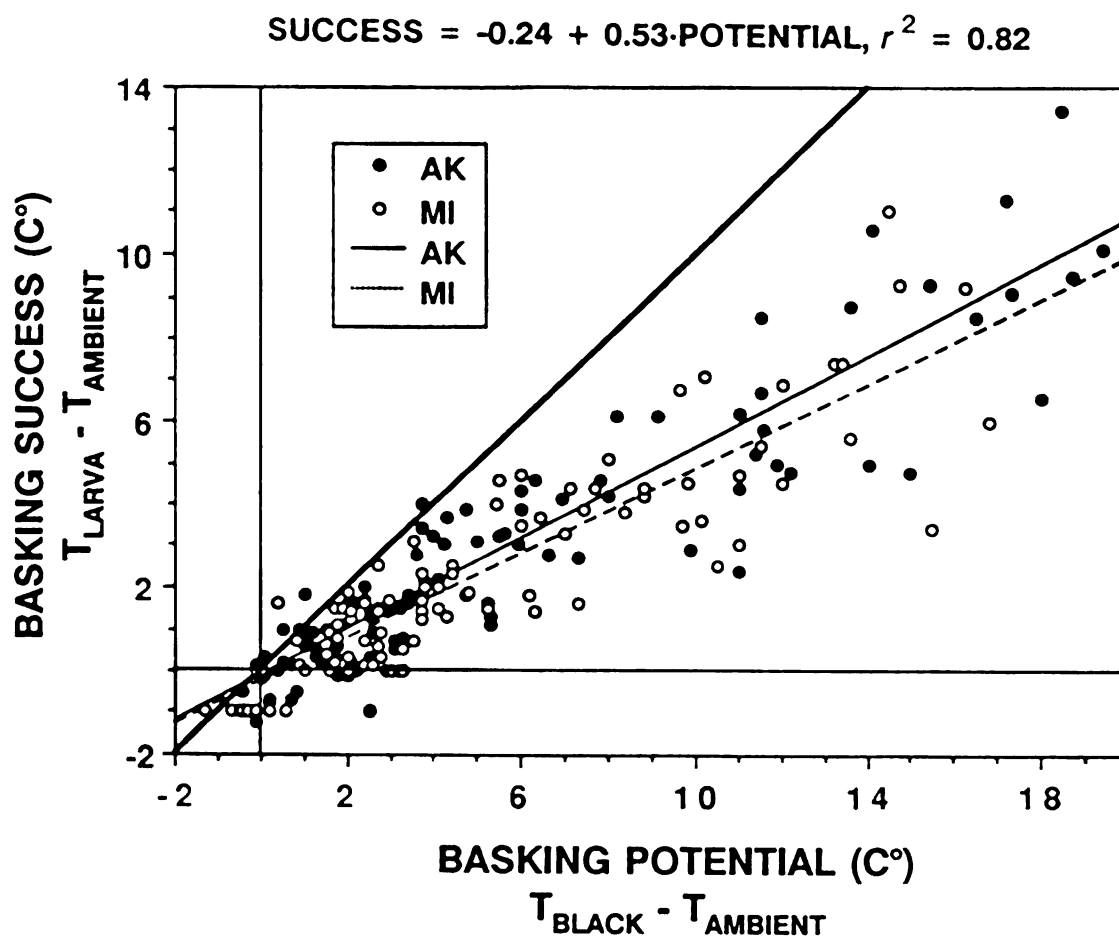


Figure 18: Basking success as a function of basking potential in fifth instar *P. canadensis* from Alaska (closed circles) and Michigan (open circles). Basking success equals the difference between larval temperature and ambient air temperature; basking potential equals the difference between the temperature of a high absorption "black body" and ambient air temperature. The heavy solid line shows the points of equality between success and potential. The pooled regression function is indicated (regressions fit to each population separately did not differ: $F_{2,112} = 1.12$, $P > 0.20$). Note that most points to the right of 6°C on the x axis represent observations of larvae on detached branches placed in full sunlight and protected from the wind.

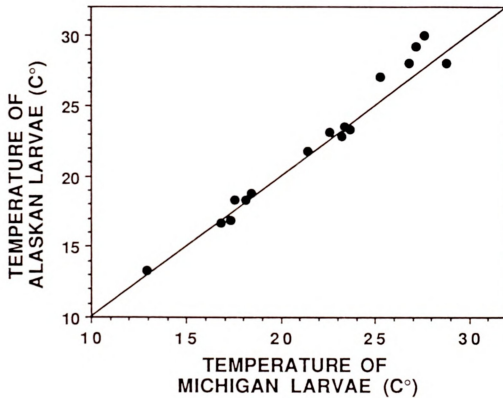


Figure 19: The temperatures of Alaskan and Michigan larvae when allowed to move and bask naturally under field conditions near Fairbanks, AK. Each point is the mean of 5-10 larvae from each population. Standard deviations ranged from 0.5°C at low temperatures to 4°C at high temperatures.

(RGR $\pm$ SE = 0.157 ± 0.006 versus 0.143 ± 0.014 mg $\cdot$ mg⁻¹ $\cdot$ d⁻¹ for field and laboratory larvae respectively; $t_{12} = 0.79$, $P > 0.20$). Over the three days of this experiment, daily minimum and maximum air temperatures in the field were: 10.5 and 24°; 10 and 20°; 8 and 17°. Larvae in the laboratory were held at constant temperatures of 17° on day 1, 15° on day 2, and 12.5° on day 3.

