TARGETING TRAITS IMPORTANT TO ADAPTATION AMID RISING GLOBAL TEMPERATURES IN THE PITCHER PLANT MOSQUITO *WYEOMYIA SMITHII*

by

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This thesis examines the pitcher plant mosquito to understand how an increase in global temperature affects invertebrates. The pitcher plant mosquito is a model organism for understanding how insects may respond to such environmental changes. The thesis includes a two part study. First the author identifies the morphological and physiological characteristics that persist under conditions of chronic heat stress. Secondly the study examines the costs and benefits of hybridity to survival in a thermally stressful environment. The thesis study found that hybridization between genetically distinct parental populations can result in outbreeding depression. Thus increasing the populations survival can be maintained by adopting a hybridization program if needed.

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CHAPTER I

INTRODUCTION

Predicting the ability of a species to evolve in response to rapid environmental change is a major concern for evolutionary biologists. Global warming, which is predicted to cause a change in the world's mean surface temperature of as much as 5°C in the next century (Root & Schneider 1993), is mainly the result of an increase in the emission of greenhouse gases since pre-industrial times. According to paleoclimatic records, even the most rapid climate changes took place between 10 and 40 times more slowly than the changes predicted to occur by the end of the next century (Peters and Darling 1985; Huntley 1990). Since the geographic range of many species ultimately is determined by climatic factors a rapid increase in global temperature threatens drastic alterations to the organization of our biosphere (Hoffman & Parsons 1991).

According to studies of past climatic changes, organisms generally respond to an increase in global temperature by migrating to remain within favorable climatic regions (Rooney et al. 1996). Models attempting to assess the effects of global warming on the geographic range of the Chinese mantid *(Tenodera sinesis)* support the migratory prediction (Rooney et al. 1996). However, habitat fragmentation caused by anthropogenic disturbance may inhibit the ability of organisms to respond to global climate change by migrating (Armbruster et al. in press). When populations do not have the option of migrating to escape unfavorable climatic conditions, the remaining options are adaptation or extinction (Lynch & Lande 1993).

Predicting a species' ability to survive stressful conditions is complex. Studies in the past have focused upon the relationship between *genetic variation* and *fitness* as an indication of population viability (Parsons 1987; Frankel and Soule 1981). Fitness is defined as the potential for an individual to contribute genes to future generations. The range of traits an organism may possess is dependent on the composition of its gene pool. A population's gene pool is the sum total of all genes (distinctive segments of DNA found in chromosomes) possessed by its individual members. A diversity of different types of the same gene, referred to as *alleles*, may create high levels of genetic variation. High levels of population genetic variation increase the likelihood that genotypes will respond successfully to stress (Schonewald-Cox 1983).

Performing crosses between two distantly related members of a species is becoming an increasingly common technique used by breeders, zoo keepers, and conservation biologists to increase overall genetic variability (Frankel & Soule 1981). A hybrid is formed as the result of matings between disparate groups or populations. Hybridization increases genetic variation because it combines alleles from two genetically distinct populations, thus widening the gene pool. Hybridization can also mask deleterious alleles (alleles with a negative effect on fitness) in the generation following the hybridization event. The result of hybridization may be an increase in fitness referred to as *hybrid vigor* or *heterosis*. However, hybridization may also inhibit adaptation to stressful environments because of the potential for *outbreeding depression*. Outbreeding depression results when hybridization break up favorably interacting sets of genes, leading to a decrease in fitness in subsequent generations (Lynch 1991; Shields 1982).

Biologists are largely ignorant of the factors that determine the ability of a species to adapt to stressful conditions (Hoffman and Parsons 1991). It is becoming increasingly apparent that maximizing variation on one level does not always result in the improvement of fitness traits that promote survival in stressful environments (Lynch 1996). Each species or population, restrained by its own genetic architecture, will respond differently to stress (Armbruster et al. 1997; Holt 1990). However, few attempts have been made to identify traits that may promote survival of species in thermally stressful conditions predicted for the next century.

While many ecological studies of global warming have examined broad-scale biogeographic patterns (Field et al. 1992; Clark 1993) or extinction within vertebrates (McDonald and Brown 1992; Dunham 1993), fewer studies have focused on the relationship between global warming and extinction of invertebrates. Invertebrates, however, are more numerous than vertebrates and play an essential role in the ecological web of life. The extinction of a single species of insect could have dramatic implications throughout the rest of the food web. For these reasons I use the pitcher-plant mosquito, *Wyeomyia smithii,* as my model organism for understanding how insects may respond to an increase in global temperature.

This study has two parts: first, I will identify the morphological and physiological characteristics that persist under conditions of chronic heat stress and second, I will assess the costs and benefits of hybridity to survival in a thermally stressful environment.

Life Cycle of W. smithii

The eastern North American mosquito *Wyeomyia smithii* maintains a symbiotic relationship with the carnivorous, purple pitcher-plant *Sarracenia pupurea*. Bacteria, algae, protozoans, rotifers, small crustaceans, mites, and two other common species of insects inhabit the water-filled leaves of the pitcher-plant along with *W. smithii* (Bradshaw 1983). Larvae of the sarcophagid (carnivorous) fly, *Blaesoxipha fletcheri,* scavenge on dead, trapped victims floating on the surface of the water; the chironomid midge, *Metriocnemus knabi*, spends its time in the detritus at the base of the pitcher-plant leaf boring into drowned victims; and the larvae of *W. smithii* filter-feed on material broken down by *B. fletcheri* and *M. knabi*. At each step in this food web, the inhabitants of the leaf provide the plant with nitrogen essential to the plant's survival.

Because the larvae of *W. smithii* can survive only within the water-filled leaf of its host, the geographic distribution for both is identical, extending from the Gulf of Mexico (about 30° N) to central Canada (about 60° N). It is within the leaves of the pitcher plant that *W. smithii* passes through its pre-adult, developmental stages (Fig. 1).

