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Changes Of Dispersal Ability In An Isolated Population

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CHANGES OF DISPERSAL ABILITY IN AN ISOLATED POPULATION

by

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Bachelor of Arts
Oberlin College, 2013

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I would like to thank Carol Boggs, for guiding me through my time here. My command of language is insufficient to express how grateful I am.

My field assistants, Justin Davies and Katherine Shultz made this possible through their hard work and tolerance of my eccentricities. May they find their own assistants to be at least as excellent.

Finally, my family. Their support is unceasing and indispensable.

ABSTRACT

Dispersal is a core mechanism in the maintenance of metapopulations. It maintains genetic diversity by connecting subpopulations and generates new populations to replace those that die out. However, as populations become more isolated, as occurs in habitat fragmentation, dispersal becomes more difficult. This should lead to selective pressure against dispersive individuals, causing a reduction in dispersal traits. Over time, this can lead to variation in dispersal traits among populations. We examine this idea using an extreme case of isolation in *Euphydryas gillettii*, a population that has remained completely isolated for forty years. By comparing this population to a baseline established using multiple populations in the native range of the species, we found that the isolated population showed characteristics indicative of relatively low dispersal.

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LIST OF ABBREVIATIONS

BT	Beartooth Mountain
KC	Kendall Canyon
GC	Granite Creek
MC	Murphy Creek
RMBL	Rocky Mountain Biological Laboratory, also Gothic, CO

CHAPTER 1 DISPERSAL IN THE LITERATURE

Dispersal is an important factor that drives gene flow and spatial distribution of populations (Zhang 2018). Dispersing individuals maintain genetic connectivity between populations and can found new populations. Both of these effects help maintain the long-term viability of a species, by reducing the chance of inbreeding depression and compensating for local extinctions.

Inbreeding depression is a well-known phenomenon, measured as a reduction in fitness as the degree of inbreeding increases in a population. Within any given population, the residents will be related to one another to varying degrees. The more closely related a mating pair are, the more inbred their offspring will be, and this effect can be compounded over successive generations. It becomes more prevalent in small populations, where individuals are more likely to be closely related due to chance. Dispersal reduces inbreeding by introducing individuals from other populations, which are less likely to be related to the local population. The rate at which this transfer occurs is called connectivity. There are a variety of metrics used to characterize it, which incorporate various combinations of distance between patches, patch quality and individual movement ability to estimate the probability of successful dispersal (Viscotti, Elkin 2009). This connectivity maintains the genetic diversity of local populations (Ficetola et al 2007), which can in turn help protect them from extinction. Saccheri et al (1998) confirmed a link between reduced heterozygosity, which is a hallmark of

inbreeding, and extinction risk for populations of *Melitaea cinxia*, even after accounting for the effects of population size, degree of isolation, and other measures.

The genetic connectivity maintained by dispersal is not the only benefit that dispersal grants. Dispersing individuals can increase the area a species occupies, thereby reducing the extinction risk of that species. It is predicted that global climate change will cause the position of suitable habitat of many species to shift poleward (Parmesan et al 1999), and dispersing individuals are what will allow species to follow this shift. There are some well-known examples of range shifts in progress, notably in barnacles. One of these, *Tetraclita rubescens* has expanded northward by several hundred kilometers since 1970 (Dawson et al 2010).

Even when a range shift is not an imminent threat, dispersing individuals can exploit recently formed habitats when they appear. This process of colonization is a major contributor to the maintenance of metapopulations which consist of habitat patches with independent population dynamics between which there is some dispersal maintaining genetic connectivity. Further, metapopulations experience periodic extinction and recolonization of subpopulations (Levins 1969). The extinction of local populations would eventually cause the extinction of the species. This is prevented by the recolonization of empty patches, which is driven by dispersal. Examples of metapopulations are fairly common in Lepidoptera, which frequently have strict larval diet requirements, limited to one or a few plant species (Saastamoinen & Hanski 2008, Hill et al 1999). This requirement limits the usable breeding habitat to areas where the hostplant is present, effectively partitioning populations. Other examples include aquatic snails (Facon, David 2006 and pond-breeding amphibians Naujokaitis-Lewis et al. 2013).

