THE EFFECTS OF RAPID CLIMATE CHANGE ON SMALL POPULATIONS OF THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

by

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A THESIS

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To determine the relative effects of rapid climate change on selection and drift in small populations, nine northern populations of the pitcher-plant mosquito, *Wyeomyia smithii*, were exposed to directional selection equivalent to 180 years of climate change, while control populations were maintained in their native climate. After three years, fitness had declined in the selected but not the control populations, indicating an adverse effect of climate change. When both selected and control populations were then reared in the selected climate, they showed no difference in fitness, indicating no genetic response to selection. Importantly, however, fitness was negatively correlated with accumulated inbreeding in both control and selected populations, pointing out that the effects of inbreeding and drift exceeded those of selection imposed by rapid climate change. Therefore, small northern populations at expanding edges of species' distributions should be most vulnerable to continued climate change.

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CHAPTER I

INTRODUCTION

At the far reaches of species' ranges, we often find small populations. These populations are formed when species are provided with the opportunity to migrate into new areas. Founded by only a subset of the original population, peripheral populations often have reduced genetic variation as a consequence of population bottlenecks. Whatever benefits range expansion gives to the species as a whole, there are persisting detriments to having the reduced population sizes found at the edges of the expansion. Two such are: *inbreeding* and *random genetic drift*. Inbreeding and drift both have the effect of reducing genetic variation, below that of the founding population, by reducing heterozygosity. Inbreeding concentrates recessive alleles found in relatives, and drift reduces alleles through random events. Although inbreeding and drift can and do occur in any population, the effect is stronger in smaller populations. Reduced genetic variation can present difficulties for small populations when they are faced with sudden selection events, such as the arrival of a new disease or predator.

Climate is a major source of environmental variation and one of the primary dictates of an organism's geographical distribution (Voigt et al. 2003). For the past 50 years, the rate of climate warming has increased. Estimates for temperature in the year 2100 range from an increase of 1.8°C to 3.4°C, numbers far exceeding the historical

fluctuation in climate dating back to 1000 CE (IPCC 2007). This unanticipated change in climate presents new selection pressures for organisms, particularly in temperate and polar regions of the Earth. Plants and animals are forced to migrate, adapt, or go extinct (Lande and Shannon 1996, Lynch and Lande 1993). Herein, we ask how rapid climate change will affect small populations and how inbreeding and drift will affect selection. We ask these questions using our study organism, the pitcher-plant mosquito *Wyeomyia smithii.*

The pitcher-plant mosquito, *Wyeomyia smithii*, can be found on the east coast of North America from 30°N to 50°N (Figure 1; Darsie and Ward 1981). Northern populations of *W. smithii* have only arisen within the past 20,000 years. The migration of *W. smithii* northward corresponds with the recession of the Laurentide Ice Sheet (Bradshaw and Holzapfel 1990). Migration has resulted in the occurrence of numerous small populations of *W. smithii* on the edges of their range, particularly in the north-west. It has been shown that the average heterozygosity of populations of *W. smithii* decreases with increasing latitude from the southern edge of the Laurentide Ice Sheet (Armbruster et al. 1998). The same pattern of decline in genetic variation with increasing latitude, has been shown in many other plants and animals, including western red cedar (O'Connell et al. 2008), poke milkweed (Broyles 1998), chalkhill blue (Schmitt and Seitz 2002), and MacGillivray's warbler (Milá et al. 2000). Small populations of *W. smithii* on the edges of post-glacial events have also been shown to suffer from inbreeding depression (decrease in fitness due to inbreeding). This has been observed by crossing north-western

edge populations with eastern post-glacial populations, and finding a recovery of fitness (Mathias 2006).