Discussion

Adaptive modifications of temperature physiology

P. canadensis populations from Alaska and Michigan have diverged in their larval temperature physiology (Figures 10-12, 14-15). Alaskan larvae are capable of growing and molting more rapidly at the low temperatures (Figures 8-9) they typically encounter. Intraspecific differentiation in insect temperature responses has also been reported in milkweed bugs (Baldwin and Dingle 1986), *Drosophila* larvae (Barnes et al. 1989), and Colorado potato beetles (Tauber et al. 1988). No differences were found in the diamondback moth (Sarnthoy et al. 1989).

Adaptation of *P. canadensis* to short cool summers has been accomplished in part through a general elevation of metabolic activity at all temperatures. Average daily metabolic rates were 36% higher in the Alaskan population (Figure 12). Consequently, Alaskan larvae had very similar metabolic rates at their average environmental temperature (0.268 mg $\cdot$ mg⁻¹ $\cdot$ d⁻¹ at 14.4°) as Michigan larvae at theirs (0.261 mg $\cdot$ mg⁻¹ $\cdot$ d⁻¹ at 18.8°) (linear interpolation of ADMR data in Figure 12). Probably as a result of this "metabolic compensation" (Scholander et al., 1953), Alaskan caterpillars were able to consume more, grow faster, and molt more quickly at low temperatures than Michigan caterpillars. The metabolic

rate of diapausing pupae was similarly elevated in the Alaskan population (35% higher rate of CO₂ production than the Michigan population; Kukal et al. 1992).

There was limited evidence for concomitant reductions in larval growth performance at high temperatures. Alaskan fourth instars feeding on *Populus tremuloides* in 1988 grew only half as fast at 30° as Michigan fourth instars, even though they grew 34% faster at 12° (Figure 10). The low growth rate of Alaskan larvae at 30° was due to high respiration rates (Figure 11), suggesting an energetic tradeoff between adaptation to low temperatures and performance at high temperatures. However, such tradeoffs were far less apparent than I would have predicted.

In 1989, Alaskan larvae grew faster (Figure 10) and survived as well (Table 12) at 30° as Michigan larvae, even though the Alaskan population never encounters sustained temperatures that warm (Figure 9). The apparent "cost" of low temperature adaptation (high metabolic rates) was still evident in these trials (Figure 12), but high consumption rates with equivalent digestive efficiency allowed the Alaskan larvae to grow faster nonetheless. As might be expected given their high maintenance metabolism, Alaskan larvae were more sensitive to host quality than Michigan larvae, especially at high temperatures. The variance among experiments in mean relative growth rate at 30° was 10-33 times higher for Alaskan compared to Michigan larvae (fourth instar SD = 0.177 versus 0.056 for Alaska and Michigan respectively; fifth instar SD = 0.097 versus 0.017; data from Figure 10). Yet this represents only a limited spectrum within generally high quality hosts; *Populus balsamifera* and *P. tremuloides* ranked one and two in a comparison of fifth instar growth rates across nine host species (Chapter 1, Figure

4). Alaskan caterpillars, as a result of their elevated metabolism, may be less able to exploit hosts of low nutritional quality. Shallow growth-temperature responses, such as on *Betula resinifera* (Figure 13), are expected to be more common as a consequence of low temperature adaptation. Even on high quality hosts, the growth rates of Alaskan larvae were less temperature sensitive than those of Michigan larvae: fifth instar Q_{10} 's of 1.99-2.16 versus 2.27-2.58 (Figure 10, 12-30°).

In general, biochemical adaptation to low temperatures can occur through two routes (Hochachka 1973, pp. 212-270): (1) increased concentrations of enzymes, which increase reaction rates at all temperatures, or (2) qualitative changes in enzyme structure that result in higher substrate affinity at low temperatures (changes in the response of K_m to temperature). The generally elevated metabolic rate of Alaskan *P. canadensis* and other high latitude animals (Scholander et al. 1953, Hochachka 1973, Block 1990) is an expected correlate of increased rates of enzyme production and turnover. Changes in enzyme structure that enhance metabolic performance at low temperatures are expected to compromise performance at high temperatures (Powers 1987), which may explain the temperature x population interaction in the duration of the fifth molt (Figure 15, Table 10). That a similar pattern was not seen in the fourth molt (where Alaskan larvae molted faster at 30° as well as 12°; Figure 14, Table 9), suggests changes of the K_m -temperature function in enzymes uniquely associated with the larva-to-pupa metamorphosis (as opposed to molt *per se*). In general, there was little evidence for shifts in the temperature optima of physiological systems. Consumption rates, assimilation efficiencies, molting rates, survival, and usually

growth rates, remained as high or higher in the Alaskan population, even at the ecologically extreme temperature of 30°. Distinct temperature optima in these same processes are often apparent over only a few degrees centigrade in aquatic insects (Sweeney and Vannote 1978, Grafius and Anderson 1979, Vannote and Sweeney 1980), presumably because their thermal environment is much less variable. Qualitative changes in enzyme structure may typically be of less importance in the thermal adaptation of terrestrial compared to aquatic insects.