Adult females oviposit their eggs inside the pitcher plant leaves. Once eggs hatch they become larvae (Fig. 1). While *S. pupurea* is considered carnivorous, the digestive juices it secretes do not effect the larvae of *W. smithii.* Larvae pass through four main stages of development. Each stage is referred to as an *instar*. *W. smithii* passes from one instar to the next when its exoskeleton becomes too small and it must molt (shed its old exoskeleton for a new one) in order to continue growing.

During the larval stage unfavorable winter conditions can induce a developmental arrest or *diapause* (Bradshaw & Lounibous 1977). Diapause is similar in nature to hibernation for a bear. Diapause is defined as a period of arrest of growth and development that enables the species to overwinter to synchronize its development cycle to that of the seasons. In most cases diapause involves the cessation of neuro-endocrine activity. The onset, maintenance, and termination of diapause in *W. smithii* is controlled by a photoperiodic time mechanism that uses the amount of daylight as an indication of seasonal shifts. *W. smithii* in diapause is protected from harsh winter conditions within the water-filled leaves of its host. When the days become longer and spring is approaching, *W. smithii* will break diapause and continue development.

When conditions are favorable and the larvae have developed to their fourth and final instar they are ready to *pupate*. Pupation is similar in nature to the cocoon stage of a moth. Pupae do not eat or change in shape. However, on the inside, *W. smithii* is going through a metamorphosis. The previously aquatic organism is getting ready to become airborne. The pupal stage lasts about a week, after which the adult mosquito will *eclose* (emerge from the pupal case) and fly out of the leaf in search of an appropriate mate to begin the life cycle again.

Evolutionary History of W. smithii

W. smithii is the only member of the genus *Wyeomyia* whose range spreads into the temperate zone (Bradshaw and Lounibos, 1977). The remaining 90 or so members of the genus *Wyeomyia* consist of tropical and subtropical species, providing evidence for the tropical origins of *W. smithii*. The recession of the latest glacial ice sheet from Northern America, 10-12,000 years ago, allowed the host plant to spread into more temperate climates, opening an entirely new range to *W. smithii*. It is therefore hypothesized that the evolution of *W. smithii* into the temperate zone proceeded in the south to north direction (Bradshaw and Holzapfel 1990). Thus, the traits of southern populations are considered ancestral, while the traits of northern populations are considered derived. Genetic, biogeographical, morphological and physiological data all support the theory that the range expansion of this species proceeded from south to north (Bradshaw and Lounibos 1977; Bradshaw and Holzapfel 1990; Armbruster et al. 1997).

The correlations between physiological traits and latitude provide some of the strongest evidence for the hypothesis that *W. smithii* migrated from south to north. Climatic patterns in central and eastern North America indicate that while mid-summer temperatures remain quite similar, winter temperatures decline more dramatically with increasing latitude. It follows that migration into northern latitudes is associated with the evolution of traits involved in the regulation of seasonal development. The critical photoperiod that regulates the initiation, maintenance, and termination of diapause is tightly correlated with latitude and altitude (Bradshaw & Lounibos 1977). The critical photoperiod is determined by the amount of daylight hours that indicate to the organism it is time to enter diapause or continue development. Northern populations have a shorter critical photoperiod, while southern populations have a longer critical photoperiod. The onset of winter in the north comes earlier in the year, when the day lengths are still long; the onset of winter in the south comes later in the year, when the day lengths are shorter. It follows then that the correlation between critical photoperiod and latitude or altitude is one hour for each 5.4 degrees north or 769 m altitude (Bradshaw and Lounibos 1977).

Southern populations diapause as fourth instars, while northern populations diapause as third instars. It is hypothesized that northern mosquitoes diapause as third instars in order to maintain the option of a second fourth-instar diapause if the variable northern climate suddenly becomes disadvantageous for development (Bradshaw and Lounibos 1975). These trends are consistent with the hypothesis that physiological traits controlling seasonal development were the primary agents of evolutionary change as *W. smithii* migrated into the temperate zone.

The divergence of morphological traits across a latitudinal gradient also provides strong support for the range expansion occurring from south to north. Anal papillae extend from the posterior section of the larvae as two long, ventral and two shorter, dorsal appendages in southern populations (Fig. 2). The two dorsal anal papillae shrink in length as one moves northward, until they are completely non-existent in the northern-most populations. The function of the anal papillae is thought to be related to basic metabolic processes such as chloride (salt) uptake (Gillet 1971) and possibly oxygen absorption. More, longer anal papillae provide a larger surface area, possibly increasing the efficiency of metabolic processes. Thus, the migration northward is associated with the loss of two dorsal anal papillae perhaps because colder, more oxygen-rich waters relax selection (to remove the selective advantage of a trait) on metabolic efficiency.

In the following experiment, I look to the length of the critical photoperiod, the stage of diapause, and the structure of the anal papillae, as indications of how *W. smithii* responded to chronic heat stress conditions reminiscent of its southern origins.

CHAPTER II

METHODS

Overview

In order to place my methods in a logical context I will provide an explanation of terms that will arise. A *population* refers to a sample of mosquitoes removed from their pitcher-plant leaves at a distinct geographic location. For example, ME refers to the sample of mosquitoes removed from a location of pitcher-plant leaves in Maine, while AL refers to a sample from Alabama (Fig. 3). A cross between two populations is always referred to with the original female parent first, so AL x ON indicates that a group of females from Alabama was mated to a group of males from Ontario. The same logic holds true for FL (Florida) x ME, and ME x FL. A four way cross was created when female offspring of one hybrid were mated to male offspring of another hybrid. I use the maternal grandmother (such as MEgm) to distinguish one hybrid from another.