To test the effects of rapid climate change on small populations, we subjected nine northern populations of *W. smithii* from Nova Scotia, Maine, Ontario and Wisconsin (all from 46°N latitude), to the southern climate of 40°N (the **experimental environment**), simulating 180 years of climate change, while simultaneously raising the same nine

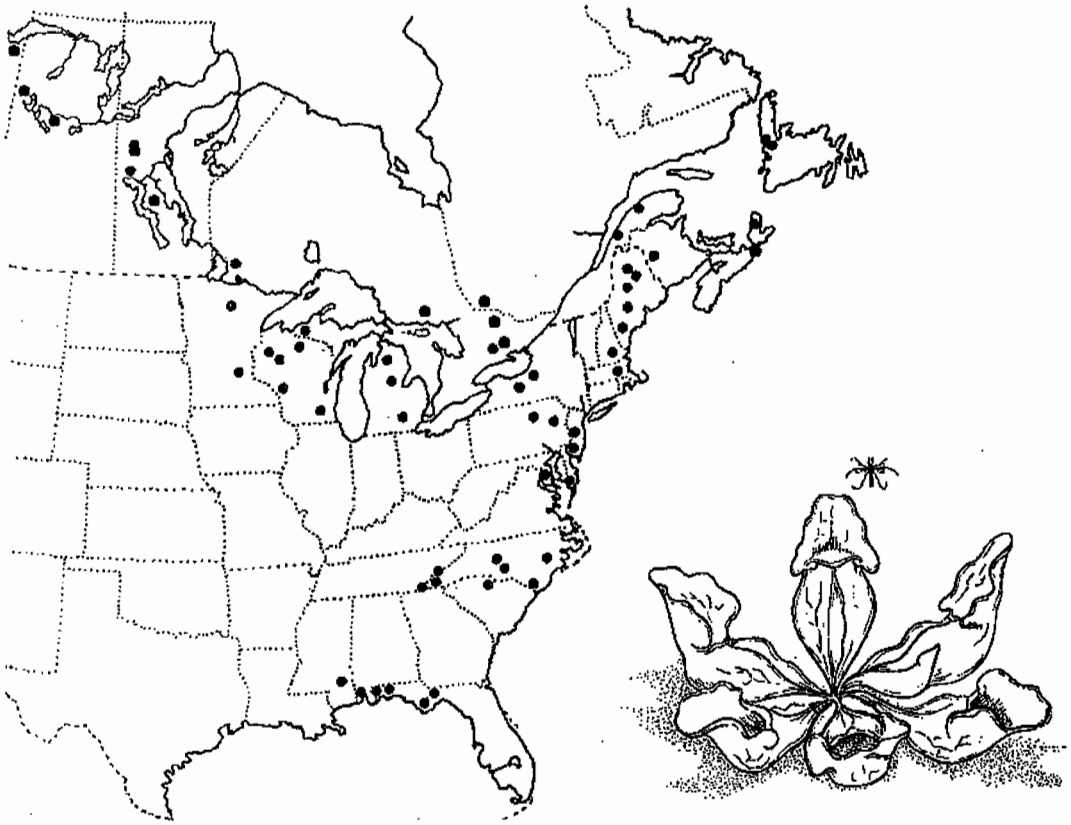

Figure 1: The range of the pitcher-plant mosquito, *Wyeomyia smithii*. Black dots indicate collection points of populations in the lab.

populations in an environment simulating their native climate (the **control environment**). This part of the experiment revealed the presence or absence of a **phenotypic response** to

climate warming. Prior research showed a detectable response to climate change in nature after five years (Bradshaw and Holzapfel 2001). It is therefore reasonable to expect that our experimental environment provided a strong enough selection differential to reveal a response.

After three years of selection in the experimental environment, we moved both control and selected populations into the experimental environment. This common garden approach allowed us to identify whether or not selection in the experimental environment provided a fitness advantage, determining the presence or absence of a **genetic response** to climate warming. Finally, we determined the effect of cumulative inbreeding on fitness.