Each habitat patch within a metapopulation has internal population dynamics that contribute to the structure of the metapopulation. As population density increases within any given patch, average reproductive success will decrease, until at a certain point the cost of remaining within that patch will exceed the potential cost of dispersing (Pulliam, Danielson 1991). At this point, attempting to move to another patch provides a chance for higher reproductive success, particularly in metapopulations. Since the productivity of different populations will vary within a given time frame, those that produce excess individuals will contribute to nearby populations. This can effectively 'rescue' populations that do poorly in that same time frame, which will help damp out population size fluctuations. These same dispersing individuals represent gene flow that can help prevent local extinctions.

Despite the benefits that dispersal offers, it presents distinct risks at the individual level. The main risk is the chance of not locating another local habitat patch once an individual departs its natal patch (Brontes et al. 2012). The longer an individual spends in the matrix between suitable habitats, the greater the risk becomes. For species that require particular resources for reproduction, such as the host plant requirement of many butterflies (Hanski et al 2002, Holdren and Ehrlich 1981), the matrix may not even be particularly hostile to adult survival. The individuals may be just as able to survive within the matrix as in the habitat patches, but the inability to reproduce within the matrix represents an opportunity cost. A final risk, curiously, is outbreeding depression. This can occur in highly heterogenous landscapes. In such landscapes, a population may have specific adaptations to the local conditions. Immigrating individuals will lack these adaptations, and the genetic maladaptation will impact any offspring the immigrant

produces (Brown 1991). Outbreeding depression is most common where dispersal rates are limited, allowing local populations to develop adaptations (Waser et al 2000).

Dispersal Adaptations

Long- and short-distance movements are often distinct, requiring different behaviors and morphologies. Short distance movements tend to be low speed with many turns, behavior that is characteristic of search patterns. These are utilized by all individuals to locate food, mates, shelter or any other necessities for continued life. Successful dispersal requires relatively long distance movements, which are usually fast and directed, and are frequently exhibited while an individual is outside a habitat patch (Van Dyck et al 2005). This is a common behavioral strategy that limits the time spent in the matrix, thereby reducing the cost of dispersal. Morphology can complement this behavioral shift. For example in the butterfly *Pararge aegeria*. Hill et al (1999) found that individuals in recently colonized patches had longer wings and larger relative thorax size, both of which contribute to flight ability. Since the patches were recently colonized, the most recent ancestors would have been dispersing individuals. This shows a clear association between movement ability as measured by morphology, and dispersal success. Detailed examples of morphological and behavioral adaptations follow.

Morphological Variation

Multiple strategies have evolved that minimize the risks and maximize the benefits of dispersal. This ranges from dimorphism, in which a species has two distinct body morphs, to continuous variation of dispersal ability within a species. Among

species, dispersal morphologies can vary considerably, as species that exhibit seasonal migration have different requirements than those that do not.

Flight dimorphism is one example of specialization, in which there are two phenotypes within the species, often flight-capable vs flightless. There are examples of these in beetles (Ikeda et al 2008) and in moths (Shi et al 2015). In these species, the flightless morph tends to have higher fecundity than the flying morph. This occurs most commonly in females and represents a distinct trade-off between dispersal and reproduction for individuals. Each morph has high fitness at the individual level under certain circumstances. The flightless morph is at an advantage when the local habitat is high quality, its higher fecundity allowing it to outcompete the flying morph. The flying morph, on the other hand, is able to abandon poor habitat in search of a higher quality patch, and will always have a higher chance of outbreeding. At the species level, the flying morph enables the colonization of new habitat, and the flightless morph contributes to the maintenance of existing populations.

