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ARE HYBRIDS MORE FIT THAN THEIR PARENTAL TYPES? A TEST USING TWO TIGER SWALLOWTAIL BUTTERFLY SPECIES. PAPILIO GLAUCUS AND P. CANADENSIS (LEPIDOPTERA: PAPILIONIDAE)

presented by

Jennifer Laura Donovan

has been accepted towards fulfillment
of the requirements for

Master's degree in Entomology

J. Mark Scriber
Major professor

Date 23 July 2001

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ARE HYBRIDS MORE FIT THAN THEIR PARENTAL TYPES? A TEST USING TWO TIGER SWALLOWTAIL BUTTERFLY SPECIES *PAPILIO GLAUCUS* AND *P. CANADENSIS* (LEPIDOPTERA: PAPILIONIDAE)

By

Jennifer Laura Donovan

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

2001

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ABSTRACT

ARE HYBRIDS MORE FIT THAN THEIR PARENTAL TYPES? A TEST USING TWO TIGER SWALLOWTAIL BUTTERFLY SPECIES *PAPILIO GLAUCUS* AND *P. CANADENSIS* (LEPIDOPTERA: PAPILIONIDAE)

By

Jennifer Laura Donovan

The ranges of *P. canadensis* and *P. glaucus* overlap and form a hybrid zone at the boreal and temperate forest transition zone in the eastern half of the United States, between 41° and 44° north latitude. It has been documented that laboratory hybrid adults of *P. glaucus* and *P. canadensis* are fertile, produce normal gametes, and back-cross or F-2 offspring (Hagen et al. 1991). It has also been shown in the laboratory and the field that prezygotic barriers are weakly effective at preventing cross fertilization (Deering 1998, Stump 2000). This study was designed to examine the strength of postzygotic barriers at the larval stage by simultaneously examining multiple indicators of fitness (growth rate, pupal weight, larval duration and survival to pupation) in reciprocal crosses hybrid and parental types of *P. canadensis* and *P. glaucus*. Parental type and reciprocal hybrid larvae were randomly assigned to a combination of temperature (15°C, 23°C, or 31°C) and host plant (*Liriodendron tulipifera*, *Populus tremuloides*, *Prunus serotina*). Hybrids showed patterns of significantly slower growth rates or smaller pupal weights than their parental types. The number of degree days required to complete hybrid larval development to pupation was always equal to or less than at least one of the parent types. Furthermore, in no treatment combination did hybrids ever perform less well than both parental types for any fitness trait. These results indicate that postzygotic barriers at the larval stage could potentially be weak.

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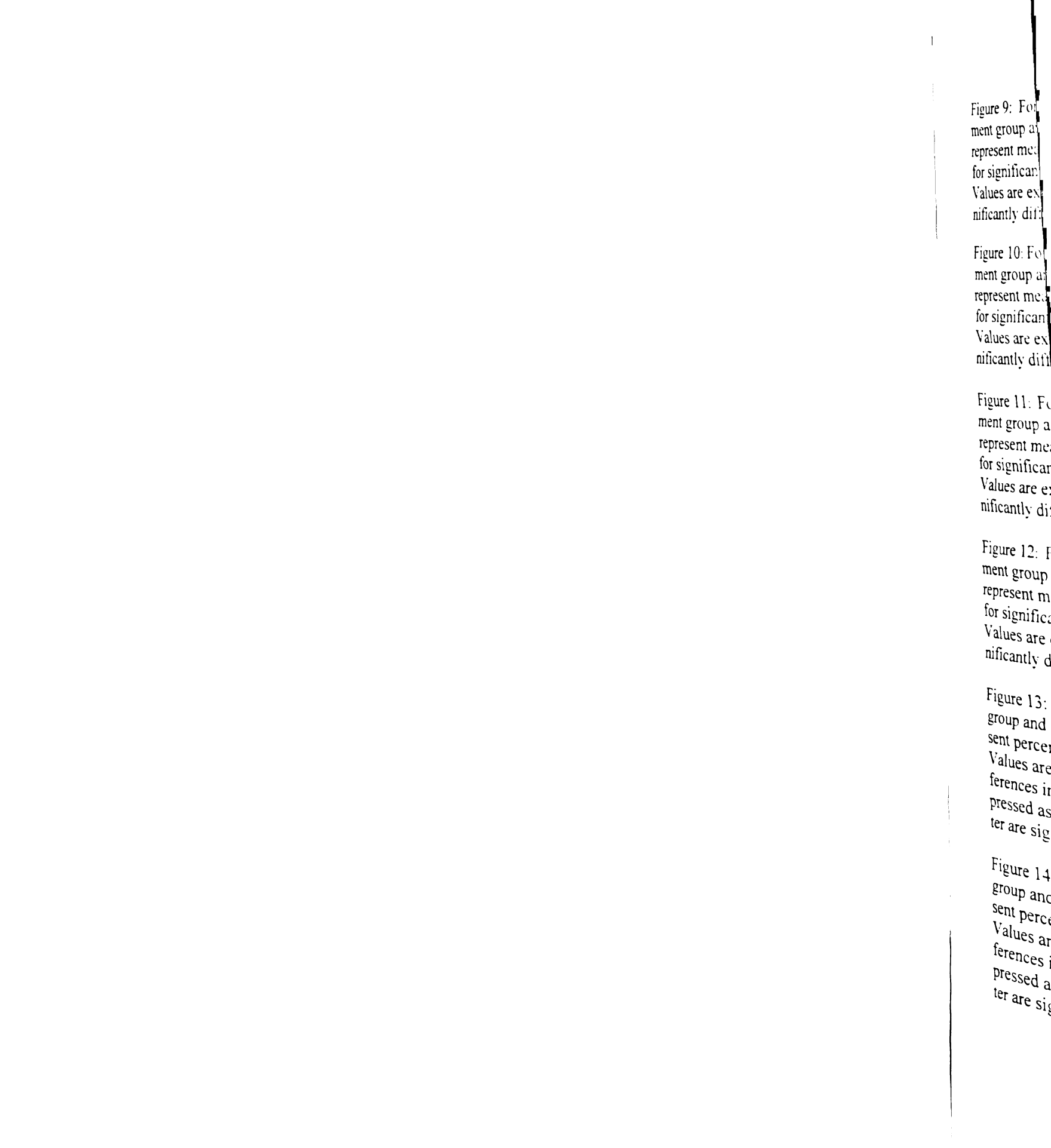

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LITERATURE REVIEW

Since Charles Darwin published On the Origin of Species in 1859, there has been constant debate over the role of hybridization in the evolutionary process. At one end of the spectrum is the view that it should play little or no role in evolution because hybrids, in general, have been considered unfit relative to their progenitors (Mayr 1963). This is illustrated by Dobzhansky (1970): "To be sure, a minority of the gene combinations formed by the hybridization of species might be fit, perhaps fit enough to spread into as yet unoccupied adaptive peaks. A majority would be relatively ill adapted in any environment." At the other end is the view championed by Arnold and Hodges (1995): "hybridization may often lead to the production of relatively fit hybrid genotypes that possess novel genetic variation and the founding of new evolutionary lineages."

This debate has been evident in the many proposed species concepts in the literature. Species concepts typically take the view that hybridization is maladaptive because the individuals involved produce fewer and/or less fertile progeny (Arnold 1997). Darwin wrote (1859) "Pure species have of course their organs of reproduction in a perfect condition, yet when intercrossed they produce either few or no offspring. Hybrids, on the other hand, have their reproductive organs functionally impotent...." More recently Mayr (1963) reinforced this viewpoint by saying "...The majority of . . . hybrids are totally sterile . . . Even those hybrids producing normal gametes in one or both sexes are nevertheless unsuccessful in most cases and do not participate in reproduction . . . when they do backcross to the parental species, they normally produce

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The four species concepts that will be discussed are: Biological, Recognition, Cohesion, and Phylogenetic. They were chosen because they are considered to be representative of all other species concepts (Arnold, 1997). They also exemplify that all species concepts consider hybridization to be "bad" because the offspring of heterospecific pairings have lower levels of fertility and/or viability and therefore effectively reduce the fitness of the parental types to zero (Arnold, 1997). Natural hybridization is also considered "bad" when it is viewed as a violation of the process of divergent evolution (Arnold 1997).

Biological Species Concept: Mayr, basing his definition of species on Dobzhansky's (1937) earlier work, defined species as ". . .groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups" (Mayr, 1942). This strict definition does not allow for the hybridization of species. Mayr dealt with this problem in two ways. First in 1942, he argued that if viable, fertile hybrids were produced, then one should consider the hybridizing forms to be subspecies or semispecies. Secondly, in 1963, he stated that if hybridization does occur, "The majority of such hybrids are totally sterile, even where they display "hybrid vigor." Even those hybrids that produce normal gametes in one or both sexes are nevertheless unsuccessful in most cases and do not participate in reproduction. Finally, when they do backcross to the parental species, they normally produce genotypes of inferior viability that are eliminated by natural selection." Mayr concludes that if hybridization does successfully occur it will most likely lead to evolutionary dead ends. Mayr (1963)

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states, "In natural populations there is usually severe selection against introgression. The failure of most zones of conspecific hybridization to broaden shows that there is already a great deal of genetic unbalance between differentiated populations within a species."

The Recognition Species Concept: Under this theory, species are defined as "that most inclusive population of individual biparental organisms which share a common fertilization system" (Paterson, 1985). Heterospecific reproductive isolation is a byproduct of adaptive evolution. The development of "Specific Mate Recognition Systems" (Paterson, 1985) occurs when an allopatric population undergoes directional selection. This selection acts upon a variety of aspects of the organism, including a subset that affects mate recognition (Paterson, 1985). Speciation with the Recognition Species Concept is therefore "an incidental effect resulting from the adaptation of the characters of the fertilization system, among others, to a new habitat, or way-of-life" (Paterson, 1985).

Strict adherence to the Recognition Species Concept leads one to conclude that reticulation can only occur between forms that are not yet species (Arnold and Hodges, 1995). Recognition Species Concept views natural hybridization between species as definitionally impossible. The Recognition Species Concept denies that these taxa are evolutionary independent because gene exchange between them indicates a common Specific Mate Recognition System (Paterson, 1985). However, this view conflicts with observations of crosses in nature that involve organisms clearly belonging to differentiated species (Templeton, 1989; Arnold, 1992).

The Cohesion Species Concept: A. R. Templeton originally put the Cohesion

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Species Concept forth. He defines species as "the most inclusive group of organisms having the potential for genetic and/or demographic exchangeability" (Templeton, 1989). Templeton felt that the Biological Species Concept and the Recognition Species Concept did not apply to either asexual organisms or individuals that belonged to syngameons (Arnold, 1997). The Cohesion Species Concept was designed to take into account all of the microevolutionary processes thought to contribute to speciation (Arnold, 1997).

Arnold (1997) best describes how the Cohesion Species Concept addresses hybridization:

> "The Cohesion Species Concept accepts that there are species that show differences in their boundaries defined by either genetic or demographic exchangeability (Templeton, 1989). For example, syngameons are a result of species having greater genetic than demographic exchangeability. Unlike the Biological Species Concept and the Recognition Species Concept, the Cohesion Species Concept does not require that these taxa be reduced to subspecific categories and thus allows for the process of heterospecific natural hybridization. Furthemore, the production of hybrid species from such heterospecific hybridization is also possible under the Cohesion Species Concept. It is important to be aware of that Templeton placed those taxa belonging to a syngameon into a category called "bad species." These species are "bad" because they demonstrate an elevated degree of genetic exchangeability. "Good species," on the other hand are "those that are well defined both by genetic and demographic exchange-

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The Phylogenetic Species Concept: The Phylogenetic Species Concept defines species as "an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent" (Cracraft, 1989). The application of this concept to determine patterns of phylogenetic relationships, define species and the process of speciation depends on the identification of the ancestral and derived states of particular characters. The pattern of branching within these cladograms is the basis for determining not only the boundaries of species (i.e. irreducible clusters; Carcraft, 1989), but also the relationships among species.

Both Hennig (1966) and Cracraft (1989) argue that species cannot arise from heterospecific hybridization because species must be of monophyletic origin not polyphyletic. Cracraft (1989) wrote: "in the majority of cases, phylogenetic species will be demonstrably monophylectic; they will never be nonmonophyletic, except through error." Hennig argues that if new species arose from polyphyletic origin that the species involved were so closely related that they could just as well be considered races of one species (Hennig, 1966). Consequently fertile offspring of hybridization events are considered evidence that the parental types are the same species.

Species concepts influence the paradigm from which researchers develop ideas for study. From the above described paradigms one would conclude that all hybrids are unfit and therefore of little evolutionary importance or that the process of hybridization is maladaptive because it is an impediment to divergent evolution (Arnold and Hodges, 1995; Arnold, 1997; Arnold, 1999) .

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There are many examples of hybrids having lower fitness than their parents. However, Arnold and Hodges (1995) have recently reanalyzed data from several studies and found that not all hybridization is "bad". Hybridization can be seen as "evolutionarily important" even in cases when the hybrids are unfit. For example, it may reinforce reproductive isolation of separate species by assortative mating (prezygotic isolating mechanisms), through selection against the hybrids (postzygotic isolating mechanisms) (Dowling and Moore 1985).

Hybridization can also be seen as important in cases where hybrids are fit. It may create a bridge between species and allow the passage of unique and beneficial gene combinations. Novel and superior adaptive gene combinations could be selected for on the basis of increased genetic variability due to introgression. This notion has been demonstrated in laboratory cultures of *Drosophila* (Nagle and Mettler 1969) and hybrid populations of *Hyalophora* moths (Collins 1984).

Definitions: Hybrid and Hybrid Zone

With the problems inherent with species definitions in relation to hybrids, I will use Harrison's (1993) definition for hybrid and Arnold's (1997) for hybrid zone.

> "Hybridization is the interbreeding of individuals from two populations, or groups of populations, which are distinguishable of the basis of one or more heritable characters."

The term hybrid will be applied to F_1 individuals and individuals of mixed ancestry. F_1 will be specified when appropriate. Using this definition has three advantages (Harrison, 1993): first, its use does not depend on reaching agreement on a

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single species concept. Second, it does not require the assignment of populations to particular taxonomic categories (e.g. races or subspecies). Third, it does not require judgments about relative fitness of hybrids or differences between parental types in "adaptive norms."

A hybrid zone will be defined simply as a place "where two populations of individuals that are distinguishable on the basis of one or more heritable characters, overlap spatially and temporally and cross to form viable and at least partially fertile offspring" (Arnold, 1997).

The *Papilio glaucus* and *Papilio canadensis* (Lepidoptera: Papilionidae) Hybrid Complex

Hybridization is relatively widespread in the genus *Papilio*. *Papilio* comprises 216 of the 557 species in the family Papilionidae and it is estimated that up to 15% of the 216 species form natural hybrids (Sperling, 1990). One of the most extensive and reliable documentations of hybridization in the genus *Papilio* is within the *machaon* group. It is thought that the dark morph of *P. zelicaon* and *P. machaon* is expressed by a gene received through introgression from *P. polyxenes asterius* (Sperling 1990). It is also suspected that *Ornithoptera allottei*, a Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), protected species, is actually a rare interspecific hybrid (Sperling 1990).