It is important to note that the experimental environment, while making a climactic advance of 180 years, retained the native photic year of Maine (46°N). The importance of this point cannot be overly emphasized. Climate change affects climate, but it does not alter day length. Day length is controlled by the rotation of the Earth about its axis, and its revolution around the sun. Some studies have involved transplant experiments wherein plants or animals are transplanted to a warmer location in an attempt to infer the effects of climate change (Etterson and Shaw 2001, Rehfeldt et al. 1999). This method is problematic because it confounds the effects of temperature and day length. Photoperiodic time measurement, the measurement of day length, is prevalent across the entire spectrum of members in the biotic world. This includes hundreds of animal taxa, from rotifers to rodents (Bradshaw and Holzapfel 2007), plants (Jackson 2009), and fungi

(Roenneberg and Merrow 2001). *Wyeomyia smithii* uses photoperiodic time measurement to anticipate the coming of summer, cuing development from larval to adult form, and to predict the onset of winter, cuing diapause (Bradshaw and Lounibos 1977). Placement of mosquitoes in the incorrect photic environment lowers fitness (Bradshaw et al. 2004). Therefore it was of primary importance not to change the photic year of the mosquitoes in the experimental environment.

CHAPTER II

MATERIALS AND METHODS

ENVIRONMENTAL ROOMS

Environmental rooms (2.5m x 5m) were run off of a SAGE controller, which controlled the temperature, humidity, and light in each room. A photoperiodic "day" for *W. smithii* starts at the onset of morning civil twilight and continues through evening civil twilight (Bradshaw and Phillips 1980). An experimental photic year is run by processor-driven controls (Auto-Matrix, Export, PA) programmed to provide day length fit to a cosine function of sunrise to sunset + two civil twilights at 46°N (Beck 1980).

The 46°N thermal year is based on maxima and minima of temperatures actually recorded in the pitcher-plant leaves through two summers and one winter (Bradshaw et al. 2004). The 40°N thermal year is based on interpolated maxima and minima of recorded leaf temperatures between 50°N (Evans 1971) and 30°N (Bradshaw et al. 2000). Daily maxima and minima are programmed as a cosine function of date, so that the thermal year lags the photic year by 25 days (winter solstice on December 21, to midwinter low temperature on January 15); similarly, temperatures during a given day are programmed as a cosine function of time of day, so that the thermal day lags the photic day by three hours as it does in the field (Bradshaw 1980, Bradshaw et al. 2000).

TEST FOR PHENOTYPIC RESPONSE TO CLIMATE CHANGE

We subjected nine northern populations of *W. smithii* to three years of selection in an environmental room simulating the climate of Maine after 180 years of warming at current rates of about 0.31°C per decade (Follard et al. 2001). This initial selection research was completed by Deder Siedler, and is the subject of his Master's thesis (2008). In brief: mosquitoes were collected from nine locations at 46°N latitude. These populations were reared for two generations in the lab to reduce parental and grand-parental effects.

Two environmental rooms were programmed. **First:** an experimental room was programmed to simulate the climate of Maine after 180 years of climate warming. The photic year of the experimental room remained the same as Maine's current photic year. **Second:** a control room was programmed where both thermal and photic years closely followed the current thermal and photic years in Maine. Both experimental and control rooms were programmed to simulate an entire year of thermal and photic changes, proceeding through winter, spring, summer, and fall, as opposed to simulating only the growing season. For each population in each growth room, census population sizes of 420-480 were used to initiate experiments.