Conserved attributes

Although Alaskan larvae had higher development rates at 12° than their Michigan counterparts, there was little evidence for changes in developmental thresholds. The minimum temperature at which a biological process occurs (T_0) can be estimated by linear extrapolation from the temperature response of developmental rate (Arnold 1959). In four experiments, the temperature responses of relative growth rate were sufficiently linear to allow reasonable extrapolation (fourth and fifth instars on *Populus balsamifera* in 1988 and *P. tremuloides* in 1989; Figure 10). Estimates of $T_0 \pm SD = 6.8 \pm 0.5$ and 7.6 ± 1.9 for Alaskan and Michigan caterpillars respectively ($t_7 = 0.80$, $P > 0.20$, paired t -test). Developmental thresholds for molting were also similar between populations (Molt₄ $T_0 = 9.6^\circ$ and 9.5° for Alaska and Michigan; Molt₅ rates were too non-linear for extrapolation), but tended to be higher than thresholds for growth (see also Ayres and MacLean 1987). Estimates of T_0 for *Papilio glaucus*, a southern sister species, range from 8-12° (Scriber and Lederhouse 1983, Ritland and Scriber 1985, Grossmueller and Lederhouse 1985). Developmental

thresholds of swallowtail caterpillars do not differ very much from Florida to Alaska, even across species.

The probability of *P. canadensis* successfully completing development in Alaska might be enhanced if larvae could regularly elevate body temperatures through basking. Lepidopteran larvae can attain temperatures 5-20° above ambient by adjusting posture and orientation, exploiting thermal heterogeneity within the environment, and minimizing convective heat losses (Casey 1976, Casey et al. 1988, Kukal et al. 1988, Weiss et al. 1988). *P. canadensis* and *P. glaucus* spend most of their time sitting on silken mats that they construct on the adaxial surface of individual leaves. Besides providing a secure footing, these mats can retard convective heat loss and contribute to radiant energy gain by turning the leaf into a parabolic reflector (Grossmueller and Lederhouse 1985). *P. glaucus* larvae also exhibit positive phototaxy within the host canopy which tends to put them in high-radiation microsites. I hypothesized that these behaviors would be particularly well developed in Alaskan caterpillars, but in fact, basking efficiency (temperature elevation as a fraction of basking potential), and average caterpillar temperatures were no different in the two populations (Figures 18-19). The opportunities for basking appear to be very rare for Alaskan swallowtails under natural conditions, apparently because of generally low light intensity at high latitudes.

Egg size and adult size

As a consequence of different provisioning strategies by their mothers, Alaskan caterpillars begin larval development 36% larger than Michigan

caterpillars (neonate mass = 1.33 versus 0.98 mg, Chapter 1), and because larval growth is nearly exponential, this relative advantage is retained throughout larval development (Figure 3). Given equal growth rates, Alaskan *P. canadensis* could attain the same pupal mass with less larval development time due solely to the difference in hatching mass.

Alaskan *P. canadensis* further shorten development time by terminating feeding and initiating pupation at a smaller mass. If we disregard the 12° treatment, where pupae were very small and survival very low, female Alaskan pupae averaged 814 mg compared to 899 mg for Michigan pupae (9% difference; Figure 9). In a different experiment (Figures 6-7), Alaskan pupae averaged 8% smaller. Field captured butterflies reveal the same pattern. Female forewing length averaged 43.7 ± 0.80 mm across 3 summers in Alaska and 46.6 ± 0.88 mm across 7 summers in Michigan (means $\pm$ SD of yearly means, each based on 10-20 butterflies). Pupal mass is related to forewing length as $M_{\text{pupa}} = -528 + 31.05 \cdot \text{WING}$ ($r^2 = 0.55$, unpublished data), which indicates that wild female pupae averaged 829 mg in Alaska versus 919 mg in Michigan (10% smaller in Alaska).

Comparing the ecological worth of temperature adaptations

Four differences between Alaskan and Michigan populations of *P. canadensis* are interpretable as adaptations to short cool subarctic summers: increased egg mass, reduced adult size, enhanced molting abilities at low temperatures, and enhanced growth rates at low temperatures. To evaluate the contribution of these hypothesized adaptations, individually and *in toto*, to

swallowtail fitness in an Alaskan environment, I incorporated them into a temperature-driven development model that input 48 years of daily temperature records and predicted the proportion of swallowtails that would successfully complete development each year.

Parameter values are summarized in Appendix 2. The model evaluated five cohorts in each year, corresponding to phenologically early, average, and late eggs (neonate hatch = 240, 280, 320, 360, and 400 degree days). The mass of hatching neonates was set at 0.98 or 1.33 mg representing Michigan and Alaskan phenotypes respectively. The size threshold for female pupation was set at 899 or 814 mg fresh pupal mass (Figure 16). Molting rates for the penultimate and final molt were calculated for each population as a function of temperature, using linear interpolation between measured temperatures and from 12° to a developmental threshold of 9.5° (based on data in Figures 14-15). Growth rates were estimated under two scenarios of host quality: a "good host" (based on *Populus tremuloides* in 1988) and an "excellent host" (based on *P. tremuloides* in 1989). Relative growth rates during the penultimate (RGR_4) and final instars (RGR_5) were calculated for each population as a function of temperature, using linear interpolation between measured temperatures and from 12° to a developmental threshold of 7.25° (based on *Populus tremuloides* data in Figures 10).

