PHENOTYPIC DIMENSIONS OF REPRODUCTIVE ISOLATION BETWEEN NORTHERN AND MELISSA BLUE BUTTERFLIES IN THE ROCKY MOUNTAINS

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✦ INTRODUCTION

Biological diversity results from speciation, which generally involves the splitting of an ancestral species into descendant species due to adaptation to different niches or the evolution of reproductive incompatibilities (Covne and Orr 2004). The diverse flora and fauna of the world, including the native inhabitants of the Greater Yellowstone Area (GYA), exist as a result of the speciation process. The central role speciation plays in generating biological diversity imbues importance to our understanding of this process. The general importance of a thorough understanding of speciation is amplified because of the current high rates of extinction on the planet. This is because a long term solution to the present extinction crisis will require maintaining the processes that create species (speciation) not simply preventing extinction. However, many central questions regarding speciation remain to be answered. One fundamental question in speciation research is whether diverging species are isolated (i.e., prevented from interbreeding) due to differences in one, a few, or many characters and whether each of these character differences results from different alleles at a few or many genes. For example, speciation and reproductive isolation might involve divergence along multiple phenotypic axes, such as mate preference, habitat use or preference, and phenology (the timing of life-cycle events). Alternatively, isolation could result from differentiation of a single character. I propose to address this question by assessing patterns of variation for a suite of characters across a hybrid zone between two butterfly species. This is possible because patterns of character variation across hybrids zones allow for inferences about reproductive isolation (Barton and Hewitt 1985).

The habitat diversity and geographic location of Grand Teton and Yellowstone National Parks provide an ideal opportunity to study speciation and hybrid zones. The topographic diversity of these parks allows a variety of different habitats (e.g. mountain forests, arid plains, meadows, alpine zones, etc.) to occur in close physical proximity. This feature allows for incipient species that have adapted to different habitats to come into contact, which provides the opportunity to study the morphological, behavioral, and genetic changes that partially isolate incipient species. The situation for studying speciation in Grand Teton and Yellowstone National Parks is further improved because these parks occupy a geographic region of North America where the ranges of many recently or incompletely reproductively isolated species overlap. This is because many incipient species that diverged in eastern and western glacial refugia during Pleistocene ice ages have since shifted their ranges to the Rocky Mountains.

My project focuses on a specific pair of incipient butterfly species, the Northern Blue butterfly (*Lycaeides idas*) and the Melissa Blue butterfly (*Lycaeides melissa*), that have come into contact in the GYA following the retreat of Pleistocene glaciers (Gompert et al. 2010). These are small butterflies in the Lycaenid family that are notable for the iridescent blue color on the dorsal surface of male wings. The Northern Blue is found in moist meadows and forest clearings from Alaska to the Rocky Mountains, whereas the Melissa Blue is found in more arid environments such as prairies and shrub-land throughout the western United States. These butterflies have several subtle differences in morphology and feed on different larval host-plants (Gompert et al. 2010). In the GYE, Northern Blue populations have a single generation per year with adults active from mid-July to early August; Melissa Blue populations have at least two generations per year with adults active in June or early July and again in late July or August (Z.G. personal observation, summer 2008 and 2009). My previous work demonstrated that Northern Blue and Melissa Blue butterflies interbreed in this region and have formed a hybrid zone (Gompert et al. 2010). This hybrid zone between the Northern and Melissa Blue is an ideal system to address the question of whether a few or many characters are typically involved in reproductive isolation.