Mosquitoes were reared in their native micro-habitat: the leaves of the carnivorous purple pitcher-plant, *Sarracenia purpurea*. Therefore, the two environmental rooms provided a close approximation of the natural environment of *W. smithii*. After three

years of selection, I calculated fitness in both control and selected populations. Fitness was defined as R_3, the 3-year cohort replacement rate:

$$R_3 = \frac{\text{inital cohort size at start of experiment (year 0)}}{\text{number of larvae hatching from overwintering parents in the 4th spring (year 3)}}$$

TEST FOR ADAPTATION TO CLIMATE CHANGE

After rearing, selected and control lines were put through two generations to minimize maternal effects of the experimental and control environments. To test for a genetic response to climate change, I raised both selected and control populations in the experimental environment. Fitness was calculated as *Ry:*

$$R_y = \frac{\text{number of larvae initiating the first growing season}}{\text{number of larvae hatching from overwintering parents in the 2nd growing season}}$$

Maintenance of populations:

Prior to placement in the experimental environment, selected and control populations were reared under lab conditions for two generations post selection, to reduce parental and grand-parental effects.

The experimental environment was set to a starting date of July 1, corresponding with the observed timing of oviposition of the first summer generation in nature. The room ran through an entire year, of all four seasons, including daily fluctuations in temperature, day-by-day changes in temperature, and day-by-day changes in photoperiod. Humidity was retained at 80% throughout the growing season.

Cohorts were reared inside the leaves of the carnivorous purple pitcher-plant,

Sarracenia pupurea, the natural micro-environment of *W. smithii*. Plants were stored 2 per 10L terrarium, in water to keep them hydrated.

Cohorts were set up in aliquots of 10, 20, or 30 larvae per leaf. Leaf sizes were chosen at 1mL per larva. Thus, a cohort of 10 larvae was placed in a 10mL leaf, and a cohort of 30 larvae was placed inside of a 30mL leaf. This larva to leaf size ratio was chosen in order to optimize against crowding (Bradshaw and Holzapfel 1989).

For each population and its control, up to 90 larvae were set up in leaves per week, per room. This addition of larvae was maintained for 4 weeks, resulting in a total of 90 larvae x 9 populations x 9 controls x 4 weeks, or 29,160 initial larvae.

Cohorts were fed on a weekly basis for the first 4 weeks of their time in the experimental environment. They were fed 100 adult fruit flies (*Drosophila melanogaster*) per 10 larvae, directly into the pitcher-plant leaf. The number of flies per feeding was split up as follows: A 10-larvae leaf, 100 flies were fed at 10 the first week, 25 the second week, 50 the third week, and 15 the fourth and final week; a 20-larvae leaf was fed 200 flies at aliquots of 25, 50, 100, 25; a 30-larvae leaf was fed 300 flies in aliquots of 38, 150, 75, 37. Feeding was split up in this manner to replicate natural prey-capture behavior of the pitcher-plant (Bradshaw 1983, Bradshaw and Holzapfel 1986).

Leaves were checked twice per week for pupating larvae. Pupae were removed from pitcher-plant leaves, and placed in 7.6L population cages where they emerged as adults. Eggs were collected from these cages thrice per week, into petri dishes of distilled water. Eggs were counted, and the dishes were scored for hatchlings at 5 and 10 day

intervals from the egg collection date. Pupal exuviae were also collected from the adult cages and sexed, in order to measure eclosion success, and the effective size of the adult population.

Populations ran through 3 to 4 generations before overwintering as larvae in the experimental environment. During overwintering, pitchers were checked every week for senescence to ensure that no larvae were lost to leakage of the pitcher-plant leaves.

During the second growing season in the experimental environment, no new larvae were added to the leaves. Larvae were fed at a rate of 1 fly per 2 larvae per week. This ratio was chosen in order to mimic passive prey capture of pitcher-plants in the spring. Pupal, egg, exuvia, and larva collection was performed in the same manner as in the first growing season. Populations were maintained until pupation ceased and all adults died.