Two host quality scenarios were used to model growth during instars 1-3 (Stage 1), but the same functions were used for all swallowtail phenotypes. Growth rate at 24° during instars 1-3 was set at $0.270 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ for the good host scenario (= growth rate for this stage on *Populus tremuloides* in 1988; Figure

3) and $0.310 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ for the excellent host scenario (= highest growth rate among nine hosts in 1988; Figure 3). Stage 1 temperature responses assume the same Q_{10} s as fourth instar larvae feeding on *Populus tremuloides* in 1988 (good host) or 1989 (excellent host).

Each cohort in each year was represented by hypothetical neonates that hatched at a specified degree day, then developed through five growth stages, at rates dictated by prevailing temperatures and stage-specific temperature responses, until they reached the overwintering pupal stage or the season ended. When they were not molting, larval mass was incremented daily as $M_{t+1} = M_t \cdot e^{\text{RGR} \cdot t}$ where M_t was the mass at the start of the day and relative growth rate (RGR) was estimated as a function of temperature. When the average daily temperature was less than the developmental threshold, but the maximum was not, development was based on proportion of the day when temperatures were above the threshold (sine function follows Watanabe 1978). Larvae grew from their hatching mass to 99 mg (Stage 1), then to 369 mg at fourth instar growth rates (Stage 2). Then growth ceased until completion of the fourth molt (Stage 3). With their mass depreciated by exuvial and respiratory losses during molt, larvae resumed growth at fifth instar rates until they reached a size threshold for pupation (Stage 4). Successful cohorts completed feeding prior to leaf senescence (15 September) and became cold-tolerant diapausing pupae (completed Stage 5) before the onset of winter (1 October).

Under the good host scenario, the Michigan phenotype was predicted to go extinct (no cohorts completed development) in 31 of 48 years under the Alaskan climate (Figure 20). Changing egg size to that of the Alaskan phenotype

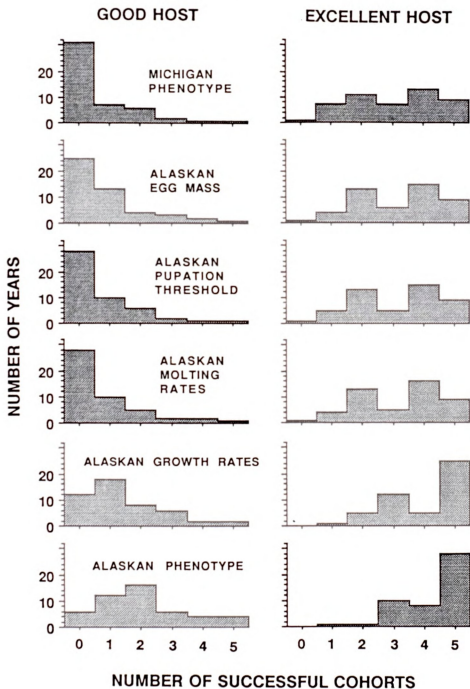


Figure 20: The predicted developmental success of various *P. canadensis* phenotypes under two scenarios of host quality during 48 seasons in interior Alaska. Possible developmental success in each year ranged from 0 successful cohorts (extinction) to 5 successful cohorts (no mortality due to incomplete development). Under each host quality scenario, I began with the Michigan phenotype (top), then changed egg mass, pupation threshold, molting rates, and growth rates (one at a time) to the condition of the Alaskan phenotype. Bottom figures show results when the four Alaskan adaptations were combined.

eliminated 6 extinctions, changes in molting physiology and pupation threshold each eliminated 3 extinctions, and changes in fourth and fifth instar growth rates eliminated 19 extinctions. The Alaskan phenotype, which incorporates all these changes, was predicted to go extinct in only 6 of 48 years. Under the excellent host scenario, extinctions were rare, but even the Alaskan phenotype still had failed cohorts in 20 of 48 years (Figure 20). This indicates that climate continues to exert hard selection (*sensu* Wallace 1968) even on the adapted phenotype under the best available conditions. Estimating the relative size of the cohorts allowed an assessment of this selection.

On a good host, only 14% of the Michigan phenotypes, compared to 42% of the Alaskan phenotypes, were predicted to reach pupation in the time available (Table 13). Thus the estimated fitness of the Alaskan phenotype was about 3.0 times higher (42/14). On an excellent host, larval success was higher (65% versus 89%), but selection against the Michigan phenotype would still be very strong (relative fitness of the Alaskan population = 1.39). Under both host quality scenarios, changes in growth rate made the largest contribution to improved fitness. Changes in egg mass made the second largest contribution (on a good host, increased egg mass resulted in a fitness of 1.21 relative to the Michigan phenotype). Changes in the size at pupation had the smallest effect on fitness. All the hypothesized adaptations of the Alaskan population contributed to successful development during Alaskan summers.

Table 13: A comparison of the value in enhanced developmental success of adaptations exhibited by Alaskan *P. canadensis*. Percent larval success indicates the average proportion of larvae predicted to complete development during 48 seasons in Alaska (based on data in Figure 20 assuming cohort sizes of 12%, 32%, 27%, 18%, and 11% for cohorts 1-5^b). Alaskan attributes were introduced into the Michigan phenotype one at a time (egg size, pupation size, molting rates, and growth rates) and then simultaneously (Alaskan phenotype).