More subtle variation in dispersal capability exists both within and between species. Insects and birds both depend on flight for the long-distance movements which comprise dispersal and there are some traits that consistently covary. Lockwood et.al. (1998) surveyed 244 species of birds, classifying them by ecological niche and migratory behavior. This study found that species that travel greater distances tend towards greater wing area and larger aspect ratio. Aspect ratio is a measure of shape, generally wing length/ wing width meaning that higher aspect ratio indicates a longer, narrower wing. This shape reduces drag at the wingtips, making this shape more efficient over distances (Lockwood et al 1998). An extreme example of a long distance

morphology is the albatross- these birds remain in flight for extended periods and are known to range from the equator nearly to the poles. They have extremely elongated wings, and a total wingspan that can exceed 3 meters, with an aspect ratio of approximately 15 (Hedenstrom, Alerstam 1998). This species occupies one end of a spectrum, the other end of which could be represented by the American Turkey. While they are capable of flight and roost in trees overnight, they are not endurance fliers. They have a much lower aspect ratio than the albatross, and the wingspan will reach little more than a meter with an aspect ratio of approximately 4 (Tobalske, Dial 2002).

Variation in these traits is visible to a lesser extent within species. The monarch butterfly, *Danaus plexippus*, is famous for its yearly migration from the breeding grounds in the US and Canada, to overwintering sites in Mexico. Not all monarchs make this trip; some on the west coast of the US overwinter in California, and there are populations in South America, the Pacific Islands and southern Florida that do not migrate at all (Altizer, Davis 2010, Zhan et al. 2014). These sedentary populations have less elongated wings, lower overall size and higher wing loading (Altizer, Davis 2010). Wing loading is mass/wing area, and measures how much lift an individual must generate per unit wing area. Lower wing loading indicates longer-distance flight. The population-level differences shown here indicate a link between life history, whether or not the population migrates in this case, and flight ability. Berwaerts et al (2002) found similar patterns in the butterfly *Pararge aegeria* by performing a direct comparison of flight morphology and acceleration capacity. They found that acceleration was positively correlated with wing length, relative thorax mass, wing area and position of wing centroid. The more distal the centroid, the greater the acceleration. This study compared

populations with different degrees of fragmentation, and showed that greater fragmentation tended to lead to stronger fliers.

Behavioral variation

Behavioral adaptations can be a powerful tool for reducing the cost of dispersal. The ability to recognize when dispersal is most advantageous or least costly will improve success rates. For example, amphibians will often move greater distances from ponds during wet seasons, when they are at a lower risk of desiccation. Palis (1997) showed a correlation between trap rates of the salamander *Ambystoma cingulatum* and damp weather, indicating that they were more mobile during these periods.

Movement patterns can increase the odds of locating another patch within a specific area. These patterns range from simple random walks to the ‘cloverleaf’ pattern employed by some ant species, in which the colony searches in a strike-and-return in a specific direction each day. This maximizes the chance of finding a fresh food source for the colony. In a flying insect, it would limit the distance they can search, but also allow the individuals to return to the natal patch repeatedly. (VanDyke et al 2005) A less conservative strategy for dispersal is to simply travel in a straight line, as quickly as can be managed. This is a remarkably common strategy across phyla, from whales to insects, and is sometimes even used to identify dispersing individuals (VanDyke et al 2005). This strategy allows the discovery of new habitat much further from the starting point than a randomized search pattern or the cloverleaf previously described. It is therefore of particular utility where habitat is highly fragmented, either naturally or otherwise.

Dispersal strategies generally refer to the choice of when to disperse, and involve the balancing of within-patch costs and the costs imposed by dispersal. A factor

that can drive this is the carrying capacity of a habitat patch. As the population density grows, the within-patch fitness costs will increase, up to the point where they exceed the cost of dispersal. In places where the cost of dispersal is low, the population need not even reach carrying capacity for dispersal to become an advantageous option. In a simulation study by Travis et al (1999), as long as within-patch costs actually existed, the population would develop a tendency to begin dispersing once the density grew high enough.