Test for effects of inbreeding

Cumulative inbreeding after three years was calculated recursively using the following equations for the inbreeding coefficient:

$$F_1 = \frac{1}{2N_1} + (1 - \frac{1}{2N_1})(\frac{1}{2N_0})$$

$$F_2 = \frac{1}{2N_2} + (1 - \frac{1}{2N_2})(\frac{1}{2N_1})$$

$$F_3 = \frac{1}{2N_3} + (1 - \frac{1}{2N_3})(\frac{1}{2N_2})$$

where N_x refers to the effective population size in year x. Effective population size is

used in lieu of census population size to control for unequal sex ratios. Effective population size is calculated using the following derivation of Sewall Wright's (1931) equation:

$$\frac{1}{2N_x}=(\frac{1}{2})(\frac{1}{4M_x}+\frac{1}{4F_x})$$

with M_x = the number of males from year x, and F_x = the number of females from year x. The equation for effective population size assumes random mating and equal contribution of each individual to the population. In reality, mating is unlikely to be random, and individuals have unequal fertilities and differences in clutch size. Female mosquitoes are monogamous, and only have one mating event in their lifetime (Clements 1963). Assuming all females mate, and each male has an equal chance to mate, I calculated the effective number of males, M', using the following equation adapted from the Poisson distribution:

$$M'=M(1-e^{-F/M})$$

with F = the number of available matings = the number of females, and M = the original number of males. Thus, when the number of females is less than the number of males, the effective number of males will be greatly reduced. For example, in a population with 80 males and 20 females, effective population size is 64. Controlling for monogamy by modifying the effective number of males, we get :

$$M'=M(1-e^{-F/M})=80(1-e^{-20/80})=17.7$$

This changes the effective population size such that:

$$N_e=4(17.7)(20)/(17.7+20)=37.6 \quad !$$

Although this modification provides stark differences in our calculation of effective population size, it must be noted that this is still an *overestimate* of effective population size because unequal fertilities and differences in clutch size were still not controlled for.

CHAPTER III

RESULTS

TEST FOR PHENOTYPIC RESPONSE TO CLIMATE CHANGE

I calculated fitness after three years in the control and experimental environments (Figure 2). In the control environment, fitness was positive, but no significant difference of log*Ry* from zero (log*Ry* should be zero if *Ry* is 1.0) was revealed by a two-tailed t-test; log*Ry* = 0.098±0.119 (t=0.819, P=0.436). This indicates that our control environment properly replicated the natural environment.

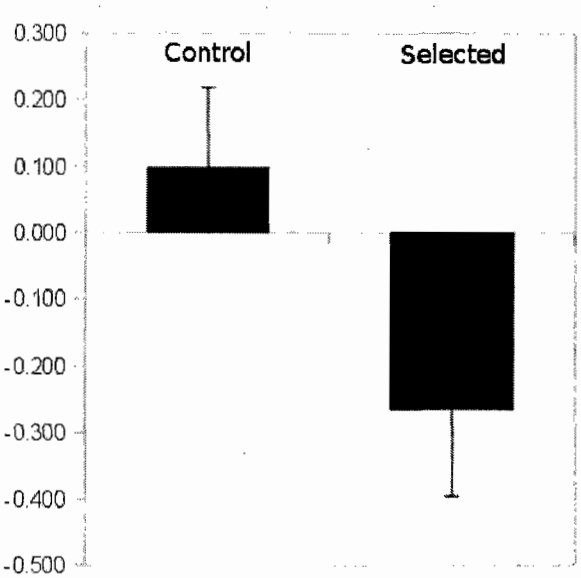

Figure 2: Mean fitness (log(*Ry*)) in control and selected lines after three years of selection. Fitness in control lines was not significantly different from zero, but selected lines had a significant decline in fitness. This shows that there was a phenotypic response to selection.

In the experimental environment, log*Ry* was significantly less than zero (log*Ry* should be less than zero if *Ry* is less than 1.0); log*Ry* = -0.265±0.131 (t=2.023, P=0.039).

Thus, rapid climate warming did, indeed, cause a selection differential because there was a phenotypic response to climate in the experimental environment.