Phenotype	Good Host			Excellent Host		
	Percent larval success	Number of Extinctions ^b	Fitness relative to MI ^c	Percent larval success	Number of Extinctions ^b	Fitness relative to MI ^c
Michigan	14	31	1.00	65	1	1.00
MI with AK egg size	17	25	1.21	68	1	1.05
MI with AK pupation size	15	28	1.06	67	1	1.03
MI with AK molting rates	16	28	1.12	69	1	1.06
MI with AK growth rates	29	12	2.03	84	0	1.29
Alaskan	42	6	3.00	89	0	1.37

^a. Cohort sizes based on the emergence phenology of female butterflies in Alaska (median = 164 and 167 degree days in two years, 10 to 90% cumulative emergence = 137 to 209 degree days), and assuming: a butterfly mortality rate of 11% per 10 degree days; constant egg production per degree day among living butterflies; and 90 degree days from oviposition to hatch (unpublished data).

^b. Number of times in 48 years when even the earliest cohort failed to complete development.

^c. Average larval success relative to the Michigan phenotype.

Tradeoffs

Because smaller pupae produce smaller less fecund adults, there is clearly a tradeoff between the probability of completing development and fitness given successful development. Two days after adult eclosion, an Alaskan butterfly that weighed 812 mg as a pupa has matured 21 eggs compared to 26 eggs for the butterfly resulting from an 899 mg pupa (Lederhouse and Ayres, unpublished data). Thus the fitness cost of pupating smaller is about 20%, compared to an apparent benefit of only 3-6% (Table 13). The actual benefits of pupating smaller are underestimated if there is appreciable larval mortality due to predation risks. In Alaska, we have recorded fifth instar mortality rates of 27%/d in 1989, 2%/d in 1990, and 5%/d in 1991. Under 1989 temperatures, lowering the pupation threshold shortened the fifth instar of the middle cohort from 14.2 to 13.4 d. Given mortality rates of 27%/d, the probability of surviving the fifth instar was 1.28 times higher with the lower pupation threshold; daily mortality rates of 5% suggest an advantage of only 1.04. Average temperatures and predation risks seem inadequate to explain the reduced fecundity that accompanies lower pupation thresholds in Alaskan *P. canadensis*. I suggest that the occasional extreme conditions (e.g., a sequence of three unusually cold summers during 1947-1949, or an outbreak of predatory wasps in 1988-1989) exert disproportionately strong selection, especially on populations that rely on an annual life history (Wigley 1985).

By virtue of producing smaller eggs, Michigan butterflies can mature about 1.59 times as many eggs per day as equal sized Alaskan butterflies (Lederhouse and Ayres, unpublished data). Compare this cost to an estimated benefit of 1.21

based on average summer temperatures in Alaska (Table 13). The actual costs of producing larger eggs may not be as great if summer temperatures frequently limit flight more than they limit vitellogenesis (if oviposition rate is not limited by egg production), but this apparent discrepancy between costs and benefits again argues for the selective strength of occasional extreme conditions. Increased egg size eliminated 6 extinctions in 48 years on good hosts (Table 13).

Model Validation

There were no obvious discrepancies between reality and results from the development model. During 1987-1990, the predicted dates of cohort initiation overlapped and followed the actual times of butterfly flight, and matched the times when eggs were hatching in the field. In 1988, two wild larvae with known hatching dates (4 July and 8 July) survived until they were collected as fifth instars on 3 August (mass of 936 and 696 mg respectively). The actual development of both larvae fell between model predictions under the good host and excellent host scenarios (predicted masses = 97 and 1166 mg for the 936 mg larva). Careful validation requires more such comparisons, but few larvae survive that long in the field. Serious inaccuracies could arise if larval temperatures are typically higher than ambient due to basking (Lamb and Gerber 1985), if foraging larvae tend to select high quality leaves (Schultz 1983), or if growth at naturally fluctuating temperatures differs from growth at a constant temperature (Taylor and Shields 1990). Congruence between field and laboratory growth rates (see Results) suggest that none of these introduce serious errors for *P. canadensis* in interior Alaska. Some parameter estimates, particularly early instar temperature

responses (Stage 1) are not as robust as would be desirable, but this has little effect on comparisons among phenotypes (Table 13).

Northern distribution limits of *P. canadensis*

There is a dramatic effect of host quality on developmental success in Alaska: average larval success of 42% versus 89% for Alaskan larvae under good host versus excellent host scenarios (Table 13). Many potential hosts are worse than our good host scenario, and few are better. Growth performance on *Populus tremuloides* in 1988, the basis for the good host scenario, was among the highest of nine host species tested (Figures 4, 6). Our excellent host scenario was based on the same tree species in 1989, suggesting that such high quality foliage may not be available in all seasons. Interior Alaska contains many tree species on which *P. canadensis* can potentially produce viable adults (laboratory experiments in Chapter 1), but on which growth rates are too low to allow successful development of any but the earliest larvae in the warmest years. Consequently, climate may select for more discriminating oviposition behavior in Alaskan populations of *P. canadensis*. Species of *Salix* and *Betula* that are suitable hosts for *P. canadensis* given adequate degree days extend throughout the circumpolar regions well beyond the occurrence of *P. canadensis*. I hypothesize that northern distribution limits are a joint function of climate and host quality. As summers become shorter, the number of potential hosts that can be realized hosts becomes increasingly restricted until even the best hosts no longer allow development. If this is a general scenario, insect distributions may respond to

climate change (Mitchell et al. 1989) almost immediately, without requiring changes in host distribution.