The final step in the dispersal process is settlement, which requires detection of suitable habitat. The ability to recognize habitat is essential, and being able to do so from a distance and home in on it provides a considerable advantage. The ability to further evaluate the habitat for quality allows for more complex decision-making, like selecting a particular habitat when there are multiple choices available (Pulliam, Danielson 1991). By selecting the best habitat available, a dispersing individual can maximize the fitness of its offspring.

Dispersal and Isolation

Habitat for many species is becoming increasingly fragmented, which can isolate populations over time (Hanski 2005, Warren et al 2001). Some effects of isolation on populations are known, such as a reduction in genetic variability and heterozygosity that occur due to drift and inbreeding (Puurinen, et al. 2004). These effects are more pronounced in smaller populations, and can even become the major drivers of evolution when populations are small enough (Welsh, 2014). One consistent effect of isolation seems to be reduction in dispersal capability. Once a population is isolated, the cost of dispersal increases to a degree correlated to the degree of isolation. This increased cost

can reduce dispersal behaviors and abilities (Schtickzelle 2006). Some examples of this include plants on islands that possess lower seed dispersal ability than their mainland counterparts (Cody, Overton 1996), and island birds that lose their ability to fly entirely. Rails (Rallidae) are one such family of birds, in which several species have evolved flightlessness independently on multiple islands in the Pacific Ocean (Silkas et.al 2002). The flight apparatus is expensive to maintain and did not provide enough of a return on the investment on the islands, and so was lost over time. The plants in question are in the Asteraceae family, which use wind-dispersed seeds. On the islands, any seed that is blown too far winds up in the ocean, where it fails. This is a more direct selection against dispersal distance, and in this case is often accompanied by an increase in seed size.

Since anthropogenic fragmentation continues to be an influence on populations, we need to make an effort to understand not only the dispersal capabilities, but also the variation of the same in species of interest. The extant variation in a species is what allows it to respond to changes that occur in relatively short evolutionary periods. By understanding it, we can make predictions about the effects that fragmentation will have, and at what point fragmentation will begin to negatively impact the future prospects of a species. We will be examining the short-term evolutionary change in dispersal characteristics that can occur with isolation.

CHAPTER 2 EVOLUTION IN AN ISOLATED POPULATION

Dispersal is a core mechanism in the maintenance of metapopulations. It maintains genetic diversity by connecting subpopulations and generates new populations to replace those that die out. However, as populations become more isolated, as occurs in habitat fragmentation, dispersal becomes more difficult. This should lead to selective pressure against dispersive individuals, causing a reduction in dispersal traits. Over time, this can lead to variation in dispersal traits among populations. We examine this idea using an extreme case of isolation in *Euphydryas gillettii*, a population that has remained completely isolated for forty years. By comparing this population to a baseline established using multiple populations in the native range of the species, we found that the isolated population showed characteristics indicative of relatively low dispersal.

As a general rule, dispersal behaviors and capabilities decrease when a population experiences isolation, the effects of which can be observed in as little as twelve generations (Cote et. al. 2017). This effect is likely caused by the increased cost of dispersal that isolation generates. Isolation is the result of some sort of barrier to movement, for example distance or newly built human structures. The barrier increases the risk associated with dispersal, and places a selective pressure on dispersing individuals. As this cost increases, dispersing becomes less and less advantageous. Therefore, the more isolated a population is, the less dispersive we would expect it to be.