A *line* refers to a population of mosquitoes in the laboratory. For example, in my experiments "ME select" indicates a subgroup of the sample from Maine that went through ten generations of selection in a thermally stressful environment, while "ME control" indicates another subgroup of the same sample that was maintained in an environment designed to gaurd against selection. The terms line and population are sometimes used interchangeably. A *cohort* refers to a group of individuals within each line. Dividing a population into cohorts is a way to replicate results and increase statistical power. In my experiments I used fourteen lines (seven selected and seven control) with five cohorts per line, for a total of seventy cohorts.

A *generation* (larva to pupa, pupa to adult, and adult to egg) lasts for approximately two months in the laboratory (Fig. 1). My experiments lasted for a total of six months (two complete generations, plus two months on short day). Placing populations on *short days* (eight hours of light and sixteen hours of dark), where the amount of light is equal to the amount they would experience on a winter day, induces diapause. This allows the researcher to synchronize development in all lines before beginning a new experiment.

Collection and Selection

My experiment used lines previously selected for population persistence under conditions of thermal stress, in addition to lines raised in parallel under optimal conditions. Specifics on methods for collection of the populations, the establishment of experimental lines, and the selection regime are provided in Armbruster et al. (in press). Briefly, approximately 2000 larvae were collected from four original locations (Ontario, Maine, Florida, and Alabama) during spring, when 100% of the population was present as diapausing larvae in pitcher-plant leaves. After four generations in the laboratory, four parental populations were used to create six 2-way hybrids which, in turn, were used to create four 4-way hybrids (Fig. 3). Each of the fifteen lines was then divided: one half was subjected to selection for persistence under conditions of chronic thermal and nutrientlimiting stress and one half was maintained under optimal conditions as unselected controls. Control lines were maintained under optimal conditions at a constant 21° C on short day (L:D = 8:16) as diapausing larvae and intermittently run through a generation approximately every four months.

The lines subjected to selection were placed in an environment designed to maximize stress near the threshold of population persistence under ecologically realistic conditions. Temperature was regulated to a warm:cool = 35:18° C (mean=27° C) smooth, sine wave daily thermoperiod. Humidity varied between 60% and 80% in a smooth sine-wave function, with low humidity occurring at the peak temperature each day. After ten generations of selection, seven of the original fifteen populations had gone extinct. The surviving parental lines were from Maine and Alabama (Fig. 3). The surviving 2-way hybrids were the grandchildren of mothers from Alabama mated to fathers from Ontario, mothers from Florida mated to fathers from Maine as well as the reciprocal cross of mothers from Maine mated to fathers from Florida. The surviving 4-way hybrids were the grandchildren of male ON x AL hybrids mated to female ME x FL hybrids and male ME x FL hybrids mated to female AL x ON hybrids. The seven surviving lines, along with their controls, were then placed on short day (L:D= 8:16) at a constant temperature of 21° C in order to initiate diapause and synchronize development. Surviving lines and their controls were run through two generations to eliminate parental effects (non-genetic or environmental effects of parents on offspring) before assessing the response to selection. At the termination of the selection experiment all lines were placed on short day.

Before the initiation of the present experiment, each surviving selected line and its control were run through one generation to build up the population size. Each population was split into five cohorts of approximately 50 larvae per cohort. Each cohort was run through one additional generation. Their offspring, which varied between 300 and 350 larvae per cohort (1,500-1,750 larvae per line or approximately 21,000 total mosquitoes), became the subject of the current analysis.

Analysis of Diapause Characteristics

All lines were monitored for pupation from the day they were placed on short days to the day they were scored for instar of diapause. Larvae were maintained in plastic petri dishes (150 by 25 mm), half filled (80 ml) with distilled water. Each dish contained approximately 25 larvae, which were fed an emulsion of ground, sifted, guinea pig chow, and freeze-dried brine shrimp (3:1 by vol.) once per week. All lines were subjected to thirty-six short days from the date of oviposition in order to insure diapause. Approximately 300 larvae from each of the 70 cohorts were scored for instar of diapause and total pupation. Scored dishes of larvae were stored on short day until randomization for the critical photoperiod experiment began.

Four astronomically increasing , light-sealed, photoperiod cabinets were set to appropriate starting and stopping times and checked twice per day for three days to ensure proper programming. The boxes were programmed to add three minutes per day indefinitely: one minute and thirty seconds in the morning and one minute and thirty seconds in the evening. Starting times were chosen according to past studies in the laboratory of critical photoperiod: twelve hours of light per day for the hybrids, fifteen hours for the northern parent, and eleven hours for the southern parent (Lair et al. 1997).

Experimental animals were removed from short day and randomized. Larvae from every oviposition date within each cohort were placed into a tray and swirled. Twenty five larvae were randomly selected and placed into one of two dishes containing 80ml of distilled water and 2ml of food. Ten dishes from each line were placed in their corresponding photoperiod box. For three months, every dish was checked on Monday and Thursday for pupae, fed 2ml of food, and cleaned as needed. The termination of the experiment was marked by the end of pupation in all lines.

Anal Papillary Phenotypes

Thirty fifth-instar larvae from each cohort were preserved. Fixed larvae were placed on a slide, ventral side down, in a drop of water, between two cover slips (Fig. 2). A third coverslip was placed over the top and the dorsal anal papillae were measured from right to left, using an ocular micrometer lens of a calibrated dissecting scope. The larvae were then turned dorsal side down and the ventral anal papillae were measured. Ten larvae from each of the 70 cohorts were measured.

Statistical Treatment of the Data

At the completion of the experiment, all data were run through a statistical test called Analysis of Variance. The objective of the statistical test was to disprove the null hypothesis, which stated: The patterns in my data are no different from what I may expect of randomly distributed numbers. The Analysis of Variance averaged the variance about the mean (Sum of Squares), taking into account the degrees to which the model is allowed to vary (degrees of freedom). These numbers were used to create an "F value" which correlated to values in a probability table. From the F-value and the probability table I was able to estimate the chances that my results were due to random sampling alone. When the probability was less then 0.001, my results were "very highly significant." When the probability was less then 0.01., my results were "highly significant." When the probability was less then 0.05 my results were "significant." Any probability above 0.05 was not considered significant. In some cases, the data were transformed using mathematical manipulation in order to put it in a form that I could analyze using statistics.