Regional specialization allows *P. canadensis* to maintain a broader climatic distribution than would otherwise be possible. It seems unlikely that a population with Michigan attributes could be sustained in the Fairbanks area (Figure 20, Table 13). In spite of geographic differentiation, many larvae in many years apparently still fail to complete development during the short subarctic summers (estimated mortality of 58% on a good host). Evolutionary divergence in the thermal physiology of *P. canadensis* is not trivial, but Alaskan swallowtails do not approach the low temperature capabilities of truly high-latitude herbivores such as the tenthredinid sawfly, *Dineura virididorsata* (Matsuki and MacLean 1990), or the geometrid caterpillar, *Epirrita autumnata* (Ayles and Maclean 1987), which are capable of feeding, growing, and molting at temperatures just above 0°C. Further adaptation in *P. canadensis* may be limited by gene flow from southern regions or by even more fundamental constraints arising from their common ancestry with the basically tropical Papilioninae (Scriber 1973, Scriber et al. 1991, Kukal et al. 1992).

SUMMARY AND CONCLUSIONS

Important physiological attributes of *P. canadensis* appear to differ markedly in their evolutionary lability (Table 14). Neonate mass and the duration of the fifth molt differed by more than 3 standard deviations between populations, while many other traits differed by less than 0.5 standard deviations. Although they seemed about equally important *a priori*, differences in summer temperature regime appeared to be a more potent force in local adaptation (divergence of $> 1SD$ in 5 of 8 traits) than differences in host community (divergence of $< 1SD$ in 7 of 7 traits).

The ability to consume, detoxify, assimilate, and convert chemically diverse plant tissue is highly conserved within *P. canadensis* (Chapter 1). There was no detectable variation in host-use abilities between Michigan and Alaskan populations even though there is little overlap between their host communities. Michigan caterpillars grew just as well as Alaskan caterpillars on Alaskan hosts, and no better on Michigan hosts. There was also no evidence of host specialists within populations. Consequently, the genetic mosaic hypothesis was rejected with respect to detoxification systems and nutritional physiology. The explanation for extraordinary polyphagous abilities in *P. canadensis* lies not within the population structure of the species, but within the genome of any individual within the species (super-genotype hypothesis). However, there is a marked

Table 14: Summary of geographic differentiation between *P. canadensis* from Alaska and Michigan. Traits are organized by the environmental factor hypothesized to select for divergence in the Alaskan population (low summer temperatures, or unique phytochemistry of their hosts). Change is expressed in terms of percentage, standard deviations, and statistical significance.

Selective force	Alaskan phenotype	Change from Michigan phenotype		
		%	SD units	P ^b
Trait	Mean $\pm$ SD ^a			
Low summer temperatures				
Neonate mass	1.33 $\pm$ 0.10 mg	36	3.20	***
Pupal mass	814 $\pm$ 73 mg	-9	-1.16	***
Molt ₄ at 12°	6.58 $\pm$ 2.78 d	-19	-0.56	*
Molt ₅ at 12°	11.83 $\pm$ 1.83 d	-33	-3.27	***
4th instar growth at 12°	0.152 $\pm$ 0.036 mg $\cdot$ mg ⁻¹ $\cdot$ d ⁻¹	33	1.06	***
5th instar growth at 12°	0.119 $\pm$ 0.025 mg $\cdot$ mg ⁻¹ $\cdot$ d ⁻¹	57	1.72	**
Apparent digestibility at 12°	0.41 $\pm$ 0.04 mg/mg	8	0.75	NS
ECD (Growth/Assimilation) at 12°	0.38 $\pm$ 0.07 mg/mg	23	1.00	NS
Nitrogen use efficiency at 12°	0.71 $\pm$ 0.09 mg/mg	-9	-0.78	NS
Survival at 12°	25 $\pm$ %	-7		NS
Basking efficiency at 12°	0.54 $\pm$ C°/C°	6		NS
Host phytochemistry^c				
1st instar growth	0.517 $\pm$ 0.160 mg $\cdot$ mg ⁻¹ $\cdot$ d ⁻¹	12	0.34	+
Middle instar growth	0.268 $\pm$ 0.022 mg $\cdot$ mg ⁻¹ $\cdot$ d ⁻¹	0	0.03	NS
5th instar growth	0.184 $\pm$ 0.050 mg $\cdot$ mg ⁻¹ $\cdot$ d ⁻¹	-4	-0.17	NS
Consumption	1.65 $\pm$ 0.24 mg $\cdot$ mg ⁻¹ $\cdot$ d ⁻¹	4	0.26	NS
Apparent digestibility	0.30 $\pm$ 0.05 mg/mg	-5	-0.31	NS
ECD (Growth/Assimilation)	0.39 $\pm$ 0.11 mg/mg	-2	-0.06	NS
Nitrogen use efficiency	0.55 $\pm$ 0.11 mg/mg	-10	-0.55	NS
Survival	59 $\pm$ %	3		NS

a. MSE^{0.5}

b. + 0.10 > P > 0.05; * P < 0.05; ** P < 0.01; P < 0.001

c. Performance is compared on 7 Alaskan host species not naturally encountered by the Michigan population.

discontinuity in host-use abilities between *P. canadensis* and its presumed progenitor, *P. glaucus*. *P. canadensis* grows faster and survives better than *P. glaucus* on a broad spectrum of boreal host species, and fares substantially worse on at least two southern tree species that are important hosts of *P. glaucus*. I hypothesize that the nutritional physiology of *P. canadensis* diverged rapidly from its ancestral type at the time of speciation and has been little changed since. This is consistent with models of stepwise evolution (Ehrlich and Raven 1964) and punctuated equilibria (Eldredge and Gould 1972). Even strong natural selection may be inadequate to alter the nutritional physiology of herbivores. Changes in boreal tree communities that are expected to accompany global change are unlikely to lead to changes in the nutritional physiology of *P. canadensis*.