Since we are using a butterfly as our model animal, we approached this problem by examining morphological characteristics associated with long distance flight,

specifically aspect ratio, wing loading, relative thorax size and centroid position. A larger aspect ratio and lower wing loading correlates with long-distance flight in between-species comparisons (Turlure et al 2016). Aspect ratio is a measure of shape, with higher values indicating a longer, narrower wing. This is thought to reduce drag at the distal portion of the wing. Wing loading measures how much weight each square unit of wing must lift in order to achieve flight, meaning that individuals with lower wing loading will expend less effort in sustained flight. The thorax in flying insects is mostly filled with flight muscle, and therefore makes a good measure of allocation of bodily resources to flight. Norberg and Leimar (2002), found relatively heavier thoraxes in specimens of *Melitaea cinxia* that were from more dispersive populations.

The centroid of a shape is its center of mass. Any polygon, if made of a single, uniform material, will balance on its geometric centroid. In butterflies, the position of the wing centroid gives some information about where the majority of the wing area is. When it is more distal from the body, it provides a lift advantage, much like using a longer paddle in a canoe. We also directly measured endurance of individuals. This approach is reasonable, given that dispersal is difficult to measure directly, and the characters listed above have been linked to flight ability in butterflies (Berwearts et al 1998, 2002).

The system we used to study dispersal capabilities with isolation is *Euphydryas gillettii*, a butterfly whose native range stretches from Wyoming, Utah, and Idaho into Canada. Its population structure is driven by some very specific habitat requirements, the most important of which are the presence of the hostplant (*Lonicera*

involuncrata), and exposure to morning sunlight. Females lay eggs on the undersides of leaves near the top of the plant, most often on leaves that catch morning sunlight (Williams 1981). Placing the eggs on leaves that catch the morning sun brings them up to a metabolically active temperature earlier in the day, and overall can shorten the incubation time by as much as ten days (Williams 1981, Bonebrake et al 2010). This specificity of their habitat requirements ensures that the populations will be patchy at best.

This species is of particular interest because we have a unique case of complete isolation to study. In 1977, a population of *E. gillettii* was introduced to Gothic, Colorado (38°57'34.34"N, 106°59'34.51"W; 9500m asl) and has since fluctuated between 20 and 10,000 individuals (Holdren & Ehrlich 1981; Boggs et al 2006; unpublished data). Upon introduction, it was the only population of *E. gillettii* in the area. The population has since spread from a single, discrete population to a slightly larger patchy population with a small amount of dispersal throughout. Additionally, a second small population was colonized between 2007 and 2011, approximately 1.75km. from the original introduction site. Despite this spread, it remains isolated from the rest of the species.

We sampled four populations in the native range to establish a baseline of variation in dispersal characteristics. All of these are expected to be less isolated than the introduced population in Colorado, and represent the natural variation in the species. If isolation does decrease dispersal capabilities, we expect that RMBL will have lower endurance, thorax size, and aspect ratio, and higher wing loading as compared to the native sites. We also examined the change in dispersal characteristics over time at RMBL using archived photos. This will allowed us to determine whether there is a consistent

change in dispersal characteristics in the expected direction, and whether there is a great deal of variation from year to year, which could indicate plasticity as a factor.

Materials and methods

All specimens were captured in July 2017 using hand nets and were kept in glassine envelopes for ease of transport until processing (Appendix 1 for map of capture sites). Each was photographed using a Canon ELPH 180 under a layer of plexiglass with a grid consisting of 1x1 mm. squares for scale. All pictures were taken at the highest resolution possible (5151x3864 pixels) with the butterfly occupying as much of the frame as possible. Specimens were weighed to within .001 g. on an OHAUS model SPX123 portable electronic balance. We recorded the wing wear of each individual, which is an estimate of relative age, and ranges from 1-5 in increments of 0.5, where a 1 is an individual still damp from adult eclosion. The next two increments are determined by the rigidity of the wing tips, as the butterflies can begin to fly before the wings fully harden. After that, an additional 1.5 can be added for degrees of scale loss and tearing of the wing edges (Watt et al.1979). We then marked the butterflies with individual numbers written on the hindwing with an ultra-fine point Sharpie™. This prevented us from including the same individual twice.