To approximate normal distribution, frequencies of pupation on short day and diapause as fourth instars were subjected to arcsine-square-root transformation prior to analysis. To reduce heteroscedasticity, critical photoperiod was subjected to log transformation prior to analysis. All analyses were performed using the SAS ANOVA (Analysis of Variance) GLM (General Linear Model) procedure (SAS Institute 1985). The dorsal anal papillae, ventral anal papillae, percent pupation on short day, percent diapause as fourth instars, and critical photoperiod were subjected to a two way ANOVA with selected lines (control and selected) and parents (AL and ME) as treatments (fixed effects) and five cohorts per cell. The same statistical procedure was used for the hybrids (ALxON, FLxME, MExFL, ALgm, and MEgm).

CHAPTER III

RESULTS

The results of the statistical test were broken down into three different parts: among parents or hybrids, between selected and control lines, and the interaction between selection and parent or hybrid. The interaction term describes the effect of one treatment as it relates to another treatment. For example, in my experiment selection had a greater effect on the population from Maine than it did on the population from Alabama. Therefore, there was a significant interaction between the parent and the effect of selection. Without a significant interaction term selection would have affected both populations the same.

As shown in Fig. 4 and 5, statistical analysis showed a significant effect of selection on dorsal anal papillae, ventral anal papillae, fourth instar diapause, and critical photoperiod, but no significant effect of selection on pupation on short day (Table 1). The statistical analysis showed a significant line-by-parent interaction for dorsal anal papillae, fourth instar diapause, and critical photoperiod. However, no significant line-by-parent interaction was found for ventral anal papillae and pupation on short day. There was a significant effect of the parent on dorsal anal papillae, ventral anal papillae, fourth instar diapause, and critical photoperiod, but no significant effect of the parent on pupation on short day.

As shown in Table 2, the statistical analysis of the hybrid populations demonstrated a significant effect of selection on dorsal anal papillae, fourth instar diapause, critical photoperiod, and pupation on short day (Fig. 4 and Fig. 5). However, there was no significant effect of the hybrid on ventral anal papillae. There was a significant effect of hybrid on dorsal anal papillae, ventral anal papillae, pupation on short day, fourth instar diapause, and critical photoperiod. There was a significant hybrid-by-line interaction for dorsal anal papillae and critical photoperiod, but no significant hybrid-by-line interaction for ventral anal papillae, pupation on short day, and fourth-instar diapause.

CHAPTER IV

DISCUSSION

Response to Heat Stress

The results of this study indicate that selection for population persistence under conditions of chronic heat stress caused a reversion to the southern, ancestral morphology and physiology in northern and hybrid populations of *Wyeomyia smithii*. In the northern selected line, thermal stress evoked the quiescent (dormant or inert), ancestral phenotype for dorsal anal papillae and stage of diapause (Fig. 4 & Fig. 5). Traits already present in the northern selected line, the ventral anal papillae and the critical photoperiod, shifted towards the southern phenotype. The same shift towards the southern phenotype was observed in the hybrid lines for critical photoperiod, stage of diapause and dorsal anal papillae. In comparison, the southern selected and control lines failed to demonstrate any consistent shift. From these results I conclude that the southern phenotypes (longer anal papillae, shorter critical photoperiods, and fourth instar diapause) provide an adaptive advantage in heat stress environments.

This study was designed to identify the morphological and physiological change caused by lifetime environmental stress. It is likely that the actual mechanism of change was direct selection on each of the traits individually. Another alternative is that selection acted directly on one of the traits and caused a change in the other traits because they a were genetically linked. The tight correlation of physiological and morphological traits with latitude (Bradshaw and Lounibous 1977) indicates that some of the divergence is a result of adaptation to different temperature regimes. This, in turn, supports the idea that southern conditions would impose direct selection for southern traits. My findings provide fuel for future experiments to verify the actual mechanism responsible for evoking the observed response.

My results verify that chronic heat stress created selection for longer dorsal and ventral anal papillae, shorter critical photoperiods, and fourth instar diapause (Fig. 4 & Fig. 5). The significant interaction term verifies that selection acted stronger on the northern parent from Maine a on the southern parent from Alabama. The different patterns of response to selection between the parents is a reflection of their unique underlying genetic architecture. I therefore conclude that some of the geographic variation for anal papillae, critical photoperiod, and stage of diapause observed in nature is correlated with temperature tolerance and that these traits will be important to survival amid rising global temperatures.

A similar case of adaptation to thermally stressful environments in the laboratory that replicated biogeographic variation in nature is documented in the fruit fly *Drosophila*. Anderson (1966) showed that, even when reared at the same temperature, populations of *Drosophila pseudoobscura* previously maintained at different temperatures for six years demonstrated smaller body sizes in the heat-adapted lines and larger body sizes in cold-adapted lines. This evidence is supported by Parson's (1980) study demonstrating that populations of *Drosophila* from different temperature regimes show the same divergence of body size.

The re-emergence of quiescent, dorsal anal papillae in the northern population is evidence that the loss of a phenotype does not necessarily connote the loss of genetic information (Fig. 4). My data suggest that dorsal anal papillae in *W. smithii* is a threshold trait (a trait that is affected by a continuous distribution of some underlying trait, but which is expressed discontinuously at the phenotypic level), similar to bristle number in *Drosophila* and digit number in mammals (Rendal 1967; Lande 1978). The rapid re-appearance of the two dorsal anal papillae is characteristic of threshold traits that evolve in spurts of rapid change. Perhaps thermal stress imposed selection for the ancestral phenotype of four long anal papillae because longer papillae increase respiratory efficiency in warmer, less oxygen-rich waters. The theory of threshold traits implies that the expression of anal papillae is controlled by an underlying, continuous character. A continuous character is one that is controlled by many genes of small effect. The distribution of possible genotypes is continuous rather than discrete, graphically illustrated as a bell curve. While I do not know what the underlying character actually is, I can imagine it as a hormone controlling the expression of anal papillae. Selection under conditions of physiological stress pushed production of the hormone beyond a certain "threshold," resulting in the expression of two dorsal anal papillae in generations subsequent to selection.