It has been documented that *P. glaucus* and *P. canadensis* are hybridizing species (Hagen et al. 1991). Laboratory hybrids of *P. glaucus* and *P. canadensis* are fertile, produce normal gametes, and participate in reproduction (Hagen et al. 1991).

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These two species form a hybrid zone between 41° and 44° North latitude and extends from New England through the Great Lakes region (Hagen and Scriber 1991) (Figure 1).

The fact that these two species can hybridize so easily begs the question why are they considered different species? It has been generally accepted that Hagen et al., (1991) convincingly described *P. glaucus* and *P. canadensis* as separate species based on several differing heritable characteristics (Table 1). There are differential host suitabilities between the parental types. *P. canadensis* has low larval survival on tulip tree and high survival on quaking aspen. Conversely, *P. glaucus* has low survival on quaking aspen and high survival on tulip tree.

The *P. glaucus* female is polymorphic. Where *P. glaucus* in sympatric with the toxic *Battus philenor* swallowtail a *P. glaucus* dark morph is present within the population. This dark morph is absent in *P. canadensis* females. It is thought that there is a sex linked suppressor for the dark morph carried by *P. canadensis* males.

There are also fixed differences in three diagnostic allozyme loci: Hexokinase (Hk), Lactate dehydrogenase (Ldh), 6-Phosphogluconate dehydrogenase (Pgd).

Pupal diapause determination is also different for the two species. Diapause is environmentally determined for *P. glaucus* and obligate for *P. canadensis*.

This evidence also meets the criteria of Arnold's (1997) definition of a hybrid zone: "where two populations of individuals that are distinguishable on the basis of one or more heritable characters, overlap spatially and temporally and cross to form viable and at least partially fertile offspring".

Figure

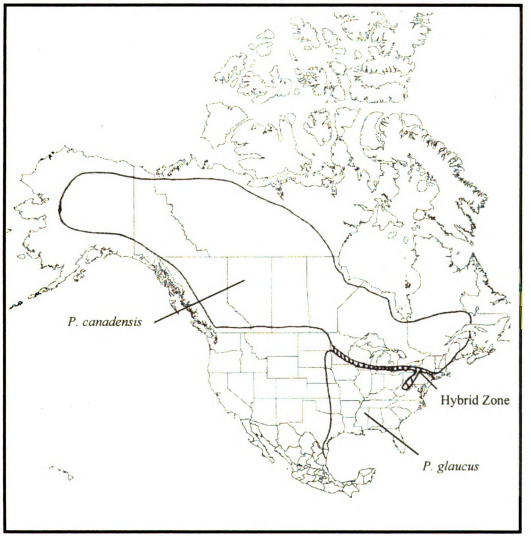

Figure 1: Ranges and hybrid zone of *Papilio canadensis* and *Papilio glaucus*.

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Table 1: Differing Heritable Characteristics of *Papilio glaucus* and *Papilio canadensis* (Adapted from Hagen et al 1991).

Character	***P. glaucus***	***P. canadensis***	**Inheritance**	**Ref.**
Physiological/Developmental				
Environmental determination of pupal diapause	Yes	No	X-linked	1,2
Lower lethal temperature for diapausing pupae	-23°C	-23°C to -27°C	?	3
Larval survival on aspen foliage	Very low	High	Polygenic	4,5,6
Larval survival on birch foliage	Low	High	Polygenic	5,7
Larval survival on tulip tree foliage	High	Low	Polygenic	4,5
Polymorphism for female color	Present	Absent	Y-linked	8,9
Suppression of melanic color	Absent	Present	X-linked	1,9,10
Biochemical				
Hexokinase (*Hk*) alleles	"100"	"110"	Autosomal	11
Lactate dehydrogenase (*Ldh*) alleles	"100"	"80," "40"	X-linked	1, 7, 11
6-Phosphogluconate dehydrogenase (*Pgd*) alleles	"100," "50"	"125," "80," "150"	X-linked	1, 7, 11

1. Hagen and Scriber (1989); 2. Rockey et al. (1987); 3. Scriber (1994); 4. Scriber (1986); 5. Scriber (1988); 6. Scriber et al. (1989); 7. Hagen (1990); 8. Clarke and Sheppard (1962); 9. J. M. Scriber, et al. (1996); 10 Scriber et al. (1987); 11. Hagen et al. (1991).

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INRODUCTION

Conventionally, research using hybrids has had one of three emphases (Arnold, 1997): First, hybrids are used to study the systematics of a particular group of organisms. Secondly, they have been used to understand the mechanisms that limit gene flow, with the assumption that the development of barriers to gene flow is equivalent to the process of speciation . Thirdly, individual examples of natural hybridization are thought to be evolutionarily important and therefore worthy of study.

Arnold (1992) suggests:

> "The most informative approach for estimating the adaptive effects of hybridization and introgression is to measure the relative fitness of different hybrid and parental genotypes under identical experimental or natural conditions."

Arnold and Hodges took a careful look at the question of whether or not hybrids are fit or unfit relative to their parents. They reviewed research that looked at 44 cases of hybridization and found that only 13 hybrid classes demonstrated an overall lower fitness than their parental types (Arnold and Hodges, 1995). They concluded that the general pattern of hybrid fitness is not that hybrids are uniformly unfit but that the hybrids are as fit as the two parental taxa or demonstrate a higher level of fitness than at least one the parents (Arnold and Hodges, 1995).

In this paper Arnold and Hodges (1995) suggest that "simultaneous measurements of fitness components for hybrid and parental individuals allow direct comparisons of fitness among parental and hybrid classes and among different hybrid

classes."

With these ideas in mind I conducted an experiment that simultaneously measured multiple components of fitness in the larval stage of the Tiger Swallowtail butterflies, *P. glaucus* and *P. canadensis* and their reciprocal hybrids. The fitness indicators that I measured or calculated were: average growth rate, average pupal weights, average number of degree days from hatch to pupation and larval survival to pupation.

These fitness indicators were measured on larvae from four genotypic classes: *P. canadensis*, *P. glaucus*, *P. canadensis x P. glaucus* and *P. glaucus and P. canadensis*. The two types of hybrids, *P. glaucus x P. canadensis* and *P. canadensis x P. glaucus* were chosen because they are easily made, through hand-pairing, in the laboratory. Three host plants were chosen: tulip tree, black cherry and quaking aspen. The host plants were included in the study because they all are present within the hybrid zone and in some part of the two parental types ranges. Tulip tree is a preferred host of *P. glaucus* and *P. canadensis* has low survival on it (Scriber, 1988). Quaking aspen is a preferred host of *P. canadensis* and *P. glaucus* has low survival on it (Scriber, 1988). Black cherry is mutually suitable for both parental types.

Three temperature treatments were used: 15°C, 23°C and 31°C. These temperatures approximate the lower and upper limits of survivorship of both parental species.

There were four main objectives of this study:

Objective 1: To determine if hybrid larvae of *P. glaucus* and *P. canadensis* will need significantly different number of degree days from hatch to pupation.

Objective 2: To determine if hybrid pupae of *P. glaucus* and *P. canadensis* have significantly different weights.

Objective 3: To determine if hybrid larvae of *P. glaucus* and *P. canadensis* have significantly different growth rates from hatch to pupation than parental type larvae.

Objective 4: determine if hybrid larvae of *P. glaucus* and *P. canadensis* have significantly different survival rates to pupation than parental type larvae.

Determining the fitness of hybrids relative to their parents will allow us to make predictions about what to expect from hybrids if they are naturally occurring and begin to asses their evolutionary importance. If we find that hybrids are unfit, this could cause the reinforcement of reproductive isolation. If they are fit, hybrids might be occupying adaptive peaks or allowing the passage of novel beneficial gene combinations from one parental species to another.

METHODS

Research was conducted that measured multiple components of fitness of the larval stage of Tiger Swallowtail butterflies, *P. glaucus* and *P. canadensis,* and their hybrids. Larvae of *P. glaucus*, *P. canadensis* and their reciprocal hybrids were randomly assigned to host plant/temperature treatments (3 temperatures: 3 hostplants) and then reared in growth chambers.

Sources of Broods

Parental types: Parental types were obtained from the offspring of wild females collected from outside the hybrid zone. *P. canadensis* females were collected from several counties in Michigan: Charlevoix County, Emmet County, Cheboygan County, and Isabella County. *P. canadensis* were also collected from Bennington County Vermont and Clark County Wisconsin.

In 1999, *P. glaucus* females were collected from Warren County Missouri. In 2000, *P. glaucus* females were bought as pupae from a breeder in Pennsylvania. Pupae were allowed to emerge and were then handpaired with wild *P. glaucus* males from St. Joseph County, Michigan.

Hybrids: In 1998, wild females were collected for laboratory stock, from several sites in Michigan: Cheboygan County, Dickinson County and Isle Royale. Females were placed in oviposition arenas and eggs were harvested daily. Caterpillars were reared under diapause inducing conditions: 12:12 light/dark and at 25°C. Pupae were stored in 24 hour dark and 4°C conditions until spring of 1999. In May of 1999,

pupae were brought out of diapause conditions and were allowed to emerge in cylindrical screen cages and then handpaired with wild *P. glaucus* males. In 2000, *P. canadensis* pupae were supplied by Howard Romack. He raised larvae from Bennington County, Vermont on Black Cherry (*Prunus serotina*). Pupae received from Romack were allowed to emerge and then handpaired with wild *P. glaucus* males.

In 1999, offspring of *P. glaucus* females that were collected in Highland County, Florida in April of that year, were handpaired to wild *P. canadensis* males. In 2000, *P. glaucus* pupae from Southeast Pennsylvania were bought as pupae from a breeder from Pennsylvania. Adult females emerged and then were handpaired with wild *P. canadensis* males.

Handpairing

All pairings were initiated by hand, after methods described in Clarke & Sheppard (1956), using laboratory-reared, virgin females. All males that were used for pairings were wild caught. After being handpaired, coupled pairs were put into a cylindrical screen cage and monitored for separation. If pairs uncoupled with in 5 minutes they were paired again or discarded. Matings were considered successful if they lasted longer than 30 minutes (Stump, 2000).

Oviposition

All females that were wild caught and handpaired were placed individually in a round plastic oviposition arena, 10 cm in height and 27 cm in diameter, with a 3 choice host plant arrangement: *Liriodendron tulipifera*, *Populus tremuloides* and

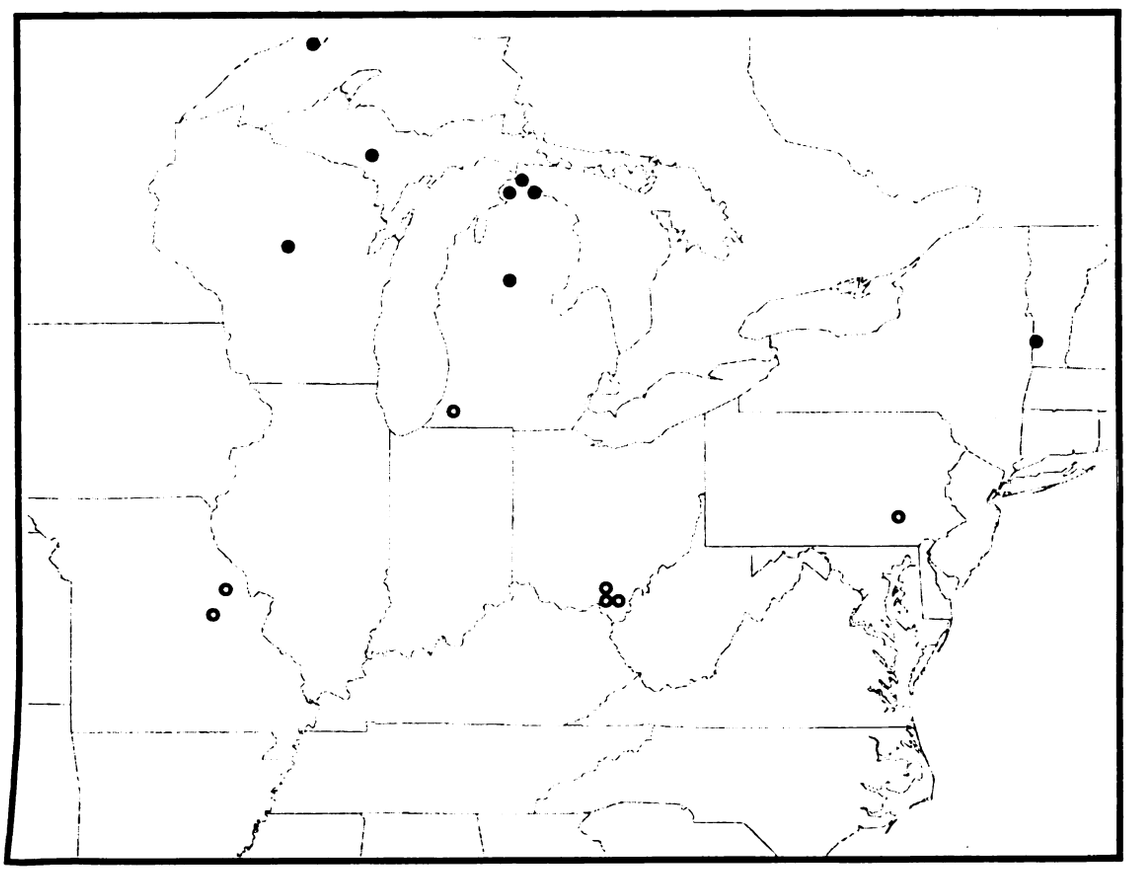

Figure 2: Collection sites for *P. canadensis* and *P. glaucus*.
P. canadensis (solid circle)
P. glaucus (open circle)

Fig

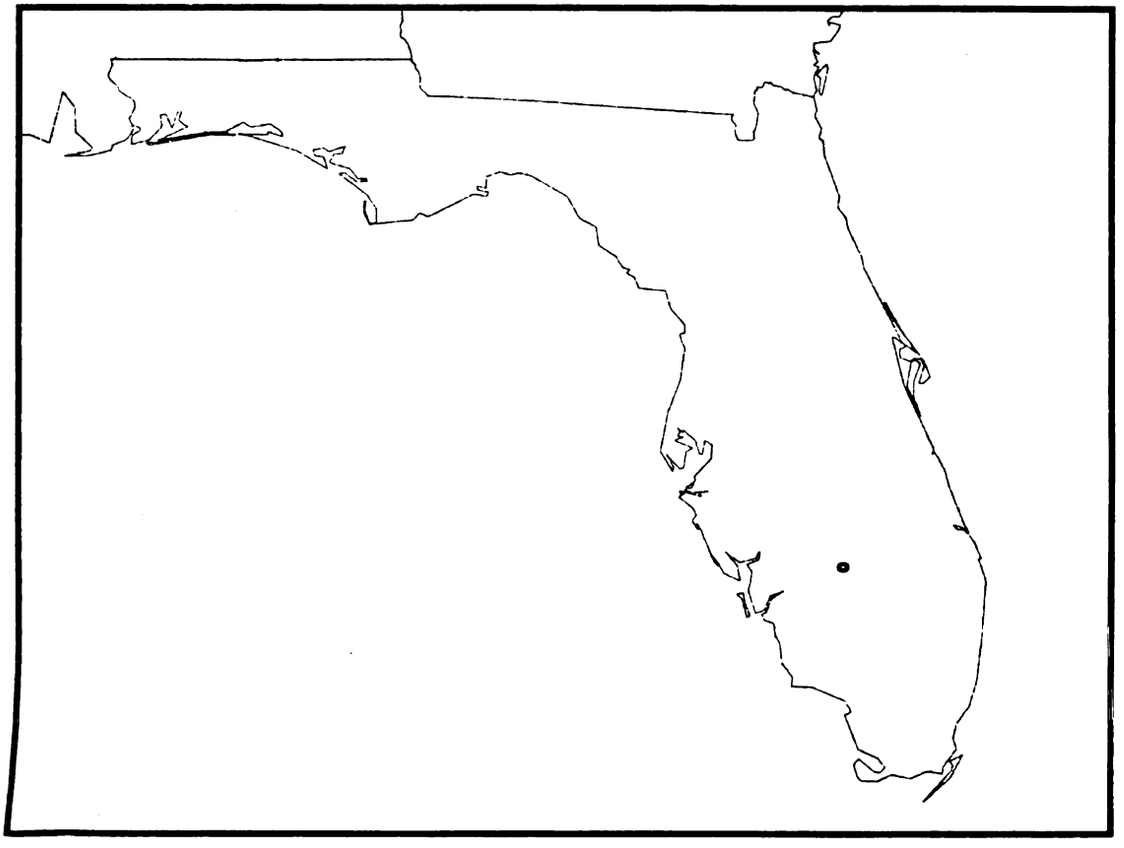

Figure 3: Florida collection site for *P. glaucus*.