Endurance testing at the native sites was done by placing individuals in a small (approx. .0028m³) enclosure, and using a paintbrush to gently flick the individuals off of whichever surface they attempted to land on. This continued until either attempts to fly ceased, or the trial reached 20 minutes. Manpower in the field was limited, so we

truncated the trial at 20 minutes in order to make sure we could test all captured individuals before sunset. Since temperature is known to affect metabolism, we measured air temperature within the enclosure and performed all trials in shaded areas to prevent additional radiative warming. As ectotherms, this procedure should couple the body temperature closely to air temperature. In Gothic the trials were performed using a 2-liter plastic jar, which was shaken to trigger flight, and the tests were done indoors. We excluded any endurance trials that were performed below 23C, as we had a high proportion of individuals that refused to even attempt flight below that point, which makes the data below that temperature questionable. Further data from Boggs (unpub) indicates that the thermal optimum is between 30-40C internal temperature. In those data, the minimum temperature for flight was 26C, though crawling began at 20C.

Raw body measurements were taken from the photos of specimens, using ImageJ and all wing measurements were taken from the forewings. Wing length was measured as the distance from the hinge of the wing to the tip of the costa, width as the greatest distance between the leading and trailing edges of the wing. Thorax width was measured as the distance between the wing joints on either side. Wing area was obtained by outlining each wing with the free-form polygon tool in ImageJ. For undamaged wings, we also digitized nine landmarks (Figure 2.1) to characterize the wing shape. This was used to determine the location of the centroid; more distal centroid position has been linked to flight ability in other butterflies (Berwearts et al 1998, 2002).



Figure 2.1 Measurement Diagram. Wing length and width and thorax width marked with solid lines. The shape characterization landmarks are marked on the right wing.

We captured a total of 71 individuals over four populations in the native range. Of these, sixty were female, leaving us with insufficient numbers of males to draw any meaningful comparisons among the populations. Of those females, four were excluded due to significant damage to the wings which prevented measurement. This left 56 females from the native range for comparison with 20 concurrent samples from RMBL. The analysis of change over time at RMBL used 3 females and 5 males from 2011, 18 females and 5 males from 2013 and included the 20 males from 2017 that were excluded from the previous analysis.

All raw measurements were entered into Excel for organization, and wing loading, aspect ratio and relative thorax size were all calculated there before importing

the data into R for analysis. Thorax size is normalized in order to give an estimate of allocation to flight muscle, which we cannot get from the raw size. Formulae used follow.

wing loading=mass/(total wing area)
aspect ratio=(wing length)/(wing width)
relative thorax size=(thorax width)/mass
relative centroid position=(distance between wing hinge and centroid)/(wing length)

R version 3.0.3 was used for statistical analysis of morphology and endurance. We ran an ANOVA on each using site as an explanatory factor. In the analysis of wing loading and thorax size, wing wear was included as an additional predictor, since it is known that mass decreases with age in butterflies as eggs are laid. Posthoc pairwise comparisons between sites were performed using Tukey's HSD.

Results

Endurance.

This direct measure of flight ability showed no significant differences among populations ($F_{4,48}=1.428$, $P=0.239$), and no significant effect of temperature ($F_{1,48}=1.285$, $P=.264$) or wing wear ($F_{1,48}=1.737$, $P=0.194$). (Figure 2.2)