My data indicate that hybrid lines had enough genetic diversity to respond to selection for southern phenotypes (Fig. 3). The significant difference between the selected and control hybrid lines in every case, except the ventral anal papillae, is the result of an overall shift towards the southern phenotype. The absence of a shift in the length of the ventral anal papillae is due to the fact that there was little original variation between northern and southern populations. The significant and nonsignificant differences for hybrid and hybrid-by-hybrid line interaction are expected of populations with distinct genetic architectures, and are therefore, relatively unimportant to my overall results.

Previous studies with *W. smithii* indicate that persistence under conditions of thermal stress is independent of the geographic location of parental populations and levels of hybridity (Armbruster et al. in press). Armbruster's results demonstrate that the fitness of hybrid populations declined in thermally stressful conditions. These results appear to contradict my own findings, which indicate that northern and hybrid populations responded positively to selection for southern traits. However, Armbruster et al. also found that resistance to larval and adult heat shock increased in the selected lines. The dichotomy between stress-resistance and long term population persistence may be due to the fact that while survivorship under transient stress is controlled by relatively few factors, long term population persistence is dependent on many genes and evolves more slowly. This strongly suggests that my results are correlated with short-term stress-resistance, rather than long-term persistence under conditions of environmental stress.

Constraints and Benefits of Hybridity

While hybridity did not constrain the ability to shift towards the southern phenotype, my results indicate that it did inhibit normal initiation and maintenance of diapause. I observed the total absence of the ability to maintain diapause in a significant proportion of the hybrid control lines and in an even greater proportion of the hybrid selected lines (Fig. 5). My hypothesis is that creating a hybrid in the laboratory between distant populations of the same species eventually leads to a breakdown in the physiology involved in initiation and maintenance of diapause. I interpret this breakdown, which is not present in either of the parental populations, as the latent effects of outbreeding depression. Outbreeding depression occurs as recombination between genetically distinct parental populations eventually breaks up groups of favorably interacting genes (Dobzansky 1948). We observed the beginnings of outbreeding depression in the control lines that experienced eight generations after the hybridization event and the more advanced effects of outbreeding depression in the selected lines that experienced fourteen generations after the hybridization event. While numerous studies have examined the potential consequences of outbreeding depression (Dobzhansky 1948; Lynch 1990), scientists are largely ignorant of the extent to which it truly influences successful hybridization. Armbruster et al. (in press) suggest that in populations of *W. smithii*, hybridization eventually constrains rather than facilitates adaptation to stressful environments. The present study, which indicates that outbreeding negatively affects the physiology of the resulting organism, further supports this claim.

Summary

In contrast to studies that focus on the relationship between levels of genetic variation and fitness, my study identifies the traits that will become important to survival in thermally stressful environments, as well as identifies the hazards involved with laboratory-induced hybridization. My study shows that chronic heat stress causes a shift towards southern phenotypes, shorter critical photoperiods, fourth instar diapause, and longer anal papillae, indicating that some of the geographic variation for these traits found in nature is correlated with temperature tolerance and that these traits will be important to survival amid rising global temperatures. The re-emerge of quiescent anal papillae in the northern population suggests that the loss of a phenotype does not necessarily imply the loss of genetic information. Therefore, stress-resistant phenotypes may be buried within the genetic architecture of organisms, only to re-emerge with a radical environmental change. Finally, my results indicate that hybridization between genetically distinct parental populations can result in outbreeding depression. Therefore, conservation biologists attempting to increase a population's chances for survival amid rising global temperatures should adopt hybridization programs only as a last resort.

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TABLE 1

ANOVA of Pupation on Short Day, 4th Instar Diapause, Critical Photoperiod. Ventral and Dorsal Anal Papillae to Show the Effects of Selection in Two Parental Populations

Treatment	Sum of Squares	df	Mean Square	F
Pupation on short day				
Between Parents	6.48	1	6.48	2.67
Between Lines	6.48	1	6.48	2.67
Parent-by-Line Interaction	6.48	1	6.48	2.67
4th instar diapause				
Between Parents	15209.26	1	15209.26	1746.12***
Between Lines	2115.41	1	2115.41	242.86***
Parent-by-Line Interaction	3970.28	1	3970.28	455.81***
Critical photoperiod				
Between Parents	0.025	1	0.025	3335.16***
Between Lines	0.00112	1	0.0011	148.03***
Parent-by-Line Interaction	0.0026	1	0.0026	354.11***
Dorsal anal papillae				
Between Parents	151.8	1	151.8	57.85***
Between Lines	2726.11	1	2726.11	1038.91***
Parent-by-Line Interaction	230.52	1	230.52	87.85***
Ventral anal papillae				
Between Parents	20	1	20	9.36**
Between Lines	320	1	320	149.71***
Parent-by-Line Interaction	7.68	1	7.68	3.6

*P<.05
**P<0.01
***P<0.001

TABLE 2

ANOVA of Pupation on Short Day, 4th Instar Diapause, Critical Photoperiod, Ventral and Dorsal Anal Papillae to Show the Effects of Selection in Five Hybrid Populations