In contrast, there was conspicuous divergence in many attributes related to the temperature physiology of *P. canadensis* (Chapter 2; Table 14). Alaskan caterpillars consume more, grow faster, and molt quicker than their Michigan counterparts at low temperatures typical of the Alaskan environment. Changes in reproductive allocation (Alaskan females lay fewer, larger eggs) and adult size (Alaskan larvae initiate pupation at a smaller size) represent additional adaptive responses to cool subarctic summers. Evaluation of fitness tradeoffs in egg size and adult size suggest that the occasional extreme years are more important than the average years in determining the optimal phenotype. Analysis of 48 years of Alaskan weather records indicates that *P. canadensis* with the attributes of the Michigan population could not occur as far north as Fairbanks. Regional adaptation of temperature physiology allows *P. canadensis* to maintain a broader geographic distribution than would otherwise be possible. In spite of low

temperature adaptations, the northern distribution of *P. canadensis* is probably still constrained by low summer temperatures. Tree species that are suitable hosts given sufficient degree days occur throughout the Holarctic, well beyond the northern limits of *P. canadensis*. Consequently, climatic warming would be predicted to yield ecological changes (e.g. rapid northern distribution extensions) as well as evolutionary changes (e.g. larger adults, smaller eggs, and lower metabolic rates in the Fairbanks population).

Since its origins as a discipline, a central problem of evolutionary biology has been to appreciate the potential of natural selection while understanding its constraints (Darwin 1859). Natural history studies have long indicated that attributes such as host-use abilities and temperature responses differ between taxa inhabiting different environments; these patterns suggest that the traits are ecologically relevant, but tell us little about their evolutionary lability. These traits could be of such physiological and genetic complexity that major alterations only come about in the evolution of new genera or families, or they could be very plastic characteristics that are critical in local adaptation. The first scenario emphasizes constraint and extinction (the physiology of the organism restricts its distribution), while the second emphasizes adaptive potential. Patterns of punctuated equilibria, as revealed by the fossil record for some morphological traits (Eldredge and Gould 1973), have given new legitimacy to arguments for evolutionary constraint within species. However, we cannot easily generalize from whorl patterns in snail shells to the physiological and behavioral attributes that interest ecologists. Even in the absence of a fossil record, the increasingly sophisticated tools of comparative biology offer a means of discriminating

between conserved and labile traits (Feder 1987, Huey 1987). My results suggest that swallowtail temperature responses are relatively labile compared to host-use abilities (Table 14). Analysis of variation within and between other taxa will test the generality of these patterns, suggest other generalizations, and allow inferences about the evolutionary history of key ecological traits. In combination with better knowledge of gene flow and the genetic architecture of traits, this research program should clarify the role of adaptation and constraint in the interactions of organisms with their environment.

APPENDICES



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.: 1991-10

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

Adaptation and constraint in Papilio canadensis:
geographic variation in nutritional physiology
and temperature responses

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Matthew P. Ayres

Date 14 November 1991

*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

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	
<u>Papilio canadensis R & J</u>	AK: Fairbanks 15-VI-1989 M. P. Ayres					2	10		MSU
<u>Papilio canadensis R & J</u>	MI: Gogebic Co. T50N R45W sec 21 10-IV-1988 M. P. Ayres					1	6		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Matthew P. Ayres

Date 14 November 1991Voucher No. 1991-10

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

Robert L. Dietrich
Curator15 Nov. 1991
Date

Appendix 2

First instar growth rates of *P. canadensis* from Alaska, *P. canadensis* from Michigan, and *P. glaucus* from Ohio, tested on tree species from Alaska, northern Michigan, and Southern Michigan. Except as noted, SE is based on the number of families, and *N* equals the number of families.