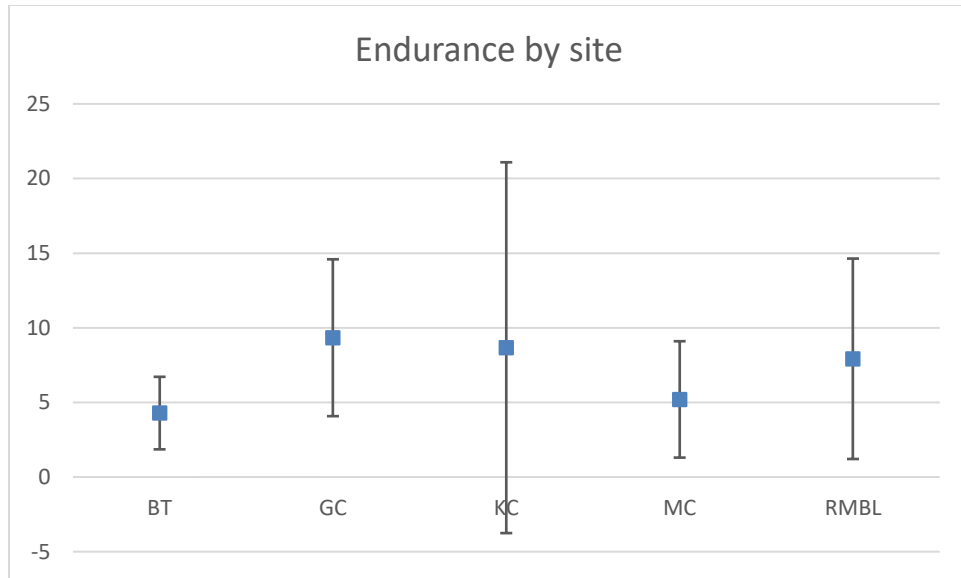


Figure 2.2 Average and 95% confidence intervals of endurance for each site measured as minutes spent attempting to fly.

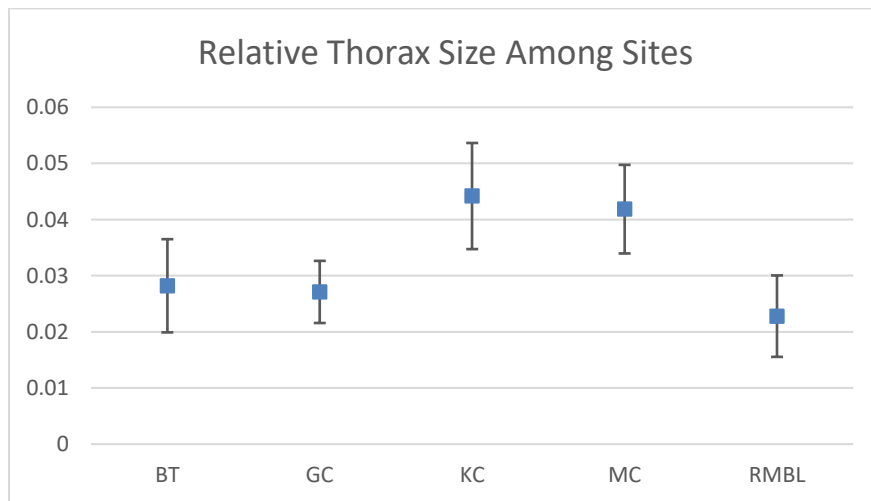


Figure 2.3 Relative thorax size for each site. Higher values are expected to correlate with greater dispersal.

Our analysis of relative thorax size showed that site ($F_{4,66}=15.1$, $P<.0001$) and wing wear ($F_{5,66}=2.72$, $P=.027$) both had significant effects (Table 2.1). Within the native sites, Granite Creek (GC) and Beartooth Lake (BT) formed a distinct

pair, as did Kendall Canyon (KC) and Murphy Creek (MC) (Figure 2.3). MC and KC had larger relative thorax sizes, which we expect to have a positive correlation with flight ability. As wing wear increased, relative thorax size also increased, most likely due to decreasing mass with age. Our isolated population fell on the lower end of the distribution for the native range, being statistically indistinguishable from BT and GC (Table 2.2). We constructed 95% confidence intervals for the differences between RMBL and the two similar native sites, GC and BT. Both intervals excluded zero, but the differences were too small to be significant.