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Prunus serotina. The arenas were placed on a rotating platform (10 times per hour), 0.7 – 1.0 meter from 6-8 100-watt incandescent lights on a 6 hour light: 6 hour dark: 6 hour light: 6 hour dark cycle (Scriber, 1993). The 6 hours light alternating with 6 hours of dark is to maximize the number of eggs laid by the female. If the females were on a 24 hour light cycle they would over heat and die in the oviposition arenas. The females were fed daily with a 20% honey/80% water solution. Females were allowed to oviposit until death. Eggs were collected and counted daily. Thirty-six larvae from each brood were used in the experiment.

Larval Experiments

After hatching, 36 larvae from each brood of *P. glaucus*, *P. canadensis* and their reciprocal *hybrids, P. canadensis x P. glaucus* and *P. glaucus x P. canadensis* (PCPG and PGPC), were randomly assigned to a host plant/temperature treatments.

In 1999, there were 5 (180 larvae) broods of *P. canadensis*, 6 (216 larvae) broods of *P. canadensis x P. glaucus*, 4 (144 larvae) broods of *P. glaucus x P. canadensis* and 2 (72 larvae) broods of *P. glaucus*. In 2000, there were 5 (180 larvae) broods of *P. canadensis*, 4 (144 larvae) broods of *P. canadensis x P. glaucus*, 4 (144 larvae) broods of *P. glaucus x P. canadensis* and 5 (180 larvae) broods of *P. glaucus*.

Rearing

The survival of *P. glaucus, P. canadensis* and their reciprocal crosses was studied on three host plants: *Liriodendron tulipifera*, *Populus tremuloides* and *Prunus*

serotina and under three different temperature regimes (15°C, 23°C, 31°C).

Using a randomized complete split block design with temperature being the whole plot and genotype and host plant being the split plots, 36 larvae from each brood were randomly assigned to a temperature and a host plant treatment. Each larva was placed in a Petri dish that was lined with paper towel and contained the appropriate host plant. To maintain turgidity, the stems of the leaves were inserted into a water filled aquapic™. Leaves were changed every other day or more frequently if required.

Hatch date, weights every 10 days, pupation date, pupal weight, emergence date, sex, and death date, were recorded.

Source of Leaves

Pesticide free host plant foliage was collected from the same East Lansing sites in both years. Branches were cut and transported to the laboratory. To maintain turgidity branches were placed in 10 gallon buckets filled with water. To ensure freshness of the leaves, all leaves for larval feeding were used within 24 hours of being cut.

Adult Emergence

After pupation a pupae were put into a cylindrical screen cage to emergence. The pupae were weighed and then placed back in the chamber where larval rearing took place. Pupae were allowed to stay in these conditions for emergence for at least 31 days. If they hadn't emerged after 31 days they were placed in overwintering storage conditions (3°-5°C/total darkness). When emergence occurred, each adult was sexed and then frozen at -80° C for possible future allozyme work.

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Statistics and Calculations

To determine if there were significant differences between pupal weight, growth rate. larval duration and survival until pupation between parental types and hybrids a standard split plot design. Temperature as whole-plot with two split plots: genotype (*P. glaucus, P. canadensis, P. glaucus x P. canadensis, and P. canadensis x P. glaucus*) and host plant (black cherry, quaking aspen and tulip tree). The experiment was blocked by temperature. Each one of these indices was measured or calculated for every individual of parental type and reciprocal hybrids.

Pupal weight, growth rate and larval duration data were analyzed using an analysis of variance (Proc glm: SAS Institute Inc. 1990). Treatment means were compared using a least squares means comparison.

Time from hatch to pupation, expressed in number of degree-days (to see number of days from hatch to pupation refer to appendix 4), was calculated using the following formula: {(average daily temperature – threshold temperature) x number of days from hatch to pupation} using a threshold temperature of 10°C (Ritland and Scriber, 1985). Growth rate was calculated by dividing pupal weight by the number of days from hatch to pupation.

Percent survival to pupation was analyzed using a nonparametric chi-square analysis (Proc npariway wilconxon anova: SAS Institute Inc. 1990).

The experiment was run twice over two consecutive summers: 1999 and 2000. Statistically a full model was ran and reported so that ultimately all effects and interactions could be compared but for the purposes of this thesis comparisons were only made within a year and treatment combination . .

RESULTS

All comparisons were made within a treatment group and within a year. For example, comparisons were made between the hybrids and parental types on black cherry at 15°C in 1999. No comparisons were made between years, across temperatures or between host plants. Sample sizes and data used in the graphs are reported in a table at the end of each section.

Objective 1: To determine if hybrid larvae of *P. glaucus* and *P. canadensis* will need significantly different number of degree days from hatch to pupation.

In 1999 there was a significant effect of temperature at $P<0.01$. In 1999 and 2000 there were significant effects of genotype at $P<0.0001$/ $P<0.0001$ and host plant at $P<0.0001$ and $P<0.0001$ on the time from hatch to pupation (Tables 2 and 3).

In both years, when PcPg larvae were fed black cherry, at all temperatures (15° C, 23°C and 31°C), they needed significantly fewer degree days to reach pupation than *P. canadensis* (Figure 4, 5 and 6). When PcPg larvae were compared to *P. glaucus* there was no difference (Figure 4, 5, and 6). Except in two cases (PgPc larave in 1999 at 15°C and 31°C needed significantly fewer degree days to reach pupation than *P. canadensis*), PgPc larvae did not differ from either parental types (Figure 4, 5 and 6).

In both years, when fed quaking aspen at 15°C, 23°C and 31°C (with the exception of PgPc at 23°C in 2000) , the surviving larvae did not differ from each other (Figure 4, 5, and 6). In 2000 PgPc larvae needed at least 43 more degree days to reach

pupation on quaking aspen than *P. canadensis* larvae (Table 4).

On tulip tree at 15 °C and 23°C, in both years the hybrid larvae needed as many or fewer degree days to reach pupation as their parental types. For example, in 1999 and 2000 at 23°C, PcPg larvae needed at least 55 and 27 respectively fewer degree days to reach pupation than *P. canadensis*(table 4). At 31°C in both years the surviving larvae did not differ statistically from each other in the number degree days needed to reach pupation (Figure 6).

Over all hybrids at each host plant and temperature treatment combination and in both years, needed the same or few number of degree days to reach pupation at the parental types.

Table 2: F-values (Type III SS) from ANOVA of 1999 degree day analysis. $P<0.05$. The model was a Split plot design with temperature as the whole plot and genotype and hostplant as the split plots. To obtain the correct F- value for temperature Block*Temperature is used as the error term.

Sources of Variation	DF	SS	MS	F	Pr<F
Block	1	25.94	25.94	0.01	0.92
Temperature	2	85695.27	42847.64	91.44	0.01
Block * Temperature	2	937.19	468.60	0.17	0.84
Genotype	3	76272.36	25424.12	9.32	0.0001
Hostplant	2	233002.64	116504.32	42.73	0.0001
Genotype * Hostplant	5	37556.85	7511.37	2.75	0.019
Temperature * Genotype	6	29214.71	4869.12	1.79	0.10
Temperature * Hostplant	4	20623.37	5155.84	1.89	0.11
Temperature * Genotype* Hostplant	8	12708.69	1588.56	0.58	0.79

Table 3: F-values (Type III SS) from ANOVA of 2000 degree day analysis. $P<0.05$. The model was a Split plot design with temperature as the whole plot and genotype and hostplant as the split plots. To obtain the correct F- value for temperature Block*Temperature is used as the error term.

Sources of Variation	DF	SS	MS	F	Pr<F
Block	1	3087.39	3087.39	1.25	0.2646
Temperature	2	134884.45	67442.23	1.14	0.47
Block * Temperature	2	118035.70	59017.85	23.96	0.0001
Genotype	3	90914.81	30304.95	12.30	0.0001
Hostplant	2	230076.29	115038.14	46.71	0.0001
Genotype * Hostplant	5	72928.56	14585.71	5.92	0.0001
Temperature * Genotype	6	22695.06	3782.51	1.54	0.17
Temperature * Hostplant	4	2675.38	668.85	.27	0.90
Temperature * Genotype* Hostplant	8	12070.34	1508.79	.61	0.77

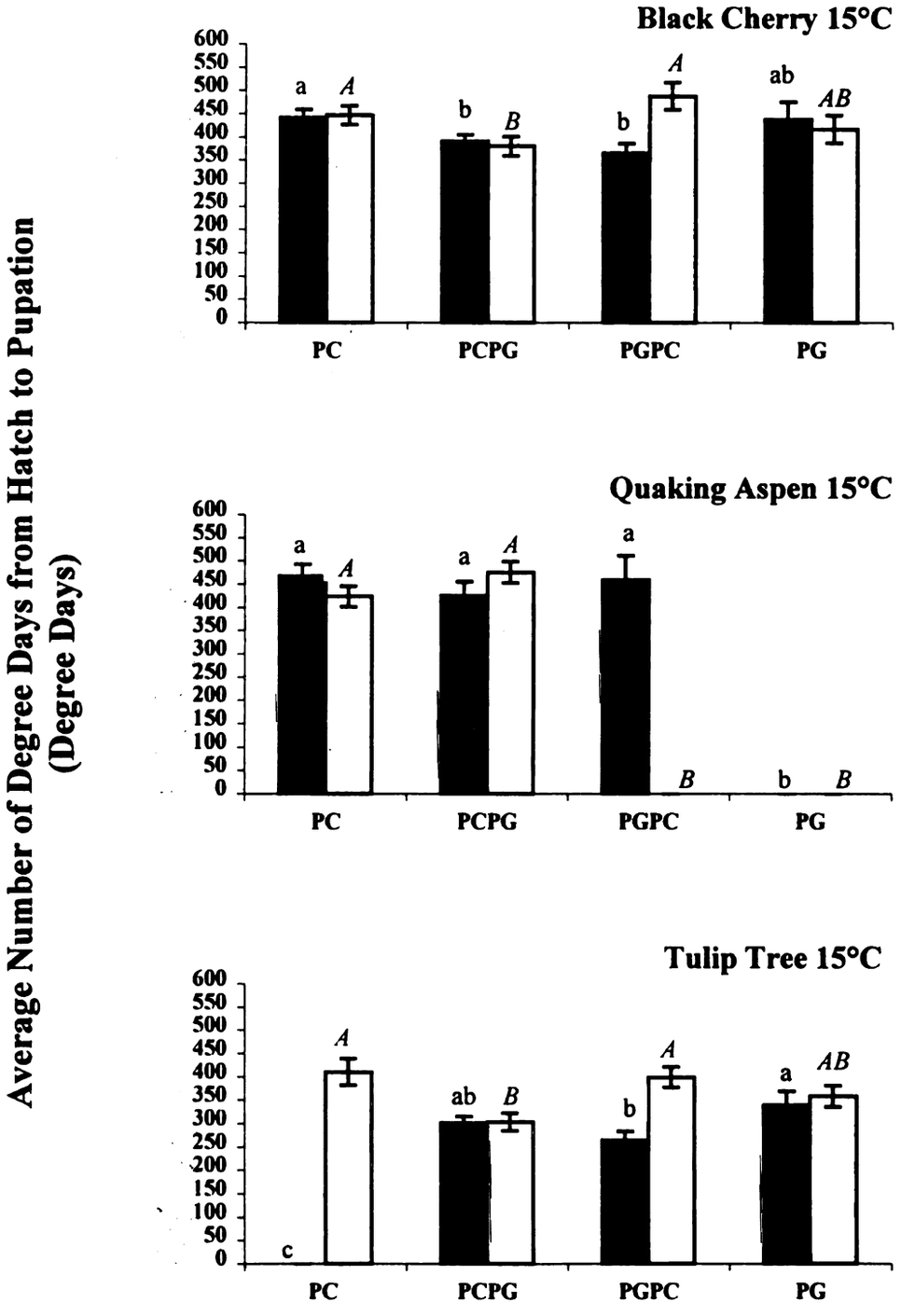

Figure 4: Average number of degree days from hatch to pupation at 15°C. All comparisons are made within a treatment group and with in a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