Treatment	Sum of Squares	df	Mean Square	F
Pupation on short day				
Amoung hybrids	242.31	4	60.58	6.20**
Between lines	443.42	1	443.42	45.40***
Hybrid-by-Line Interaction	101.1	4	25.27	2.59
4th instar diapause				
Amoung hybrids	1146.35	4	286.58	8.66***
Between lines	718.82	1	718.82	21.72***
Hybrid-by-Line Interaction	317.31	4	79.33	2.4
Critical photoperiod				
Amoung hybrids	0.00068	4	0.00017	5.71**
Between lines	0.00174	1	0.00174	58.42***
Hybrid-by-Line Interaction	0.00043	4	0.000106	3.58
Dorsal anal papillae				
Amoung hybrids	220.43	4	55.11	5.46*
Between lines	441.64	1	441.64	43.74***
Hybrid-by-Line Interaction	175.24	4	43.81	4.34
Ventral anal papillae				
Amoung hybrids	139.84	4	39.96	6.63**
Between lines	9.33	1	9.33	1.77
Hybrid-by-Line Interaction	47.02	4	11.76	2.23

*P<.05
**P<0.01
***P<0.001

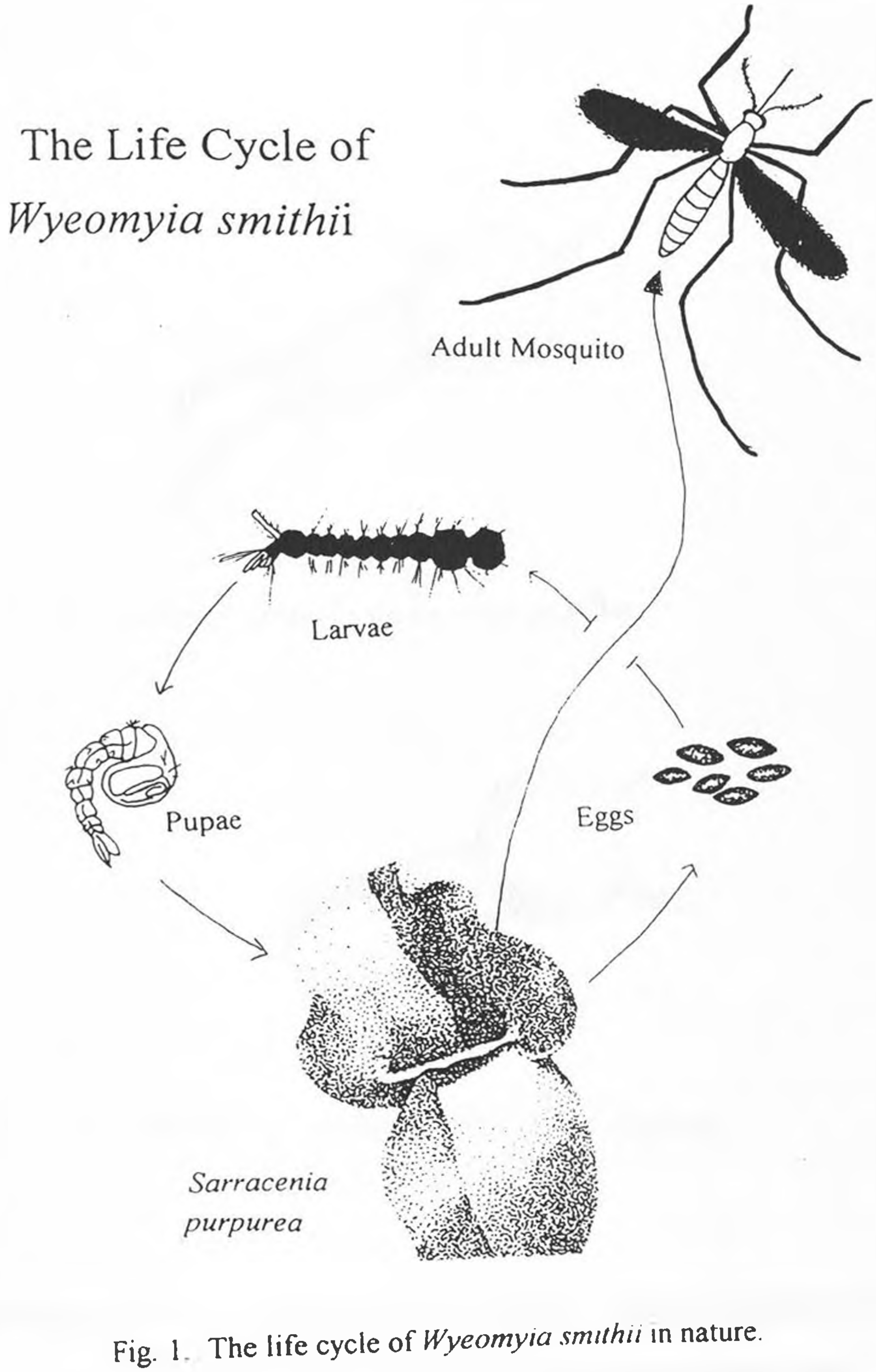
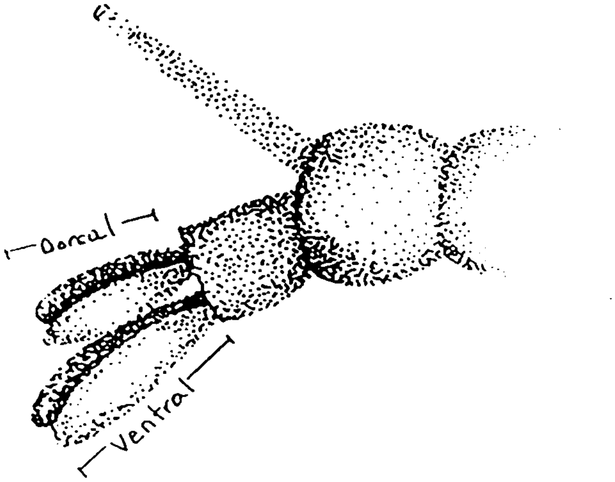


Fig. 1. The life cycle of *Wyeomyia smithii* in nature.