The objective of this project is to identify specific characters that contribute to isolation between Northern Blue and Melissa Blue butterflies and determine whether these constitute a few or many characters. I will be focusing on the following characters: male genitalic morphology, wing pattern morphology, male mate preference, female oviposition preference, diapause emergence conditions, and diapause initiation conditions. Data from morphological characters have been previously reported and published (see Gompert et al. 2010 for details). For each character, I will first determine whether differences exist between Northern and Melissa Blue butterfly populations. Characters that do not differ are unlikely to contribute to reproductive isolation. Character differences will be assessed using a series of field and lab experiments coupled with Bayesian parameter estimation. For those characters that differ between Northern and Melissa Blue populations, we will also collect experimental data across the hybrid zone. We will contrast the geographic pattern of variation across the hybrid zone for each of these characters with the pattern of variation for neutral genetic markers using analytical methods derived from geographic cline theory (Barton and Hewitt 1985). Characters with significantly narrower geographic transitions between Northern Blue-like and Melissa Blue-like than expected given patterns of neutral molecular markers will be identified as likely being involved in reproductive isolation. The number of characters with geographic patterns of variation that indicate a potential contribution to reproductive isolation will provide a preliminary answer to the question of whether these species are isolated by a few or many characters. Because these butterfly species still hybridize, the estimate of the number of characters involved in isolation will underestimate the number of characters necessary for isolation between these species to be complete. The proposed research will be followed by extensive genetic mapping of these characters and population genetic analyses to verify these results.

✦ METHODS

I intend to assay male mate preference, female oviposition preference, and diapause initiation in two Melissa Blue populations (Lander, WY lat: 42.65°N long: 108.36°W; Victor, ID lat: 43.66°N long: 111.11°W), two Northern Blue populations (Bunsen Peak, WY lat: 44.93°N long: 110.72°W; Siyeh Creek, MT lat: 48.77°N long: 113.72°W), and five populations from the Rocky Mountains hybrid zone (Bull Creek , WY lat: 43.30°N long: 110.55°W; Blacktail Butte, WY lat: 43.64°N long: 110.68°W; Mt. Randolf, WY lat: 43.86°N long: 110.40°W; Shadow MT., WY lat: 43.70°N long: 110.61°W; Hayden Valley, WY lat: 44.68°N long: 110.50°W). We will estimate male mate preference for Northern Blue and Melissa Blue female wing patterns by assaying approximately 25 male butterflies from each of these populations. This will be done by placing pairs of model females (made by printing high quality wing patterns from photographed butterflies) with Northern Blue and Melissa Blue wing patterns at each population in the presence of males searching for mates. The behavior of individual males will then be recorded, specifically we will record which model individual males approach. Similar experimental designs have been effective for testing male mate preference in butterflies (Jiggins et al. 2001, Fordyce et al. 2002). Approaching males will be captured using insect nets and retained for future morphological and genetic analyses. We will use Bayesian statistical models to estimate mate preference for each population and test for differences in this preference between Northern Blue and Melissa Blue populations.

I will also test for variation in female oviposition preference (i.e., female preference for laving eggs on alternative larval host plants). Approximately 25 females will be collected from each of the 11 populations. Each female will be placed in a small oviposition arena (made from plastic cups) with equal amounts of plant material from a Northern Blue host plant (Astragalus miser) and a Melissa Blue host plant (Medicago sativa). Females will be allowed to lay eggs on these plants for 48 hours. I will then remove each female and count the number of eggs laid on each host plant species. Eggs will be retained for the next set of experiments (see next paragraph) and female butterflies will be retained for future morphological and genetic analyses. This experimental design has been used previously to assess oviposition preference in the Northern and Melissa Blue. Females generally lay between 10 and 100 eggs. As above we will use Bayesian statistical models to estimate oviposition preference for each population and test for differences in this preference between Northern Blue and Melissa Blue populations.

Finally, I will conduct two experiments to test for potential causes of differences in adult flight season and number of adult broods (i.e., two components of phenology) between Northern Blue and Melissa Blue populations. The first of these experiments will test for differences in the number of cold days required for eggs to complete their dormant stage (diapause emergence), which should affect the onset of the adult flight. The second experiment will test for differences in whether eggs laid by adults enter diapause or directly develop as larvae (diapause initiation), which should affect the number of adult broods per year. The diapause emergence experiment will involve maintaining the collected eggs (from the oviposition preference tests, above) at 2-4° C to simulate winter beginning in mid-September. Monthly, from February to May of the following year we will place five eggs from each female in a growth chamber kept at 25° C with 16 hours of light and 8 hours of dark and record the proportion of larvae from each female hatching after being removed from winter conditions. We will use Bayesian models to estimate the effect of family and population on the proportion of larvae emerging after 5, 6, 7, or 8 months of simulated winter. All larvae that hatch will be reared to adults for the diapause initiation experiment. We will rear larvae at 25° C under either 16 hours of light and 8 hours of dark or 10 hours of light and 14 hours of dark to determine sensitivity of entering diapause to photoperiod. Individuals from each family will be split evenly between the two treatments. Adults from each treatment will be mated and we will record the proportion of each reared and mated females' eggs that develop directly into larvae versus entering diapause. Similar to diapause emergence experiment, we will use Bayesian models to estimate the effect of family, population, and environmental treatment on the proportion of eggs entering diapause. These results will be contrasted between Northern and Melissa Blue populations.