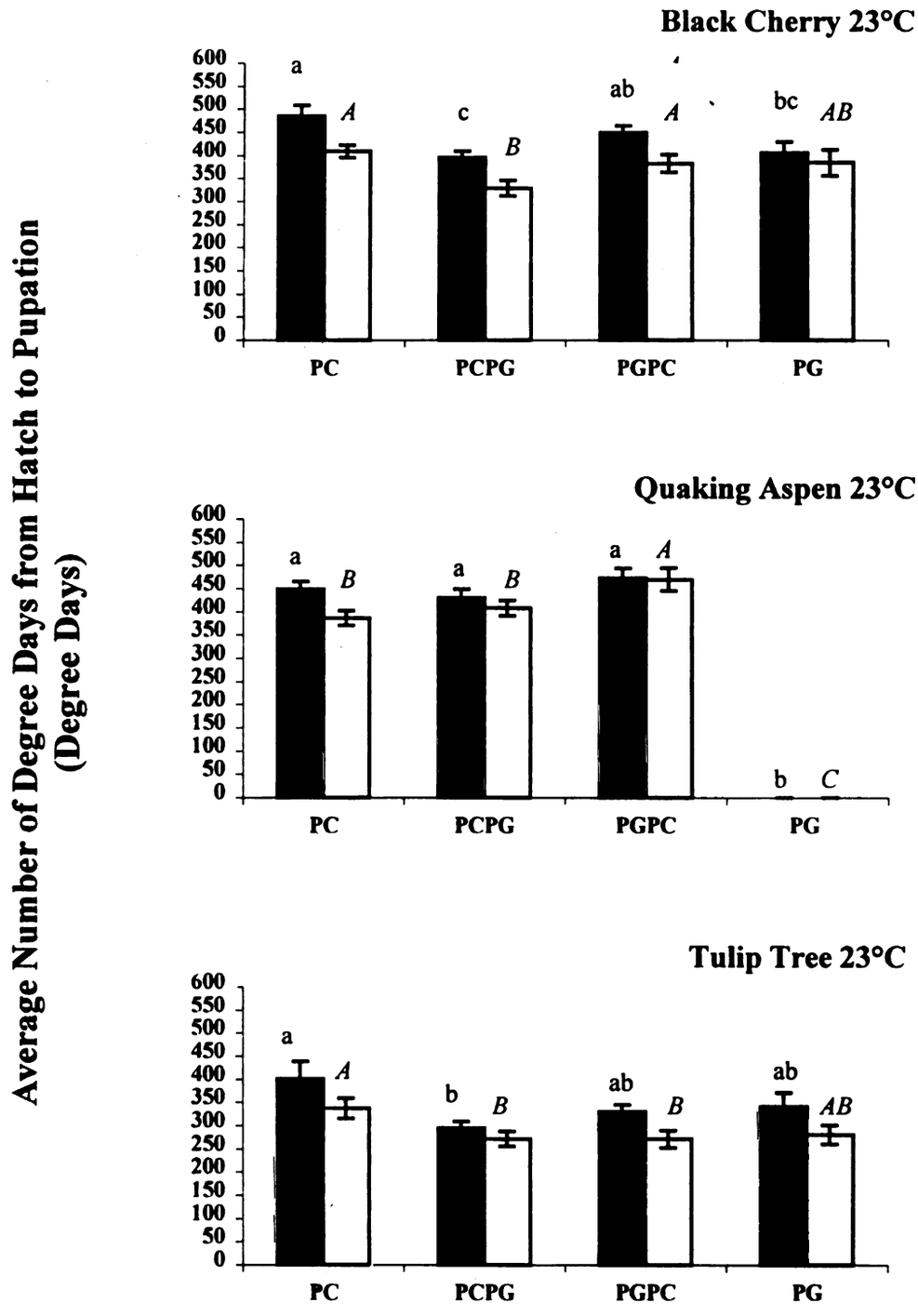

Figure 5: Average number of degree days from hatch to pupation at 23°C. All comparisons are made within a treatment group and with in a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

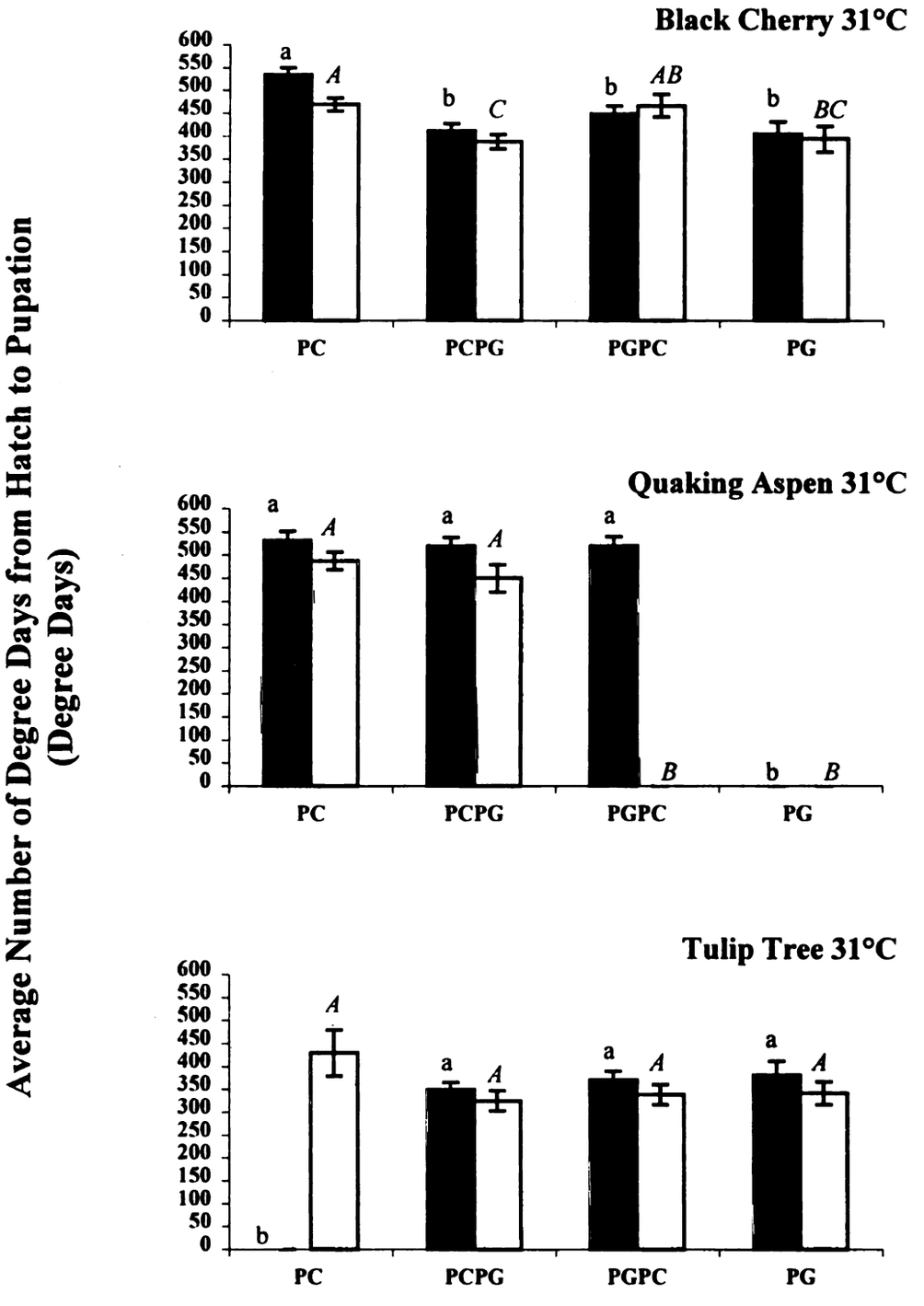

Figure 6: Average number of degree days from hatch to pupation at 31°C. All comparisons are made within a treatment group and with in a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Table 4: Average Number of Degree Days From Hatch to Pupation with ± 1 standard error. **** Denotes no survival until pupation. n=number of larvae that survived until pupation.

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000
Black Cherry 15°C	441±18 n=8	447±20 n=6	391±15 n=13	380±21 n=6	365±21 n=6	488±29 n=3	438±38 n=2	417±30 n=3
Black Cherry 23°C	486±22 n=6	409±14 n=13	397±12 n=18	330±17 n=9	450±15 n=12	383±19 n=7	406±23 n=5	385±29 n=3
Black Cherry 31°C	535±15 n=13	470±14 n=13	412±16 n=11	388±16 n=10	449±18 n=8	467±25 n=4	406±26 n=4	395±29 n=3
Quaking Aspen 15°C	466±26 n=4	423±22 n=5	424±30 n=3	475±23 n=5	458±53 n=1	****	****	****
Quaking Aspen 23°C	449±17 n=10	386±16 n=10	430±19 n=8	408±17 n=9	472±21 n=6	470±25 n=4	****	****
Quaking Aspen 31°C	532±19 n=8	487±19 n=7	519±19 n=8	450±30 n=3	520±20 n=7	****	****	****
Tulip Tree 15°C	****	410±29 n=3	301±13 n=16	304±19 n=7	264±19 n=8	399±22 n=5	339±30 n=3	358±22 n=5
Tulip Tree 23°C	401±37 n=2	337±22 n=5	296±13 n=16	272±16 n=10	330±15 n=12	271±19 n=7	342±30 n=3	281±20 n=6
Tulip Tree 31°C	****	429±50 n=1	350±16 n=11	325±22 n=5	370±20 n=7	338±22 n=5	381±31 n=3	341±25 n=4

Objective 2: To determine if hybrid pupae of *P. glaucus* and *P. canadensis* have significantly different weights.

In 1999 and 2000 there was a significant effect of genotype on pupal weight at P<0.0001/P<0.0001 (tables 5 and 6). In 1999 there was a significant effect of host plant (P<0.0001) on pupal weight (Table 5).

In general, when larvae were fed black cherry, the hybrids produced pupae that were as large as the parental types. The only exception was in 2000 at 23°C and 31° C , PcPg had significantly small pupae than *P. glaucus* (Figure 7, 8, and 9).

On quaking aspen, across all temperature treatments (15°C, 23°C and 31°C), there was only one case were a hybrid produced pupae that were significantly smaller than the surviving parental type (Figure 7, 8, and 9). PcPg's pupae, in 2000, were at least .109g small than *P. canadensis* pupae (Table 7).

By and large, when larvae were fed tulip tree they produced pupae that were statistically similar is size to at least one of the parental types. PcPg, in 1999 at 15°C, and in 2000 at 23° and 31°C, produced pupae that were significantly smaller than *P. glaucus* (Figure 7, 8 and 9). In all other cases, (except one, PgPc pupae in 1999 at 31°C were significantly larger than the surviving parental type: *P. glaucus*) there were no differences in pupal weights of the hybrids compared to the parental types.

Even though there were three cases where in one year hybrids produced pupae that were significantly smaller than the surviving parental type (tulip tree 15°C, tulip tree 31°C and quaking aspen 23°) this did not occur in both years and the other parental type did not have any larvae that survived at those treatment levels. In all other cases hybrids produced pupae that were statistically the same or larger as at least one

of the parental types.

Table 5: F-values (Type III SS) from ANOVA of 1999 pupal weight analysis. $P<.05$. The model was a split plot design with temperature as the whole plot and genotype and hostplant as the split plots. To obtain the correct F- value for temperature Block*Temperature is used as the error term

Sources of Variation	DF	SS	MS	F	Pr<F
Block	1	.001	.0.001	0.06	.8058
Temperature	2	0.54	0.27	8.40	.1063
Block * Temperature	2	0.06	0.03	1.38	0.25
Genotype	3	1.66	0.55	23.67	0.0001
Hostplant	2	0.58	0.29	12.35	0.0001
Genotype * Hostplant	5	0.35	0.07	2.99	0.01
Temperature * Genotype	6	0.052	0.008	0.37	0.90
Temperature * Hostplant	4	0.17	0.04	1.87	0.12
Temperature * Genotype * Hostplant	8	0.14	0.02	0.76	0.64

Table 6: F-values (Type III SS) from ANOVA of 2000 pupal weight analysis. $P<.05$. The model was a split plot design with temperature as the whole plot and genotype and hostplant as the split plots. To obtain the correct F- value for temperature Block*Temperature is used as the error term

Sources of Variation	DF	SS	MS	F	Pr<F
Block	1	0.002	0.001	0.09	0.77
Temperature	2	1.89	0.94	645.79	0.002
Block * Temperature	2	0.002	0.001	0.07	0.94
Genotype	3	0.51	0.16	7.54	0.0001
Hostplant	2	0.1	0.05	2.26	0.10
Genotype * Hostplant	5	0.27	0.05	2.44	0.04
Temperature * Genotype	6	0.4	0.06	2.99	0.01
Temperature * Hostplant	4	0.09	0.02	1.00	0.41
Temperature * Genotype * Hostplant	8	0.11	0.01	0.6	0.77

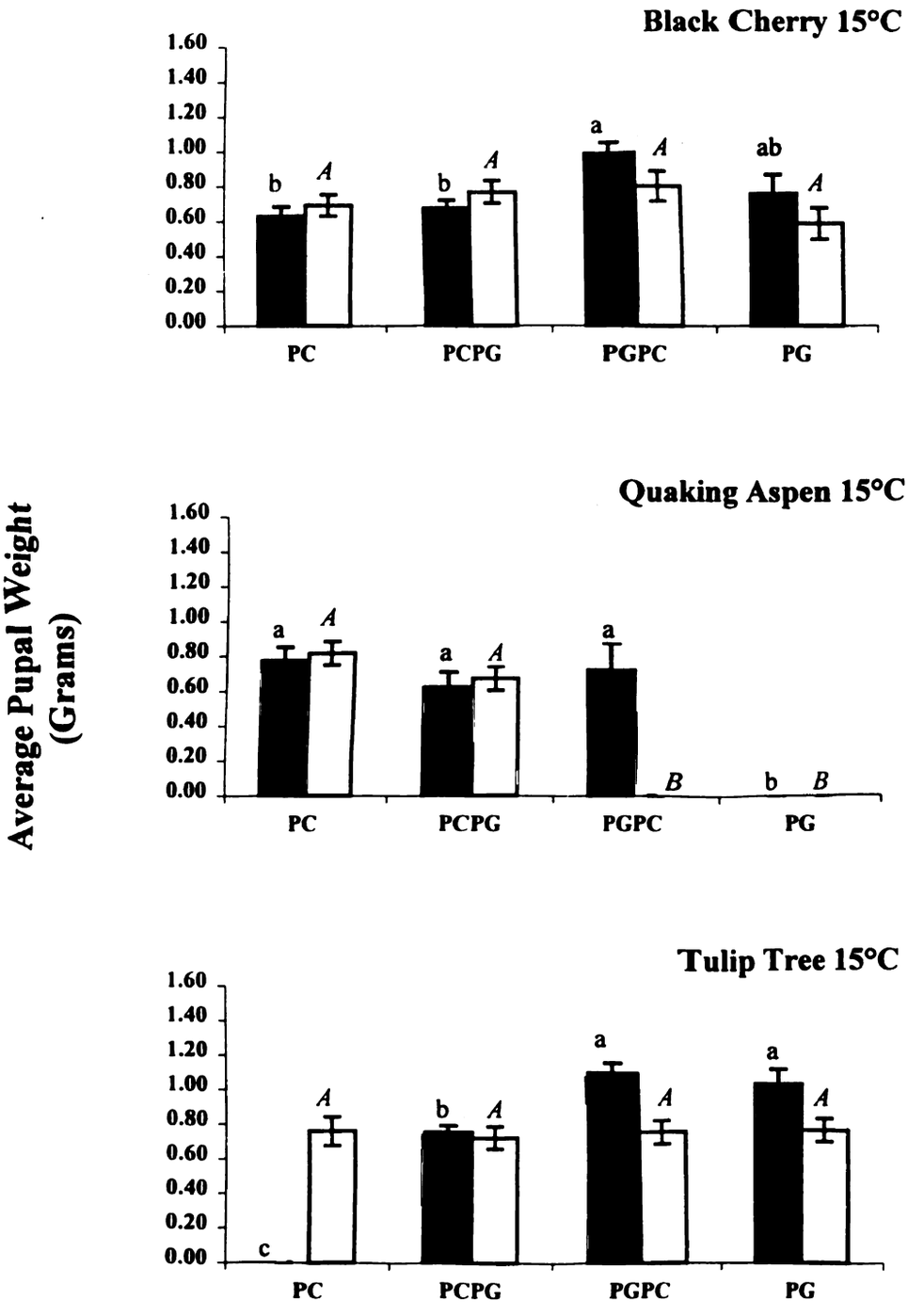

Figure 7: For average pupal weight at 15°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