Test for adaptation to climate change

To test for a genetic difference in control and selected lines, I raised both lines simultaneously in the experimental environment, and then compared fitness (*Ry*) (Figure 3). There was no significant difference in fitness between the control and selected lines showed by a t-test for paired comparisons: *Ry(selected-control)* = -0.644±0.852 (t=0.756 P=0.236). Therefore, there was no genetic change induced by the climate warmed environment.

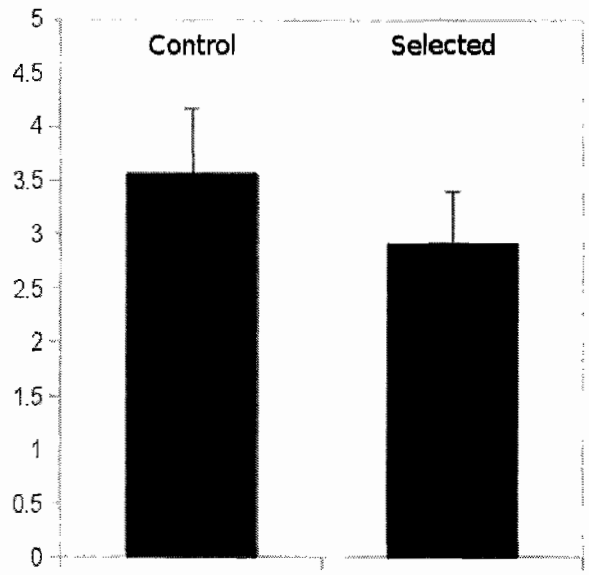

Figure 3: Fitness in control and selected lines after rearing in the common garden. There was no significant difference in the fitnesses, which indicates that there was no genetic response to the experimental environment.

TEST FOR EFFECTS OF INBREEDING

To discover the effects of inbreeding on fitness, we first calculated the coefficients of inbreeding for each population in the selected and control lines. When regressed on fitness, there was a strong correlation between the coefficient of inbreeding and log*Ry* (Figure 4). This correlation was found in both experimental and control populations. Fitness was negatively correlated with inbreeding (ANCOVA: $F_{1,17} = 31.43$; $P < 0.001$) but fitness did not differ between selected and control lines ($F_{1,17} = 2.78$; $P = 0.117$) or their interaction with inbreeding ($F_{1,17} = 2.81$; $P = 0.116$).