Geographic patterns of hybridization (i.e., geographic clines) provide insight into the processes affecting hybridization dynamics and can be used to identify characters involved in reproductive isolation. We will estimate parameters for geographic cline models using maximum likelihood for each of the characters above using the software *Cfit7*. We will then test whether these clines can be explained well by a model in which they are constrained to be concordant with the geographic cline in overall genomic composition based on neutral genetic markers (e.g., Gompert et al. 2010); models will be compared using the Akaike Information Criterion. Clines in morphological characters from our previous research will also be included in this comparative analysis. Characters involved in reproductive isolation should not be concordant with the genomic composition cline, but should instead be more narrow.

✦ RESULTS

Data analysis from the 2009 field season is on-going, and the following preliminary patterns should not yet be cited. Preliminary mate preference data have been collected from three hybrid zone populations and one Northern Blue population. These data have been analyzed using Quade tests, which are rank-based non-parametric tests. Males from the three hybrid zone populations failed to discriminate between Melissa Blue and Northern Blue female wing patterns (BCR: N = 27, P = 0.793; BTB: N = 20, P = 0.417; MRF: N = 23, P = 0.931). Conversely males from the Northern Blue population at Bunsen Peak preferred Northern Blue female wing patterns relative to Melissa Blue wing patterns (N = 14, P = 0.0499).

I have obtained preliminary oviposition preference from three hybrid zone populations and one Northern Blue populations. Females from the Blacktail Butte and Bull Creek populations failed to distinguish between *M. sativa* and *A. miser* (BTB: N = 28, P = 0.237; BCR: N = 23, P = 0.236), however, females from the Shadow Mountain population preferred *A. miser* relative to *M. sativa* (SHA: N = 27, P = 0.011). Females from the Kings Hill Northern Blue population had a marginally significant preference for *A. miser* relative to *M. sativa* (KHL: N = 21, P = 0.061). We have not yet obtained data for the diapause experiments, nor do we have data for Melissa Blue populations.

✦ DISCUSSION

More data are necessary before any definitive conclusions can be made. These data are being collected during the summer of 2010. The

current results suggest that admixed populations do not discriminate between Northern and Melissa Blue wing pattern, but that a weak preference for Northern Blue female wing patterns might exist in Northern Blue populations. Without data from Melissa Blue populations or multiple Northern Blue populations these data are difficult to interpret, however, this pattern might suggest that preference exists in the parental populations but has broken down in the admixed populations following hybridization. Similarly, I found little evidence for oviposition preference in admixed or Northern Blue populations (with the sole exception of the Shadow Mountain admixed population). However, with so little data from parental populations it is hard to say whether this represents a lack of oviposition preference in Northern and Melissa Blue populations or a breakdown of preference following admixture in the hybrid zone.

My previous results show clear genetic and morphological (male genitalia size and to a lesser extent wing pattern elements) differences between Northern and Melissa Blue populations in the Rocky Mountains with a broad geographic transition between the character states from these parental populations across the hybrid zone in the GYA (Gompert et al. 2010). I also know that differences in host-plant use and habitat exist between these species (Scott 1986, Gompert et al. 2010). Whether differentiation is limited to these phenotypic axes or includes mate preference, oviposition preference, and aspects of diapause physiology cannot be answered at this time, but should be clearer following my field work during the summer of 2010.

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