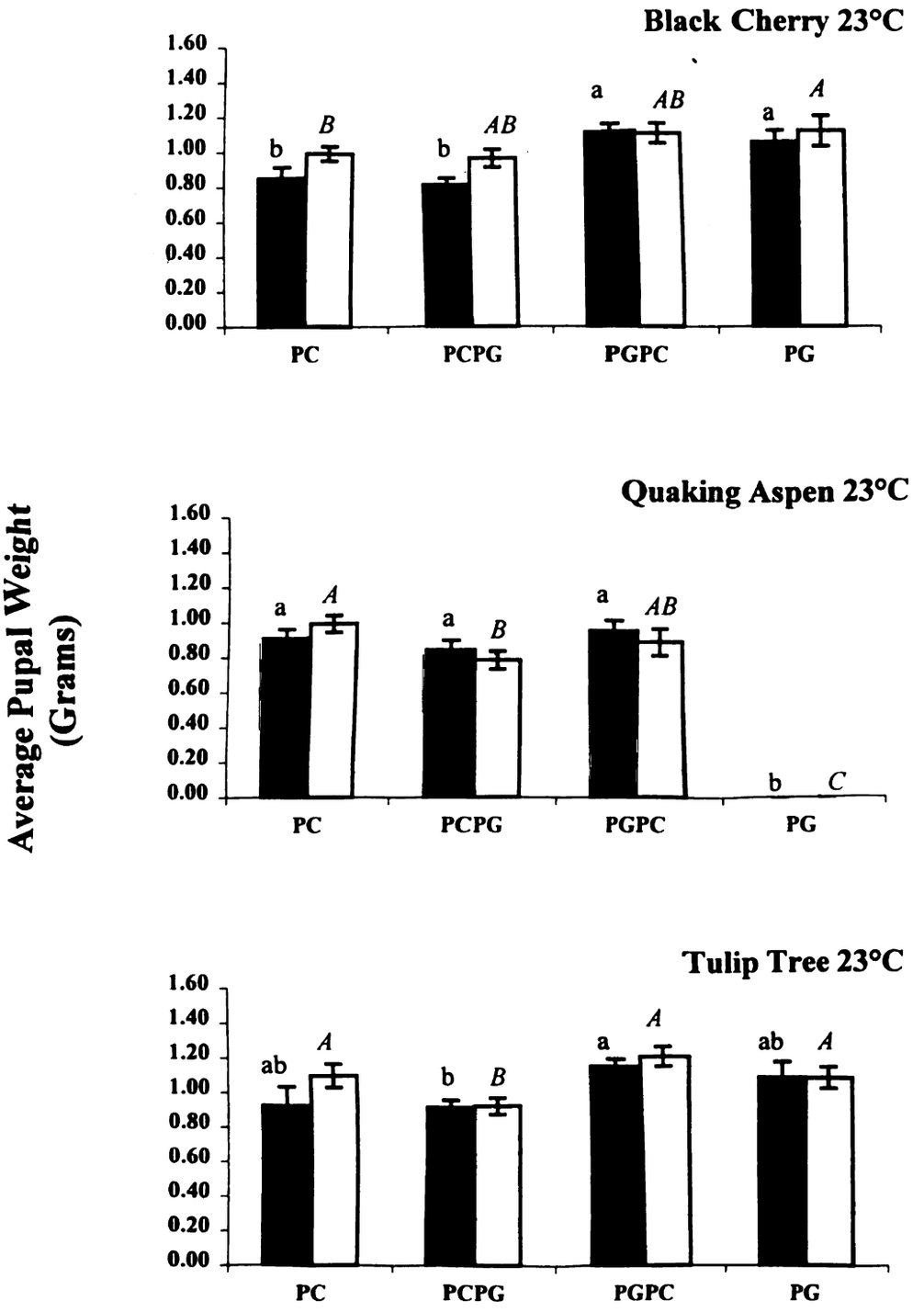

Figure 8: For average pupal weight at 23°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P< 0.05$.

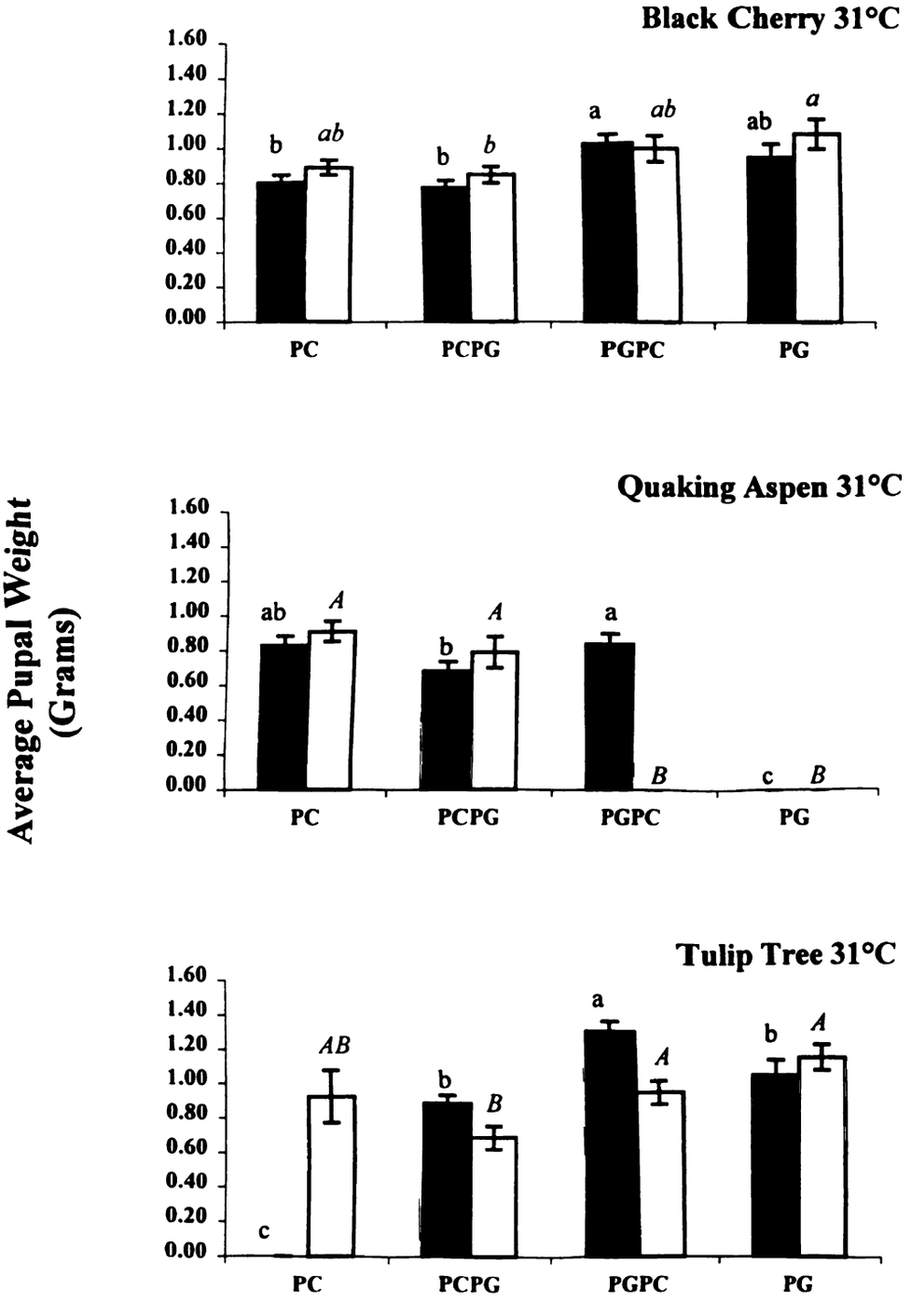

Figure 9: For average pupal weight at 31°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Table 7: Average pupal weight ± 1 standard error.
**** Denotes no survival until pupation. n=number of pupae.

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000
Black Cherry 15°C	.629±0.05 n=8	.690±0.06 n=6	.675±0.04 n=13	.768±0.06 n=6	.992±0.06 n=6	.796±.087 n=3	.755±0.11 n=2	.585±0.09 n=3
Black Cherry 23°C	.850±0.06 n=6	.991±0.04 n=13	.816±0.04 n=18	.967±0.05 n=9	1.117±0.04 n=12	1.100±.056 n=7	.052±0.07 n=5	1.117±0.09 n=3
Black Cherry 31°C	.804±0.04 n=13	.890±0.04 n=13	.772±0.04 n=11	.852±0.05 n=10	1.031±0.05 n=8	.995±.747 n=4	.945±0.08 n=4	1.081±0.09 n=3
Quaking Aspen 15°C	.776±0.08 n=4	.815±0.07 n=5	.620±0.09 n=3	.671±0.07 n=5	.720±0.15 n=1	****	****	****
Quaking Aspen 23°C	.912±0.05 n=10	.991±0.05 n=10	.841±0.05 n=8	.782±0.05 n=9	.948±0.06 n=6	.885±.075 n=4	****	****
Quaking Aspen 31°C	.830±0.06 n=8	.909±0.06 n=7	.680±0.05 n=8	.789±0.09 n=3	.837±0.06 n=7	****	****	****
Tulip Tree 15°C	****	.761±0.09 n=3	.755±0.04 n=16	.723±0.06 n=6	1.096±0.05 n=8	.754±.067 n=5	1.031±0.09 n=3	.768±0.07 n=5
Tulip Tree 23°C	.924±0.11 n=2	1.096±0.07 n=5	.917±0.04 n=16	.922±0.05 n=10	1.148±0.04 n=12	1.205±.057 n=7	1.086±0.09 n=3	1.084±0.06 n=6
Tulip Tree 31°C	****	.925±0.15 n=1	.888±0.05 n=11	.688±0.07 n=5	1.303±0.06 n=6	.948±.067 n=5	1.050±0.09 n=3	1.154±0.08 n=4

Objective 3: To determine if hybrid larvae of *P. glaucus* and *P. canadensis* have significantly different growth rates from hatch to pupation than parental type larvae.

In 1999 and 2000 there was a significant effect of temperature ($P<0.0046$/ $P<0.0131$), genotype ($P<0.0001$/$P<0.0006$) and hostplant ($P<0.0001$/$P<0.0001$) on growth rate (Tables 8 and 9).

In 1999 and 2000, of the larvae that survived to pupation at 15°C, there were no significant differences in growth rate, at any level of the host plant treatments (black cherry, quaking aspen and tulip tree), between the hybrids and the parental types (Figure 10).

In general, at 23°C they hybrid's growth rates, across all host plant treatments, were the same or faster than the surviving parental type larvae (in 1999 and 2000 *P. glaucus* did not survive to pupation on quaking aspen) (Figure 11). However, in 2000 on black cherry and in 2000 on quaking aspen and tulip tree, PcPg's growth rates were significantly slower than one of the parental types (Figure 11).

In both years on black cherry at 31°C PgPc did not differ statistically from *P. glaucus* and in 2000 it did not differ from *P. canadensis* (Figure 12). In 1999, PgPc grew an average of at least 0.012 g/day faster than *P. canadensis* (Table 10). In both years PcPg had a significantly slower growth rate than *P. glaucus* but grew at the same rate or faster than *P. canadensis*.

In both years, on quaking aspen at 31°C, the surviving hybrid larvae did not differ statistically in growth rate from *P. canadensis*.

Even though in 2000, on tulip tree at 31°C, PcPg and PgPc had significantly

Table 8: F-values (Type III SS) from ANOVA of 1999 growth rate analysis. $P<0.05$. The model was a split plot design with temperature as the whole plot and genotype and hostplant as the split plots. To obtain the correct F- value for temperature Block*Temperature is used as the error term

Sources of Variation	DF	SS	MS	F	Pr<F
Block	1	0.000001	0.000002	.05	0.83
Temperature	2	0.02	0.01	217.67	0.004
Block * Temperature	2	0.0001	0.0001	1.28	0.28
Genotype	3	0.001	0.001	12.87	0.0001
Hostplant	2	0.004	0.002	45.07	0.0001
Genotype * Hostplant	5	0.001	0.0002	4.65	0.0005
Temperature * Genotype	6	0.001	0.0001	2.48	0.02
Temperature * Hostplant	4	0.001	0.0004	8.45	0.0001
Temperature * Genotype * Hostplant	8	0.0004	0.00001	1.26	0.27

Table 9: F-values (Type III SS) from ANOVA of 2000 growth rate analysis. $P<0.05$. The model was a split plot design with temperature as the whole plot and genotype and hostplant as the split plots.. To obtain the correct F- value for temperature Block*Temperature is used as the error term

Sources of Variation	DF	SS	MS	F	Pr<F
Block	1	0.0001	0.0001	1.37	0.24
Temperature	2	0.03	0.02	75.12	0.01
Block * Temperature	2	0.0004	0.0002	3.36	0.04
Genotype	3	0.001	0.0004	6.11	0.0006
Hostplant	2	0.003	0.001	20.98	0.0001
Genotype * Hostplant	5	0.001	0.0003	4.29	0.001
Temperature * Genotype	6	0.001	0.0002	3.56	0.003
Temperature * Hostplant	4	0.001	0.0002	3.40	0.01
Temperature * Genotype * Hostplant	8	0.0003	0.00003	0.60	0.78

slower growth rates than *P. glaucus*, in 1999, they had a statistically identical or faster (PgPc had a faster growth rate than *P. glaucus* of at least 0.01g/day) growth rates than the parental types.

When hybrids are compared to their parental type they did not show any reduction in growth rate. Furthermore, across all treatment levels of host plant and temperature, the hybrids had a growth rate that was as fast or faster than at least on of the parental types.