	First instar growth rate (mg · mg ⁻¹ · d ⁻¹)								
	<i>P. canadensis</i> AK			<i>P. canadensis</i> WI			<i>P. glaucus</i> OH		
	Mean	SE	<i>N</i>	Mean	SE	<i>N</i>	Mean	SE	<i>N</i>
Alaskan hosts									
<i>Populus tremuloides</i> Michx.	0.462	0.030	7 ^a	0.409	0.052	7 ^a	0.220	0.015	9 ^b
<i>Populus balsamifera</i> L.	0.653	0.023	8 ^a	0.598	0.026	8 ^a	0.418	0.024	9 ^b
<i>Salix alaxensis</i> (Anderss.) Cov.	0.469	0.030	8 ^a	0.442	0.033	7 ^a	0.393	0.024	9 ^b
<i>Salix novae-angliae</i> Anderss.	0.724	0.023	7 ^a	0.651	0.044	7 ^a	0.398	0.015	9 ^b
<i>Salix bebbiana</i> Sarg.	0.435	0.043	8 ^a	0.371	0.035	8 ^a	0.307	0.020	9 ^b
<i>Salix glauca</i> L.	0.566	0.036	8 ^a	0.403	0.030	7 ^a	0.336	0.017	9 ^b
<i>Betula resinifera</i> Britton	0.408	0.020	7 ^a	0.381	0.033	7 ^a	0.331	0.031	10 ^b
<i>Alnus crispa</i> (Ait.) Pursh	0.454	0.071	7 ^a	0.400	0.049	7 ^a	0.177	0.022	9 ^b
<i>Alnus tenuifolia</i> Nutt.	0.560	0.020	9 ^a	0.541	0.026	7 ^a	0.347	0.029	10 ^b
Northern Michigan hosts									
<i>Tilia americana</i> L.	0.336	0.032	8 ^c	0.356	0.017	8 ^c			
<i>Prunus virginiana</i> L.	0.450	0.042	8 ^c	0.374	0.054	8 ^c			
<i>Prunus serotina</i> Ehrh.	0.464	0.050	7 ^c	0.359	0.031	6 ^c			
<i>Populus grandidentata</i> Michx.	0.477	0.030	9 ^c	0.460	0.035	8 ^c			
<i>Fraxinus americana</i> L.	0.373	0.026	6 ^c	0.447	0.026	6 ^c			
<i>Betula papyrifera</i> Marsh.	0.442	0.026	7 ^c	0.448	0.020	6 ^c			
<i>Betula alleghaniensis</i> Britton	0.162	0.029	8 ^c	0.233	0.020	8 ^c			
<i>Betula nigra</i> L.	0.598	0.062	8 ^c	0.623	0.031	7 ^c			
<i>Alnus rugosa</i> (Du Roi) Spreng.	0.461	0.045	7 ^c	0.394	0.059	6 ^c			
Southern Michigan hosts									
<i>Liriodendron tulipifera</i> L.	0.188	0.056	7 ^d	0.299	0.084	9 ^{de}	0.758	0.046	7 ^d
<i>Ptelea trifoliata</i> L.	0.550	0.047	8 ^d	0.416	0.077	9 ^{de}	0.649	0.044	6 ^d
<i>Tilia americana</i> L.	0.267	0.041	7 ^d				0.296	0.039	4 ^d
<i>Prunus virginiana</i> L.	0.340	0.030	8 ^d				0.460	0.035	5 ^d
<i>Prunus serotina</i> Ehrh.	0.384	0.055	9 ^d				0.363	0.032	6 ^d
<i>Fraxinus americana</i> L.	0.405	0.041	7 ^d				0.388	0.096	5 ^d

^a Foliage collected on 28 June 1988 from University of Alaska Fairbanks arboretum.

^b Foliage collected on 18 August 1989 from University of Alaska Fairbanks arboretum.

^c Foliage collected on 5 July 1989 from Dunn County, Wisconsin.

^d Foliage collected on 18 June 1988 from Ingham County, Michigan.

^e All larvae from a single family; *N* = number of larvae.

Appendix 3

Summary of parameters used in *P. canadensis* development model. MI = Michigan; AK = Alaska.

A. Phenology

First day of season to begin accumulating thermal sums: 1 April.

Degree days (10°C base) of neonate hatch for cohorts 1-5: 240, 280, 320, 360, 400.

Last day of permissible growth (Stages 1-4): 14 September.

Last day of permissible prepupation (Stage 5): 30 September.

B. Mass of hatching neonates

MI phenotype: 0.98 mg.

AK phenotype: 1.33 mg.

C. Stage 1, Instars 1-3

Relative growth rates at 12, 18, 24, and 30°C

Good host: 0.083, 0.116, 0.270, 0.280 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

Excellent host: 0.099, 0.223, 0.310, 0.416 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

D. Stage 2, Instar 4

Initial mass: 99 mg.

Relative growth rates at 12, 18, 24, and 30°C

MI phenotype, good host: 0.104, 0.161, 0.449, 0.533 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

AK phenotype, good host: 0.139, 0.174, 0.334, 0.279 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

MI phenotype, excellent host: 0.109, 0.268, 0.350, 0.456 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

AK phenotype, excellent host: 0.146, 0.305, 0.448, 0.617 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

Final mass: 369 mg.

E. Stage 3, Molt 4 (Instar 4 to Instar 5)

Molting rates at 12, 18, 24, and 30°C

MI phenotype: 12.3, 32.1, 52.6, 84.8 percent/day.

AK phenotype: 15.2, 48.1, 60.2, 116.3 percent/day.

F. Stage 4, Instar 5

Initial mass: 348 mg.

Relative growth rates at 12, 18, 24, and 30°C

MI phenotype, good host: 0.067, 0.127, 0.382, 0.369 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

AK phenotype, good host: 0.099, 0.181, 0.345, 0.341 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

MI phenotype, excellent host: 0.069, 0.161, 0.255, 0.375 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

AK phenotype, excellent host: 0.142, 0.214, 0.317, 0.532 $\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$.

Final mass

MI phenotype: 1798 mg (corresponds to female pupal mass of 899 mg).

AK phenotype: 1628 mg (corresponds to female pupal mass of 814 mg).

G. Stage 5, Molt 5 (Instar 5 to Pupa)

Molting rates at 12, 18, 24, and 30°C

MI phenotype: 5.61, 16.3, 18.3, 34.8 percent/day.

AK phenotype: 8.45, 23.2, 23.5, 32.3 percent/day.

H. Minimum developmental thresholds

Growth (Stages 1, 2, and 4): 7.25°C.

Molting (Stages 3 and 5): 9.50°C.

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