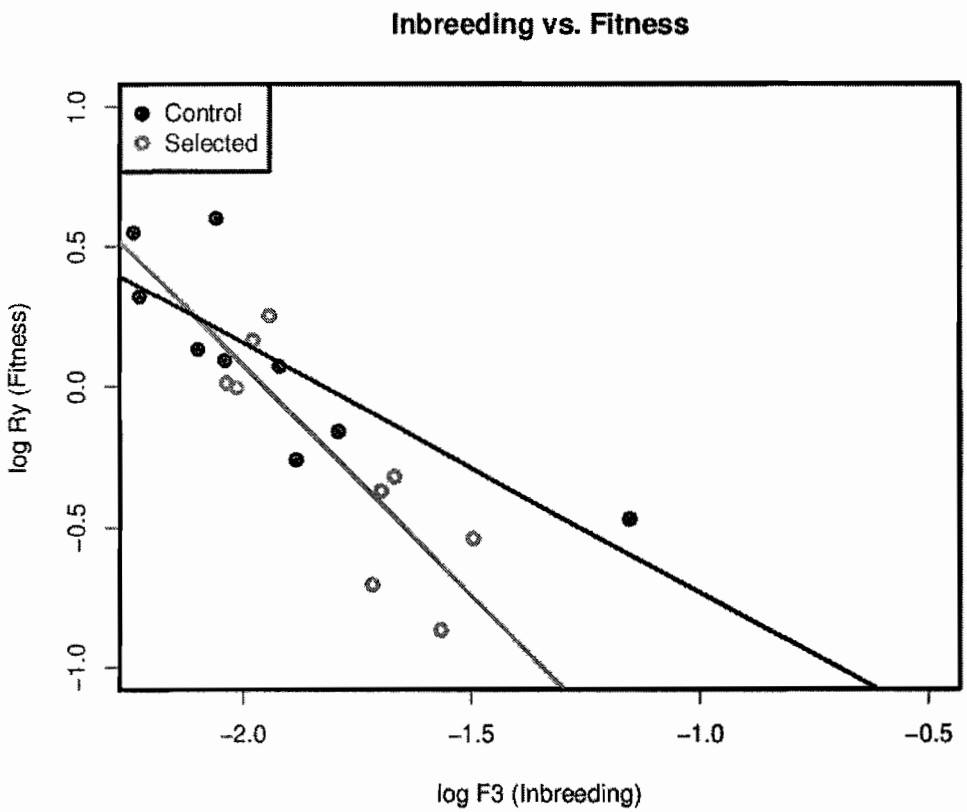

Figure 4: Fitness regressed on inbreeding in both control and selected lines raised in the experimental environment. There was a significant correlation between fitness and inbreeding in both control and selected lines. Furthermore, ANCOVA showed no significant difference in slope or intercept of the lines.

CHAPTER IV

DISCUSSION

Herein, I have found that small populations of *W. smithii* are unable to respond to selection imposed by rapid climate change because of the effects of inbreeding and drift. Rapid climate change does, indeed, impose selection on *W. smithii,* as evidenced by the decline in fitness of selected lines in the experimental environment, compared with the maintenance of fitness in control lines in the control environment over three years (Figure 2). Yet, the common garden experiment showed that there was no genetic change distinguishing selected and control lines (Figure 3). There was, however, a strong negative correlation between fitness and inbreeding (Figure 4). The relationship appeared in both selected *and* control lines, which implies that the decline in fitness is due to inbreeding and drift in small populations rather than selection. Since the relationship between fitness and inbreeding did not differ between the selected and control lines, we conclude that the effects of inbreeding and drift on fitness exceeded any selection due to differences in the climates we imposed.

This research also revealed a strong disparity in census versus effective population sizes. Despite initiating selection with reasonable census population sizes of 420-480, our calculation of N_e, controlling for monogamy, showed drastic declines in population sizes, especially when males outnumbered females. Thus, inbreeding and drift can be

important factors even in initial census population sizes greater than 400. The clear evolutionary (genetic) response of *W. smithii* to natural selection due to climate change in nature (Bradshaw & Holzapfel 2001) is therefore likely due to the larger population sizes in nature where the effects of selection are sufficient to overcome genetic drift.

This research project involved an instant 180-year climate jump rather than replicating current rates of climate warming. In nature, *W. smithii* have shown an evolutionary response to climate warming in only five years (Bradshaw and Holzapfel 2001). Therefore, the drastic climate change performed in our research has imbued in us a sense of confidence that, if *W. smithii* could respond to the selection imposed, the response should show up within the three years of selection. There is no definite reason to believe that the strong selection imposed in this research should alter the nature of the response of *W. smithii* to selection. Nevertheless, it must be taken into consideration that this instant climate change does not replicate naturally occurring climate change. However, I assert that the purpose of scientific research is to give us the ability to predict the future. We can examine the consequences of climate change in real time, *without* sullying the naturalness of our experiments, but *while* surrendering our predictive power. Meanwhile, the research elaborated herein, in concert with the greater body of research, can give us more confidence in our ability to predict the consequences of climate warming far into the future.

The rate of climate change is increasing with latitude (IPCC 2007). At the same time, peripheral populations at the edges of their distribution have been undergoing

inbreeding due to successive founder events. Even when population sizes may appear to be adequate for selection to be effective in the new environment, inbreeding and drift can be having an overriding effect. We conclude, therefore, that small northern populations should be especially vulnerable to rapid climate change due to the potential of inbreeding and drift to overcome response to selection.

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