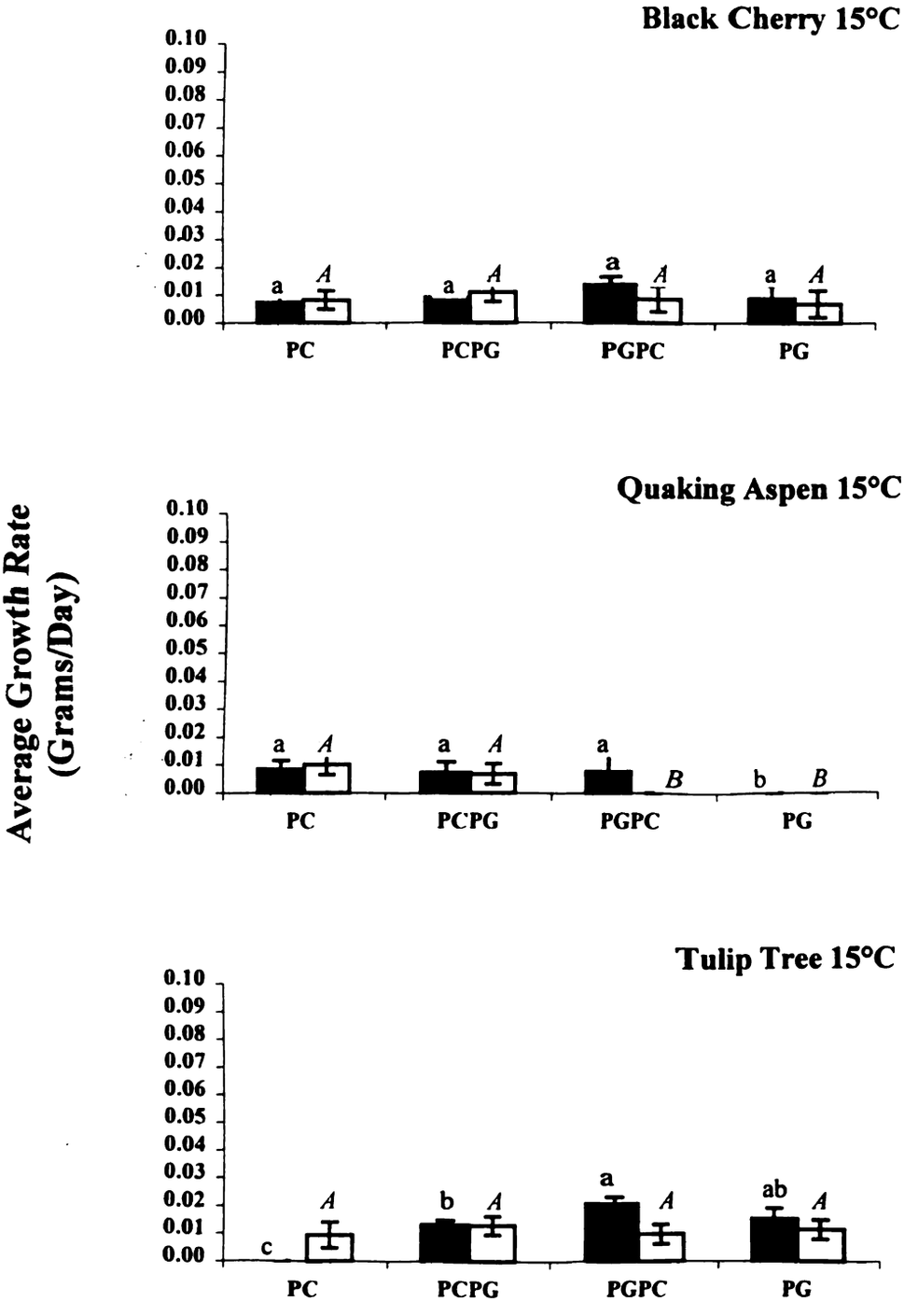

Figure 10: For average growth rate at 15°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Average Growth Rate
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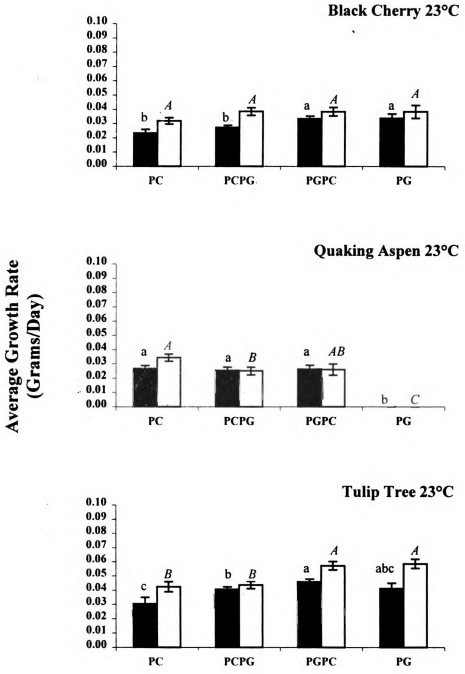

Figure 11: For average growth rate at 23°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Average Growth Rate
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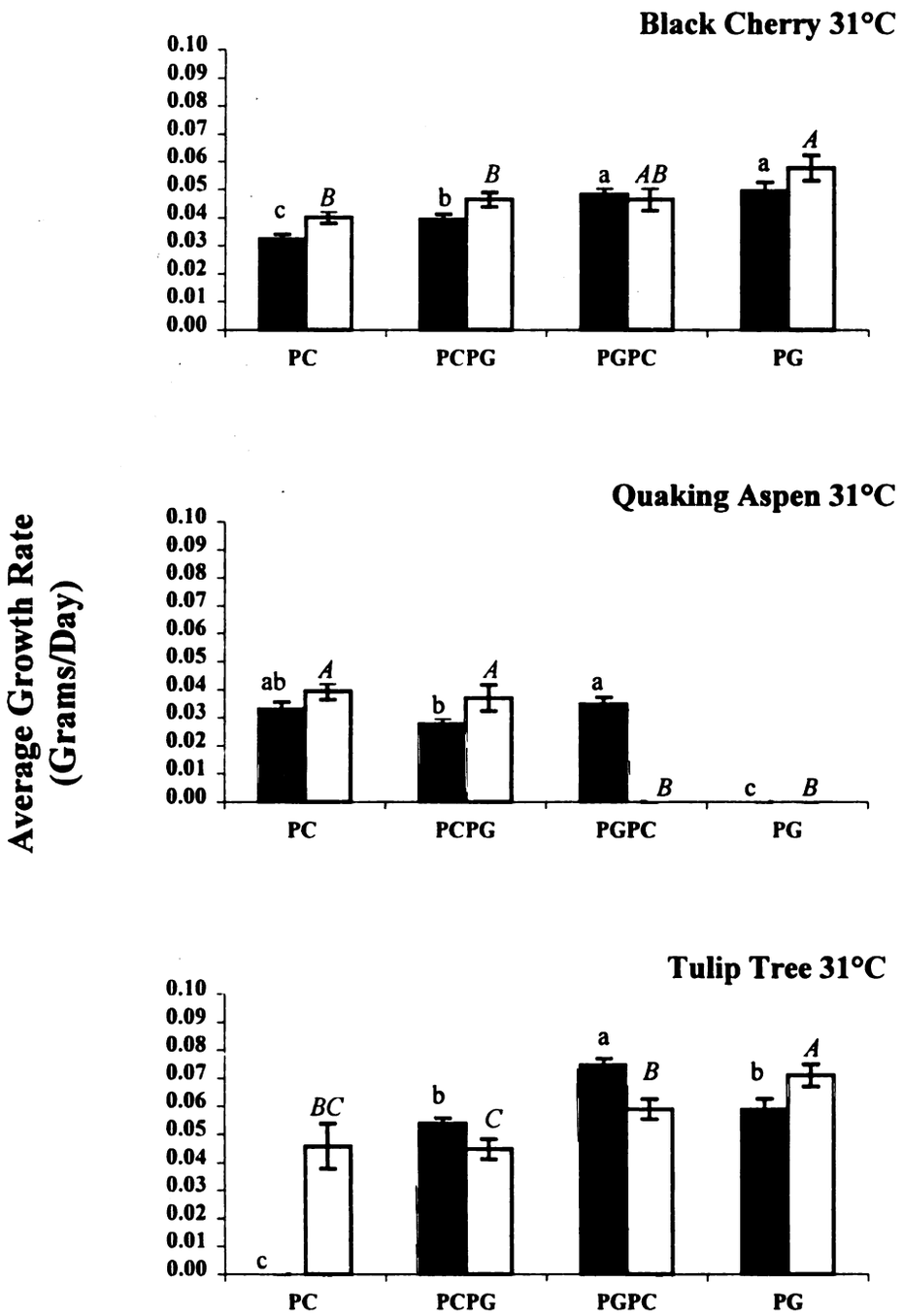

Figure 12: For average growth rate at 31°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent means from 1999 and black bars represent means from 2000. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Table 10: Average growth rate ± 1 standard error.
***** Denotes no survival until pupation. n=number of pupae.*

Table 10: Average growth rate ± 1 standard error.
**** Denotes no survival until pupation. n=number of pupae.

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000
Black Cherry 15°C	.007±0.002 n=8	.008±0.003 n=6	.009±0.002 n=13	.011±0.003 n=6	.014±0.003 n=6	.008±0.005 n=3	.009±0.005 n=2	.007±0.005 n=3
Black Cherry 23°C	.023±.003 n=6	.032±0.002 n=13	.027±0.002 n=18	.039±0.003 n=9	.033±0.002 n=12	.038±0.003 n=7	.034±0.003 n=5	.038±0.005 n=3
Black Cherry 31°C	.032±0.002 n=13	.040±0.002 n=13	.039±0.002 n=11	.046±0.003 n=10	.048±0.002 n=8	.047±0.004 n=4	.049±0.003 n=4	.058±0.005 n=3
Quaking Aspen 15°C	.008±0.003 n=4	.010±0.004 n=5	.007±0.004 n=2	.007±0.004 n=5	.008±0.006 n=1	****	****	****
Quaking Aspen 23°C	.027±0.002 n=10	.034±0.003 n=10	.025±0.002 n=8	.025±0.003 n=9	.026±0.003 n=6	.026±0.004 n=4	****	****
Quaking Aspen 31°C	.033±0.002 n=8	.039±0.003 n=7	.028±0.002 n=8	.037±0.005 n=3	.035±0.002 n=7	****	****	****
Tulip Tree 15°C	****	.009±0.005 n=3	.013±0.002 n=16	.013±0.003 n=6	.021±0.002 n=8	.009±0.004 n=5	.015±0.004 n=3	.011±0.004 n=5
Tulip Tree 23°C	.030±0.005 n=2	.042±0.004 n=5	.041±0.002 n=16	.044±0.003 n=10	.046±0.002 n=12	.057±0.003 n=7	.041±0.004 n=3	.059±0.003 n=6
Tulip Tree 31°C	****	.045±0.008 n=1	.054±0.002 n=11	.045±0.004 n=5	.075±0.002 n=7	.059±0.004 n=5	.059±0.004 n=3	.071±0.004 n=4

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Objective 4: determine if hybrid larvae of *P. glaucus* and *P. canadensis* have significantly different survival rates to pupation than parental type larvae.

Percent survival until pupation data was tested for significant differences with a Kruskal-Wallis Chi-Square test (Proc npariway wilconxon anova: SAS Institute Inc. 1990)..

When fed to mutually-acceptable host plant black cherry, both hybrid types survived as well as both parental types in all temperature treatments. Except for lower survival of *P. canadensis* at 23°C in 1999 (χ^2, P>0.02) (Figure 14) no significant differences were observed between the four genotypes of butterflies at 15°C (χ^2, P>0.66 in 1999; χ^2, P> 0.28 in 2000, Figure 13) , at 23°C (χ^2, P>0.08 in 2000, Figure 14) nor at 31°C (χ^2, P> 0.63 in 1999; χ^2, P> 0.1 in 2000, Figure 15).

In general, on quaking aspen, hybrids survived as well as *P. canadensis* and *P. glaucus* at all temperatures in both years (Figures 13, 14 and 15). However, in 2000, the PgPc hybrid larvae had a significantly lower survival than *P. canadensis* at 31°C(Figure 15).

On tulip tree at 15°C and 23°C, *P. canadensis* survival was significantly lower than the hybrids in 1999 but not at significant levels in 2000. Hybrid larvae on tulip tree survived at numerically higher levels than both *P. canadensis* and *P. glaucus* in every treatment (15, 23, 31°C) but in no case did these levels of hybrid survival reach statistical significance (Figures 13, 14, 15; table 11)

Percent Survival to Pupation

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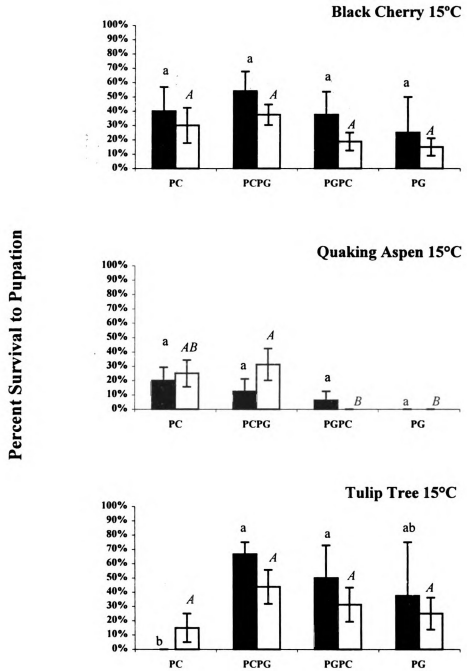

Figure 13: Percent survival at 15°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent percent survival from 1999 and black bars represent percent survival from 2000. Values are expresses as percent survival to pupation. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as an average of the family means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Percent Survival to Pupation

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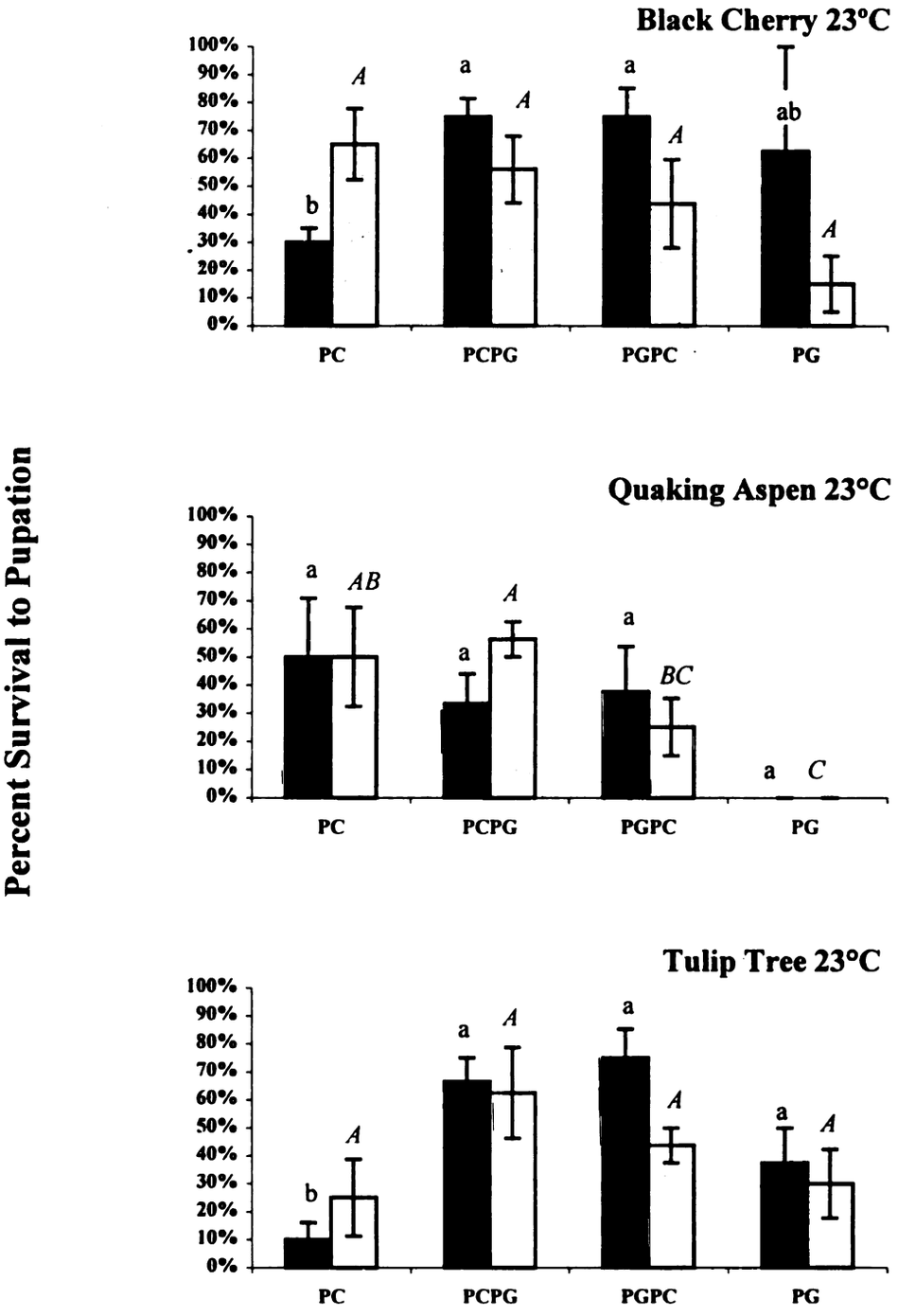