A. Southern phenotype for anal papillae

B. Northern phenotype for anal papillae

Fig. 2. The southern and northern phenotypes for anal papillae. Southern populations have two ventral and two shorter dorsal, while northern populations have only two short dorsal.

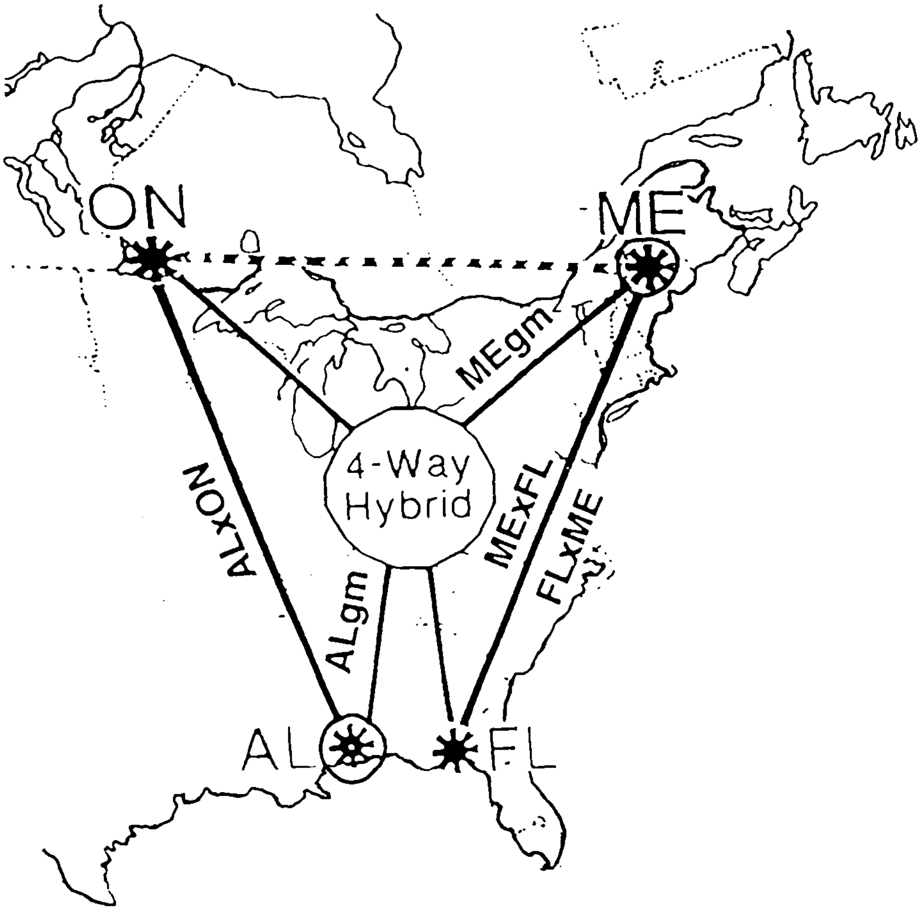


Fig. 3. This figure describes the geographic distribution of the pitcher-plant mosquito *Wyeomyia smithii*, spreading from the Gulf of New Mexico to Ontario, Canada. It also depicts the original collection sites, the methods of hybridization, and the seven surviving lines. The surviving parents are indicated by the circle/star and the surviving hybrids are labeled.