Figure 14: Percent survival at 23°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent percent survival from 1999 and black bars represent percent survival from 2000. Values are expresses as percent survival to pupation. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as an average of the family means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Percent Survival to Pupation

Figure 15: p
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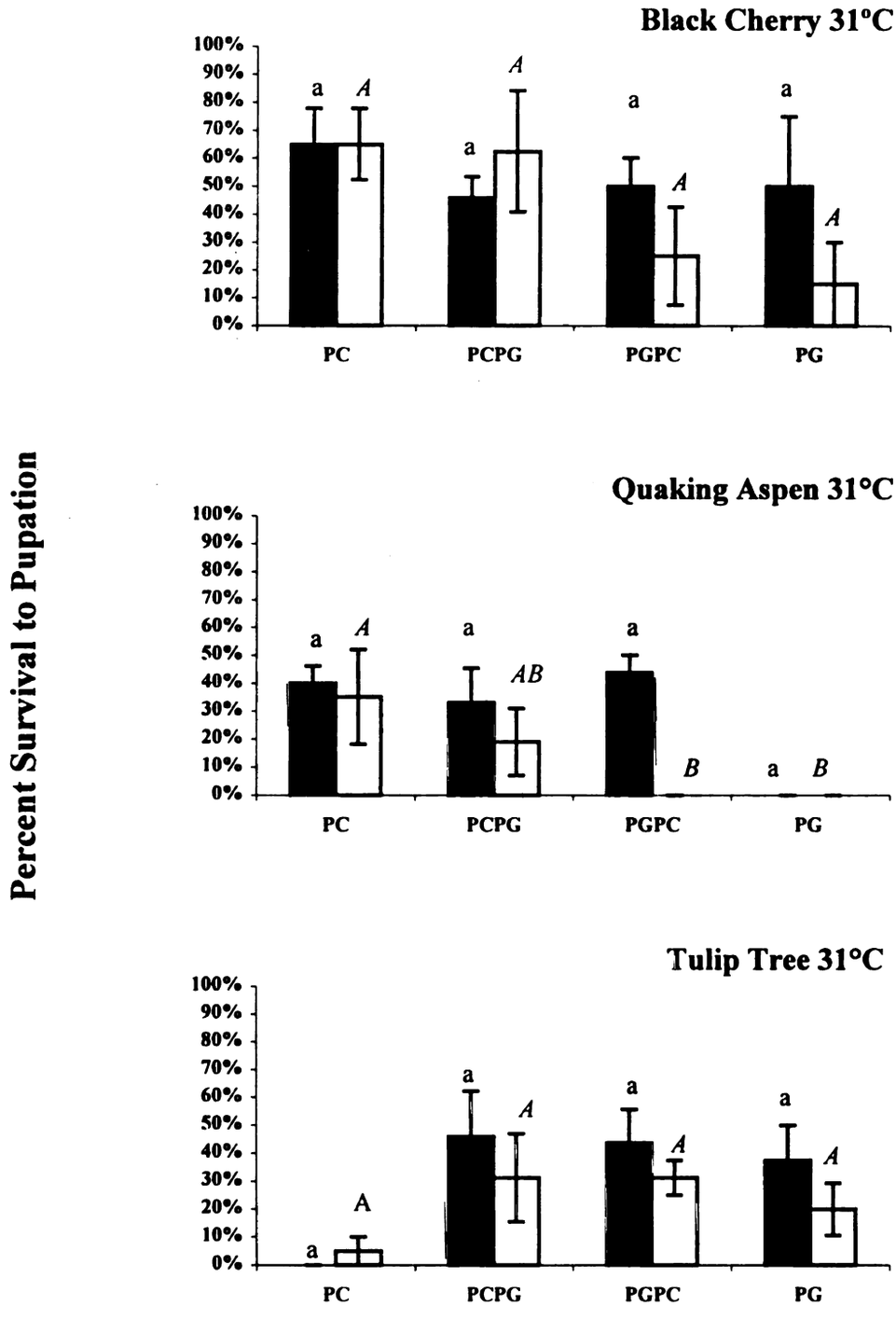

Figure 15: Percent survival at 31°C. All comparisons are made within a treatment group and within a year. Each graph represents a treatment group. White bars represent percent survival from 1999 and black bars represent percent survival from 2000. Values are expresses as percent survival to pupation. Capital letters for significant differences indicate 1999 data and lower case letters indicate 2000 data. Values are expressed as average of the family means, error bars are ±1 s.e. Bars not sharing a letter are significantly different from each other at $P<0.05$.

Table 11: Percent Survival to Pupation with ± 1 standard error.
***** Denotes no survival until pupation. n =number of families.*

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000

Table 11: Percent Survival to Pupation with ± 1 standard error.
**** Denotes no survival until pupation. n=number of families.

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000
Black Cherry 15°C	40%±17% n=5	30%±12% n=5	54%±14% n=6	38%±7% n=4	38%±16% n=4	19%±6% n=4	25%±3% n=2	15%±6% n=5
Black Cherry 23°C	30%±5% n=5	65%±13% n=5	75%±6% n=6	56%±11% n=4	75%±10% n=4	44%±16% n=4	63%±38% n=2	15%±10% n=5
Black Cherry 31°C	65%±25% n=5	65%±13% n=5	46%±16% n=6	63%±22% n=4	50%±10% n=4	25%±18% n=4	50%±13% n=2	15%±15% n=5
Quaking Aspen 15°C	20%±9% n=5	25%±11% n=5	13%±9% n=6	31%±11% n=4	6%±6% n=4	****	****	****
Quaking Aspen 23°C	50%±21% n=5	50%±18% n=5	33%±11% n=6	56%±6% n=4	38%±16% n=4	25%±10% n=4	****	****
Quaking Aspen 31°C	40%±6% n=5	35%±17% n=5	33%±12% n=6	19%±12% n=4	44%±6% n=4	****	****	****
Tulip Tree 15°C	****	15%±10% n=5	67%±8% n=6	44%±12% n=4	50%±23% n=4	31%±12% n=4	38%±38% n=2	25%±11% n=5
Tulip Tree 23°C	10%±6% n=5	25%±14% n=5	67%±8% n=6	63%±16% n=4	75%±10% n=4	44%±6% n=4	38%±13% n=2	30%±12% n=5
Tulip Tree 31°C	****	5%±5% n=5	46%±16% n=6	31%±16% n=4	44%±12% n=4	31%±6% n=4	38%±13% n=2	20%±9% n=5

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DISCUSSION

This study was an attempt to compare the fitness of parental type and hybrid larvae of *P. glaucus* and *P. canadensis* Swallowtail butterflies under different thermal and host plant regimes. I looked at four indicators of fitness: number of degree days from hatch to pupation, pupal weight, growth rate and percent survival until pupation. Over the two year period of this study, I did not observe any evidence for hybrid larvae performing more poorly than both their parental types. In fact, hybrids of both types performed at least as well as one parent and sometimes better than both parents.

It is clear that pairings between *P. canadensis* and *P. glaucus* parental types produced healthy and viable larvae. Hybrids were able to survive on host plants that their parental types did poorly on. *P. canadensis* larvae have low survival when fed tulip tree (*Liriodendron tulipfera*) and *P. glaucus* have low survival on quaking aspen (*Populus tremuloides*) (Hagen et al., 1991). Hybrids consistently produced pupae that were well within the normal size range for both species and their growth rates also fell well within the range of their parental types. Finally, hybrids don not show a significant reduction in survival of larvae to pupation.

Consequences of Hybridization

Three commonly described consequences of hybridization are : 1) reinforcement of speciation due to the evolution of pre-mating barriers to gene exchange in response to selection against the hybrids; 2) extinction of one or the other parental forms; 3) fusion of the species (Harrison 1993, Rhymer and Simerloff 1996, Arnold

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To date evidence of complete prezygotic barriers in the *P. canadensis* and *P. glaucus* hybrid complex have not been found (Deering, 1998). In 1998, Deering showed, through field tethering studies, that wild males of both *P. glaucus* and *P. canadensis* prefer females of *P. glaucus*. *P. glaucus* preferred conspecific females 94.2% of interactions and *P. canadensis* males preferred heterospecific females 82.3% of interactions (Deering, 1998).

Stump (2000) found no evidence for postpairing prezygotic barriers. He looked at four indices of success for heterospecific and conspecific matings: copulation duration, spermatophore deposition, oviposition and egg hatchability. It was shown that their was no reduction in any of the four factors of pairing success (Stump, 2000), when females were mated once. He went on to show that that their was no evidence for consistent conspecific sperm precedence in *P. canadensis* or *P. glaucus* in females mated to both a conspecific and a heterospecific male (Stump, 2000). It was found that some females continued to exclusively use sperm from the original mating others used sperm from the second mate exclusively and some used sperm from both mates (Stump, 2000).

The extinction of one of the parental types usually occurs in cases where one of the parental types is rarer than the other and the and the species that is "taking over" is introduced (Rhymer and Simberloff 1996). This in not the case with the *P. glaucus* and *P. canadensis* system. Both are native abundant species and have vast ranges (figure 1).

Incomplete prezygotic barriers and postzygotic barriers through the larval stage

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may result in the two taxa diffusing into each other with their unique characters fading away as their trait clines "decay" and become broader or less steep (Scriber 2001a in press, Rymer and Simberloff 1996; Porter et al., 1997).

It is thought that the North American *P. glaucus* group, which is comprised of *P. glaucus*, *P. canadensis*, *P. alexiares*, *P. eurymedon*, *P. rutulus*, and *P. multicaudatus*, are descendent from one common ancestor (Scriber 1996). This "proto" *P. glaucus* type, before the Pleistocene glaciations, was believed to have had a continuous range through out North America. As the glaciers expanded southward they may have spilt the proto *P. glaucus* type into two populations, one to the North in the Beringial refugia (Alaska) and one into a refuge South of the Laurentide ice sheet. It is known, though fossil records that populations of *Populus balsamifera* and *Populus tremuloides* persisted in the Beringial refugia during the glaciation, 36,000 B.P.

The isolation of the Beringial population for 25,000 years (36,000 to 9,500 years B.P.) with only Salicaceae as an host plant would have been enough time for the ability to detoxify allelochemicals produced by the Salicaceae host plant (Scriber 1988) to be selected for. This would explain the ability of *P. canadensis* to survive on quaking aspen and the loss of the ability to survive on the current southern host of *P. glaucus*, tulip tree. With the retreat of the glaciers we see a secondary contact of what are now two species of butterflies: *P. glaucus* and *P. canadensis*.

With the increased frequency of *P. canadensis* near the hybrid zone surviving on tulip tree over the last three years, other morphological and physiological traits that seem to be moving northward, possibly as a result of global warming (Scriber et al 2001b, Scriber et al 2001d) and the lack of strong prezygotic and postzygotic barriers

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Benefits of Hybridization

Traditional wisdom has generally emphasized hybrids as evolutionary dead ends for animal species e.g. Mayr (1963). However, it is important to recognize the benefits that hybridization can play. Hybridization can also allow for rapid evolutionary change in populations by creating novel and beneficial gene combinations (Rhymer and Simberloff 1996, Lewontin and Birch 1966). Botanists have recognized that this phenomenon may lead to increased fitness and adaptation to new environments in existing taxa (Rhymer and Simberloff 1966, Arnold 1997). Lewontin and Birch give an insect example in their paper "Hybridization as a Source of Variations for Adaptation to New Environments". They cite the example of *Dacus tryoni* expanding its range by hybridizing with the closely related *D. neohumeralis*. It is apparent that the two species exchange genes through hybridization. The gene exchange has not been great enough to merge the species, presumable because of selection against the hybrids, but it has been enough to incorporate foreign species genes into each other's gene pool (Lewontin and Birch 1966). This could be the case with *P. canadensis* larvae being able to survive on tulip tree.

As a catalyst for the evolutionary process by the creating novel gene combinations in the hybrids, hybrids can occupy and thrive in novel (disturbed) habitats were the parental types were unable to survive. Anderson and Stebbins (1954) proposed that four nearly concurrent events during the Cretaceous period: retreat of seas, overgrazing by dinosaurs, the diversification modern like birds which transported seeds

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long distances, and rise of flower pollinating bees and other insects all contributed to the diversification and dominance of angiosperms. They propose that these events created conditions favorable for hybridization. i.e. new habitat for colonization and bringing together of species that were previously isolated. In this case, hybridization may have acted as one of the fuels of evolution that led to the diversification of the angiosperms.

It is known that with the radiation of the angiosperms came the radiation of insects. With these conditions fostering an ideal environment for plant hybridization, it could also have been an ideal environment for insect hybridization.

Future Research

Even though this study did not show any reduction in fitness, in the laboratory, at the larval stage, hybrids may show lower fitness at any life stage in the field. Further investigation in the *P. canadensis* and *P. glaucus* hybrid complex is needed to determine what forces are at work in this system and whether or not hybrids are truly as fit as their parental types. Research in this area has been primarily done in a laboratory setting. Laboratory studies have been useful in analyzing fitness of hybrids and the role of prezygotic and postzygotic barriers in isolating closely related taxa (Gregory and Howard 1993). However, they cannot look at components of fitness that depend on external effects (Gregory and Howard 1993). Further research in the field might reveal that the hybrids are being selected against by some factor that is not replicable in the laboratory setting.

The possibility of hybrids being unfit at the pupal and adult stage needs investi-

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gation. Preliminary laboratory work has shown that hybrid pupal survival at high temperatures (30°C, 33°C, 36°C) is greater for hybrids than parental types (Scriber et al 2001c). Even with this being true, reduction of fitness could manifest itself as hybrids acting as a parasitoid/predator sink (West and Hazel 1982). It also has been shown that there is a slight Haldane effect in the slightly higher mortality of *P. glaucus x P. canadensis* hybrid female pupae (Hagen and Scriber 1995).

Whether or not hybrids are being selected as mates by either male parental type needs to be looked at. An intriguing study by Deering (1998) showed that *P. canadensis* males prefer *P. glaucus* females 82.3% of the time but no study to date has investigated male selection of hybrids as mates.

It has been determined, through electrophoretic analysis that natural hybrids and backcrosses do exist but at unknown natural frequency and fitness. I suggest that a cohort analysis, similar to the Howard et al (1993) study, of Tiger Swallowtails within and through the hybrid zone be undertaken to begin to characterize the fitness of all classes of hybrids in a field setting. This would involve repeatedly sampling and genetically analyzing different populations within the hybrid zone over the span of several field seasons. A cohort analysis would begin to answer such questions as: Are hybrids emerging synchronously with their parental counterparts? At what frequency are the hybrids present? What kinds of genetic classes are present? Are hybrids being selected as mates by heterospecifics and conspecifics or other hybrids? Are hybrids being selected against by unknown forces?

APPENDICES

APPENDIX 1

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

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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.: 2001-04

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

Are Hybrids More Fit Than Their Parental Types?
A Test Using Two Tiger Swallowtail Butterfly Species
Papilio glaucus and *P. canadensis*
(Lepidoptera: Papilionidae)

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)

Jennifer Donovan

Date 7/23/2001

*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: Include 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 SPECIMENT DATA

Appendix 1.1

Voucher Specimen Data

Page 1 of 6 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio glaucus (L.)	MICHIGAN St. Joseph Co. State Route 131 22-May-00 J. Donovan					1			
"	MICHIGAN St. Joseph Co Purgatory Road 1-Jun-00 J. Donovan						1		
"	MICHIGAN St. Joseph Co Purgatory Road 22-Jun-00 J. Donovan						1		

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Investigator's Name(s) (typed)

Jennifer Donovan

Date 23-Jul-01

Voucher No 2001-04

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

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 2 of 6 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
P. canadensis (Rothschild & Jordan)	VERMONT Bennington Co Bought Pupae from H. Romack Emerged 5 June 2000 J. Donovan						2		
"	VERMONT Bennington Co. Bought Pupae from H. Romack Emerged 27 May 2000 J. Donovan					1			
Papilio canadensis (R&J) (♀) x *P. glaucus* (L.) (♂)	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 15 June 1999 J. Donovan					1			

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Jennifer Donovan

Date 23-Jul-01

Voucher No 2001-04

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

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 3 of 6 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio canadensis (R&J) (♀) x *P. glaucus* (L.) (♂)	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 7 July 1999 J. Donovan						1		
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 12 July 1999 J. Donovan					1			
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em 22 July 1999 J. Donovan						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Jennifer Donovan

Date 23-Jul-01

Voucher No 2001-04

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

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 4 of 6 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio canadensis (R&J) (♀) *P. glaucus* (L.) (♂)	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 24 July 1999 J. Donovan					1			
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 17 July 1999 J. Donovan					1			
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 7 July 1999 J. Donovan						1		

(Use additional sheets if necessary)

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Appendix 1.1

Voucher Specimen Data

Page 5 of 6 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
P. canadensis (R&J) (♀) x *P. glaucus* (L.)(♂)	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 10 July 1999 J. Donovan						1		
P. glaucus (L.) (♀) x *P. canadensis* (R&J) (♂)	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 18 July 1999 J. Donovan					1	1		
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 28 July 1999 J. Donovan						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Jennifer Donovan

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Appendix 1.1

Voucher Specimen Data

Page 6 of 6 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Aduits ♀	Adults ♂	Other	Museum where deposited
P. glaucus (L.) (♀) x *P. canadensis* (R&J) (♂)	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 17 July 1999 J. Donovan					1			
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em. 12 July 1999 J. Donovan						1		
"	MICHIGAN Ingham Co. East Lansing - MSU Campus - Lab Culture Em 24 July 1999 J. Donovan					1			

(Use additional sheets if necessary)

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APPENDIX 2

FAMILY 15116

In 1999 one *P. canadensis* female was caught in Charlevoix county, MI. Her offspring were used as one of the *P. canadensis* families in the multiple indicators of fitness study presented in this thesis. Her off spring demonstrated high survival on Tulip Tree, a known poor host of Canadensis larvae and direct development. *P. canadensis* are obligate diapausers. It was suspected that her offspring were actually hybrid type not parental type. To confirm suspicions, diagnostic allozymes were run on the mother and her offspring. The results confirmed that female 15116 was a pure parental type *P. canadensis* and her offspring were hybrids. This has led us to believe that this is evidence of field mating between a heterospecific species. i..e. *P. canadensis* female was mated to a *P. glaucus* male in the field (Donovan et al 2001).

Consequently, *P. canadensis* female 15116's offspring were eliminated from the analysis that compared multiple indicators of fitness.

INDIVIDUALS RUN	HK	PGD	LDH
15116 male offspring	110 ?	-125/-100	80/100
15116 male offspring	110?	-125/-100	80/100
15116 female offspring	110?	-100	100
15116 female offspring	100/110?	-100	100
15116 female offspring	110?	-100	100
***P. glaucus* control**	100	-100/-50	100
***P. glaucus* control**	100	-100	100
***P. canadensis* control**	110	-125	80
15116 female mother	110	-125	80?
15116 female offspring	100/110?	-100	100
15116 female offspring	100/110?	-100	100
15116 female offspring	100/110?	-100	100

Table 12: Diagnostic allozymes ran to confirm genotypic identity of putative *P. canadensis* female mother 15116 and her offspring. Question marks indicate that resolution was difficult but best guesses were made.

APPENDIX 3

ALLOZYME ELECTROPHORSIS ANNAYLSIS OF PARENTS

Individual parents of all families used in these studies were characterized through analysis of two diagnostic enzyme loci: 6-phosphogluconate dehydrogenase (PGD) and lactate dehydrogenase (LDH). In cases where the allozymes could be visualized parental origin was as expected based on morphological characters (Table 3).

All parental butterflies and subsequent offspring were frozen at –80°C. Allozyme electrophoresis protocols followed Hagen and Scriber (1991). Specimens were prepared by grinding the proximal half of the abdomen or part of the thorax for females (using the proximal half of the abdomen or part of the thorax avoided including spermatophore proteins from male mates) or distal half of the abdomen of males in 100uL buffer (0.1M tris, 1.07M EDTA, 0.15mN NAD, .13mM NADP, 35.75mM 2-mercaptoethanol, pH 7.0). After grinding, the specimens were centrifuged for 10 minutes at 12,000 x g. Allozymes were separated by electrophoresis on thin layer cellulose acetate plates. We then stained for the enzymes LDH and PGD.

Formulae for enzyme stains followed Richardson et al. (1986). Gel scoring protocols were after Hagen and Scriber (1991). Allozymes were scored by assigning the origin (where samples were applied) a score of '0' and the most common allozyme a score of "100". All other allozymes were assigned a score relative to their location in relation to the origin and the most common allozyme. *.P glaucus'* PGD allozyme scores a -100 with a rarer allozyme at –50 and *P. canadensis* scores at –125 and a rarer allozyme at –80 (Hagen and Scriber 1991). The LDH allozyme scores at 100 for *P. glaucus*. *P. canadensis* scores at 80 and a rarer allozyme at 40. All specimens were run twice to confirm allozyme types. Every plate was run with controls: a previously scored *P. glaucus* and *P. canadensis*.

Table 13: Parental allozyme and geographical origin. Wild males denotes that females were captured in the field and mates are unkown wild males. All other females were hand pair with the male indicated. Numbers beginning with 15 are butterflies used in 1999 and numbers beginning with 16 are from 2000.
*** Indicates that allozymes were run but were not able to be visualized.

	Number	County	Origin	PGD	LDH	County	Origin	PGD	LDH
P. canadensis		Female				Male			
	15116	Charlevoix Co., MI	Wild	-125	80	Wild Mate			
	15120	Emmett Co., MI	Wild	***	80	Wild Mate			
	15155	Emmett Co., MI	Wild	***	***	Wild Mate			
	15157	Emmett Co., MI	Wild	***	80	Wild Mate			
	15160	Cheboygan Co., MI (14013)	Lab	-125	***	Emmett Co., MI	Wild	-125/ -80	80
	15201	Bennington Co., VT	Wild	-125	80	Wild Mate			
	16024	Isabella, Co., MI	Wild	-125	80	Wild Mate			
	16027	Isabella, Co., MI	Wild	-125	80	Wild Mate			
	16067	Charlevoix, Co., MI	Wild	-125	80	Wild Mate			
	16068	Charlevoix, Co., MI	Wild	-125	80	Wild Mate			
	16134	Clark Co., WI	Wild	***	***	Wild Mate			

Table 13 (cont'd)

P. canadensis x P. glaucus									
	15085	Cheboygan Co., MI (14005)	Lab	-125	***	Jackson Co., OH	Wild	-100	***
	15091	Cheboygan Co., MI (14018)	Lab	-125	***	Gallia Co., OH	Wild	-100	100
	15092	Cheboygan Co., MI (14005)	Lab	-125	***	Lawrence Co., OH	Wild	-100	100
	15101	Dickinson Co., MI (14141)	Lab	***	***	Lawrence Co., OH	Wild	-100	100
	15133	Kewennaw Co., MI (14109)	Lab	***	***	Lincoln Co., MO	Wild	-100	100
	15138	Kewennaw Co, MI (14109)	Lab	-125	80	Lawrence Co., OH	Wild	-100	100
	16023	Bennington Co., VT	Lab	-125	80	St. Joseph Co., MI	Wild	-100	100
	16030	Bennington Co., VT	Lab	***	***	St. Joseph Co., MI	Wild	-100/ -50	100
	16037	Bennington Co., VT	Lab	-125	80	St. Joseph Co., MI	Wild	-100	100
	16038	Bennington Co., VT	Lab	-125	80	St. Joseph Co., MI	Wild	-100	100
P. glaucus									
	15111	Warren Co., MO	Wild	-100	100	Wild Mate			
	15112	Warren Co., MO	Wild	-100	100	Wild Mate			

Table 13 (cont'd)

	16029	Southeast Pennsylvania	Lab	-100	100	St Joseph Co., MI	Wild	-100	100
	16100	Southeast Pennsylvania	Lab	-100	100	St Joseph Co., MI	Wild	-125/ -100	100
	16103	Southeast Pennsylvania	Lab	-100	***	St Joseph Co., MI	Wild	-100	100
	16104	Southeast Pennsylvania	Lab	***	***	St Joseph Co., MI	Wild	-100	100
	16105	Southeast Pennsylvania	Lab	-100	100	St Joseph Co., MI	Wild	-100	100
P. glaucus x P. canadensis									
	15086	Highland Co., FL (15019)	Lab	***	***	Isabella Co., MI	Wild	-125/ -80	80
	15087	Highland Co., FL (15019)	Lab	-100	***	Isabella Co., MI	Wild	-125	80
	15090	Highland Co., FL (15019)	Lab	-100	***	UMBS, MI	Wild	-125	80
	15093	Highland Co., FL (15019)	Lab	-100	100	Isabella Co., MI	Wild	-125	80
	16039	Southeast Pennsylvania	Lab	-100	100	Isabella Co., MI	Wild	-125/ -80	80
	16040	Southeast Pennsylvania	Lab	-100	100	Isabella Co., MI	Wild	-125	80
	16042	Southeast Pennsylvania	Lab	-100	100	Isabella Co., MI	Wild	-125/ -80	40
	16099	Southeast Pennsylvania	Lab	-100	100	Charlevoix Co., MI	Wild	-125	80

APPENDIX 4

AVERAGE NUMBER OF DAYS FROM HATCH TO PUPATION

Table 13: Average Number of Days From Hatch to Pupation with ± 1 standard error.
**** Denotes no survival until pupation. n=number of larvae that survived until pupation.

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000
Black Cherry 15°C	88.13±2.14 n=8	89.32±2.65 n=6	78.14±1.71 n=13	76.03±2.72 n=6	73.00±2.47 n=6	97.65±3.73 n=3	87.63±4.36 n=2	83.38±3.86 n=3
Black Cherry 23°C	37.41±2.51 n=6	31.44±1.78 n=13	30.52±1.44 n=18	25.35±2.18 n=9	34.61± 1.75 n=12	29.46±2.43 n=7	31.23±2.71 n=5	29.59±3.72 n=3
Black Cherry 31°C	25.49±1.69 n=13	22.38±1.82 n=13	19.63±1.83 n=11	18.48±2.04 n=10	21.38±2.14 n=8	22.25±3.21 n=4	19.31±3.05 n=4	18.80±3.72 n=3
Quaking Aspen 15°C	93.25±3.03 n=4	84.61±2.88 n=5	84.79±3.51 n=3	95.03±2.94 n=5	91.63±6.11 n=1	****	****	****
Quaking Aspen 23°C	34.50±1.92 n=10	29.70±2.03 n=10	33.09±2.15 n=8	31.37±2.16 n=9	36.33±2.47 n=6	36.12±3.23 n=4	****	****
Quaking Aspen 31°C	25.31±2.17 n=8	23.17±2.46 n=7	24.72±2.15 n=8	21.41±3.83 n=3	24.73±2.29 n=7	****	****	****
Tulip Tree 15°C	****	82.02±3.73 n=3	60.30±1.52 n=16	60.74±2.47 n=7	52.84±2.15 n=8	79.81±2.88 n=5	67.79±3.51 n=3	71.61±2.88 n=5
Tulip Tree 23°C	30.86±4.33 n=2	25.95±2.88 n=5	22.73±1.52 n=16	20.89±2.05 n=10	25.37±1.76 n=12	20.89±2.43 n=7	26.29±3.50 n=3	21.59±2.63 n=6
Tulip Tree 31°C	****	20.41±6.95 n=1	16.65±1.83 n=11	15.48±2.88 n=5	17.62±2.31 n=7	16.12±2.88 n=5	18.12±3.57 n=3	16.25±3.21 n=4

APPENDIX 5

NUMBER OF LARVAE THAT SURVIVED TO PUPATION

Table 15: The number of larvae that survived to pupation/the total number of larvae set up at the start of the experiment

	P. canadensis		*P. canadensis x P. glaucus*		*P. glaucus x P. canadensis*		*P. glaucus*	
	1999	2000	1999	2000	1999	2000	1999	2000
Black Cherry 15°C	8/20	6/20	13/24	6/16	6/16	3/16	2/8	3/20
Black Cherry 23°C	6/20	13/20	18/24	9/16	12/16	7/16	5/8	3/20
Black Cherry 31°C	13/20	13/20	11/24	10/16	8/16	4/16	4/8	3/20
Quaking Aspen 15°C	4/20	5/20	3/24	5/16	1/16	0/16	0/8	0/20
Quaking Aspen 23°C	10/20	10/20	8/24	9/16	6/16	4/16	0/8	0/20
Quaking Aspen 31°C	8/20	7/20	8/24	3/16	7/16	0/16	0/8	0/20
Tulip Tree 15°C	0/20	3/20	16/24	7/16	8/16	5/16	3/8	5/20
Tulip Tree 23°C	2/20	5/20	16/24	10/16	12/16	7/16	3/8	6/20
Tulip Tree 31°C	0/20	1/20	11/24	5/16	7/16	5/16	3/8	4/20

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