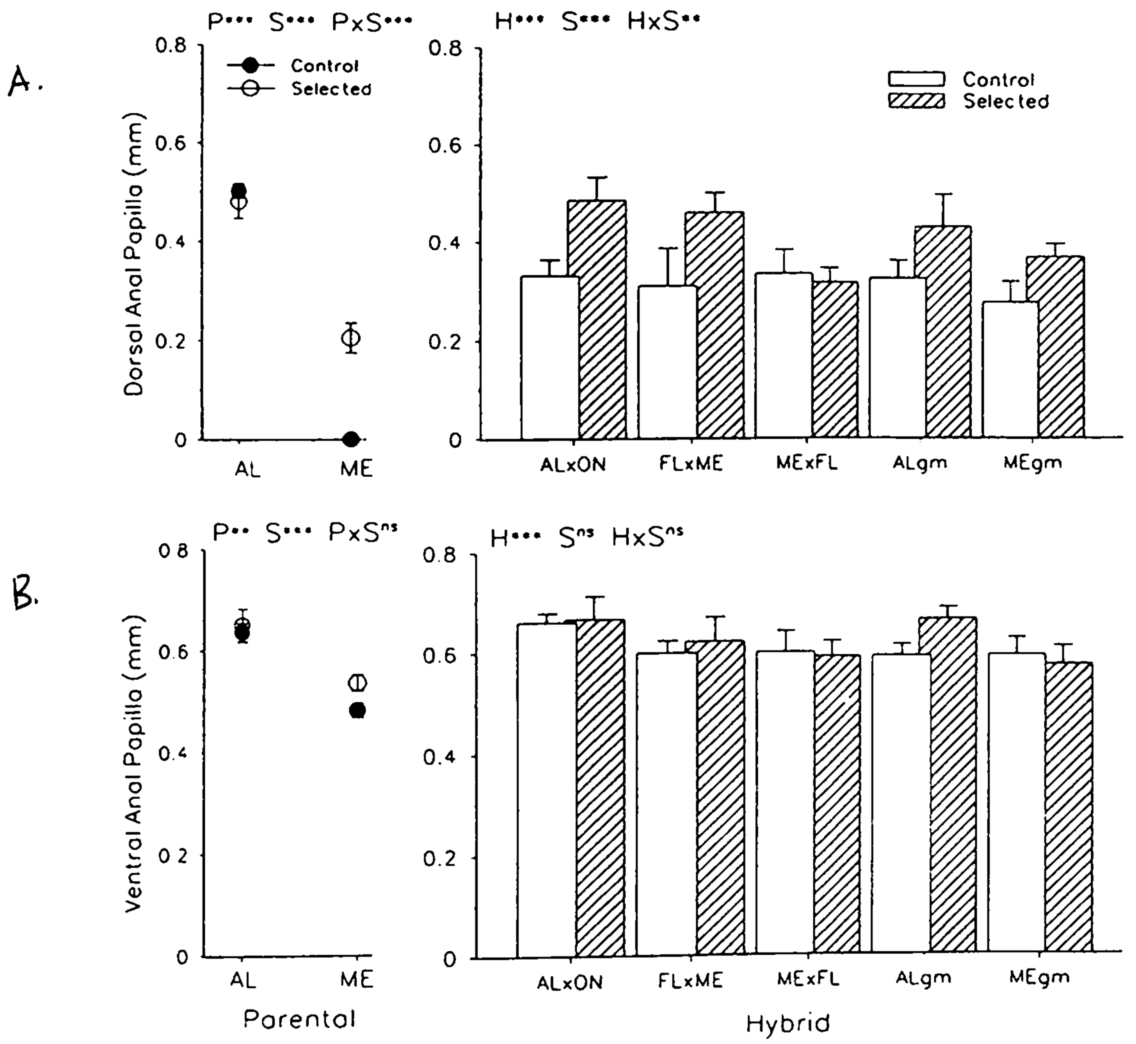


Fig. 4. Effect of selection under conditions of chronic heat stress on (A) the length of the dorsal anal papillae and (B) the length of the ventral anal papillae. In the graphs on the left, control lines are represented by closed circles and selected lines are represented by open circles.

In the graphs to the right, control lines are represented by empty bars and selected lines are represented by stripped bars. The results of the Analysis of Variance are labeled at the top left of each graph (same key as for the tables).

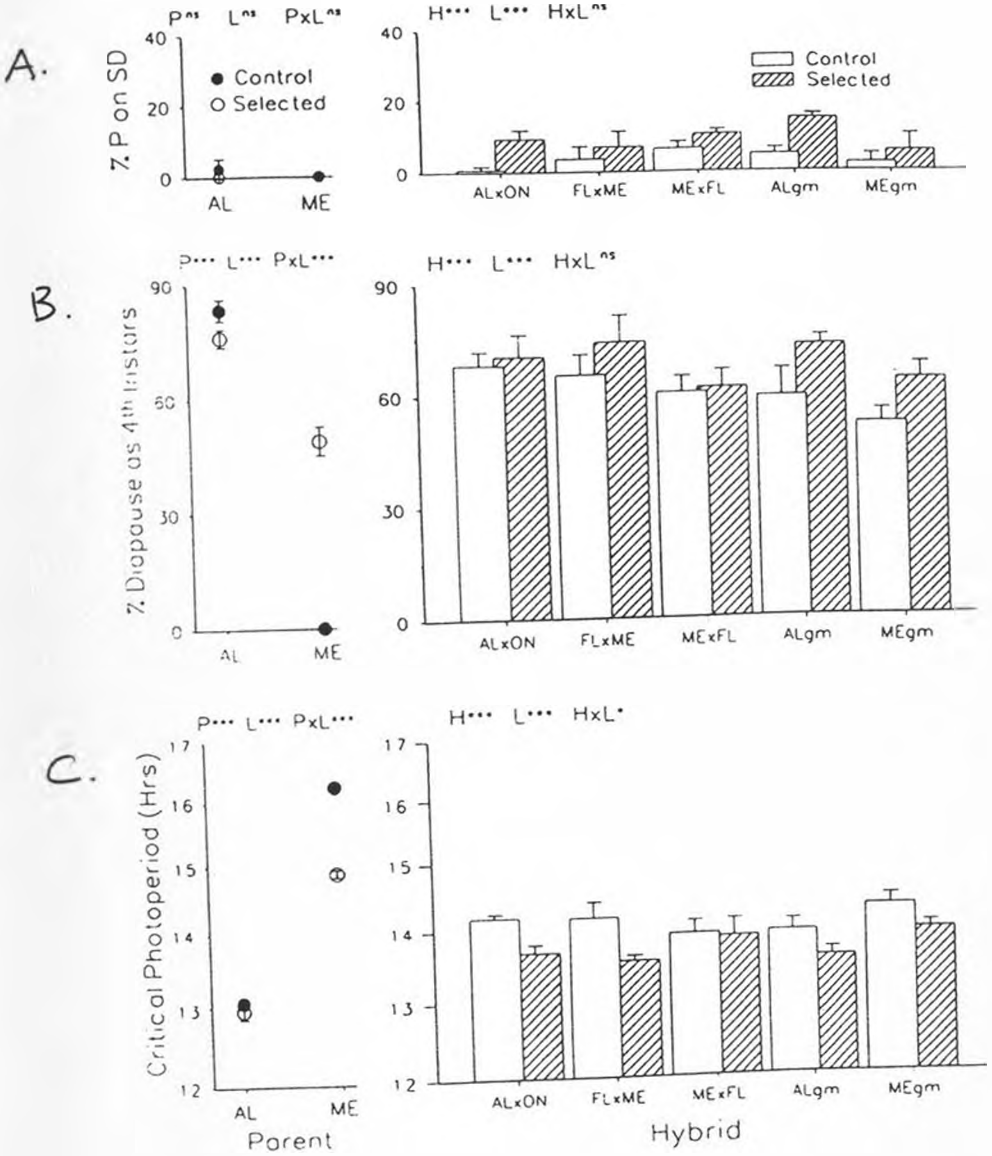


Fig. 5. Graph (A) illustrates the effect of hybridization on the percent pupation in short day. Graphs (B) and (C) represents the effect of selection on (B) the percent diapause as fourth instars and (C) the length of the critical photoperiod. In the graphs on the left, control lines are represented by closed circles and selected lines are represented by open circles. In the graphs to the right, control lines are represented by empty bars and selected lines are represented by stripped bars. The results of the Analysis of Variance are labeled at the top left of each graph.