Table 2.1 ANOVA Results

		Df	Sum Sq	Mean Sq	F value	Pr (>F)
Endurance Time	site	3	185	61.67	2.103	0.117
	temp	1	37.7	37.69	1.285	0.264
	Residuals	37	1085.2	29.33		
Thorax size	site	4	0.002576	0.000644	15.1	8.15E-09
	as.factor (wear)	5	0.00058	0.000116	2.72	0.027
	Residuals	66	0.002814	4.26E-05		
Wing loading	site	4	0.088	0.021999	7.137	7.74E-05
	as.factor (wear)	5	0.05382	0.010763	3.492	0.00734
	Residuals	66	0.20345	0.003083		
Aspect Ratio	site	4	0.04578	0.011444	4.341	0.00338
	Residuals	71	0.18717	0.002636		

Table 2.2 Results for Tukey's HSD Among Sites

Endurance	BT	GC	KC	MC
GC	0.246099			
KC	0.800638	0.999688		
MC	0.998737	0.577951	0.920034	
RMBL	0.658152	0.948352	0.999608	0.893743
Wing loading	BT	GC	KC	MC
GC	0.994745			
KC	0.177093	0.074732		
MC	0.031869	0.007323	0.993442	
RMBL	0.268972	0.260156	0.005255	0.000257
Aspect Ratio	BT	GC	KC	MC
GC	0.792354			
KC	0.033652	0.106099		
MC	0.366434	0.730938	0.818199	
RMBL	0.931634	0.176057	0.005818	0.112424
Adjusted thorax	BT	GC	KC	MC
GC	0.984285			
KC	0.000506	5.39E-05		
MC	0.001432	0.000128	0.98349	
RMBL	0.134557	0.148488	0.000001	1.7E-06
Raw Thorax	BT	GC	KC	MC
GC	0.792451			
KC	0.997888	0.848769		
MC	0.996196	0.996569	0.982079	
RMBL	0.002615	9E-07	0.230615	0.026901
centroid position	BT	GC	KC	MC
GC	0.564759			
KC	0.814093	0.292789		
MC	0.999992	0.993925	0.962013	
RMBL	0.98653	0.804314	0.622174	0.999979

In addition, RMBL females had narrower thoraces on average than females from all of the native sites, which were all fairly similar to one another (Figure 2.4, Table 2). Analysis of raw thorax size over time at RMBL shows that this is not unusual for RMBL, and that 2013 had slightly lower thorax sizes for both sexes (Figure 2.5). The variation among years within each sex is nonsignificant, $p > .9$ for both sexes.

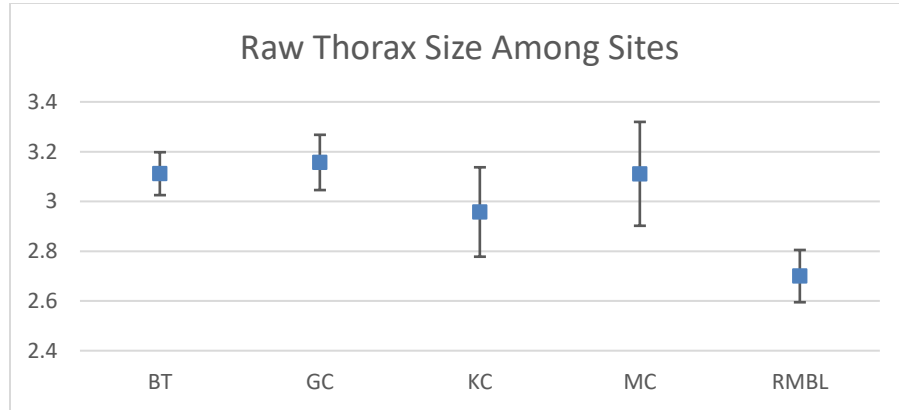


Figure 2.4 Raw thoax size shows no significant differences among the native sites, and is significantly smaller at RMBL

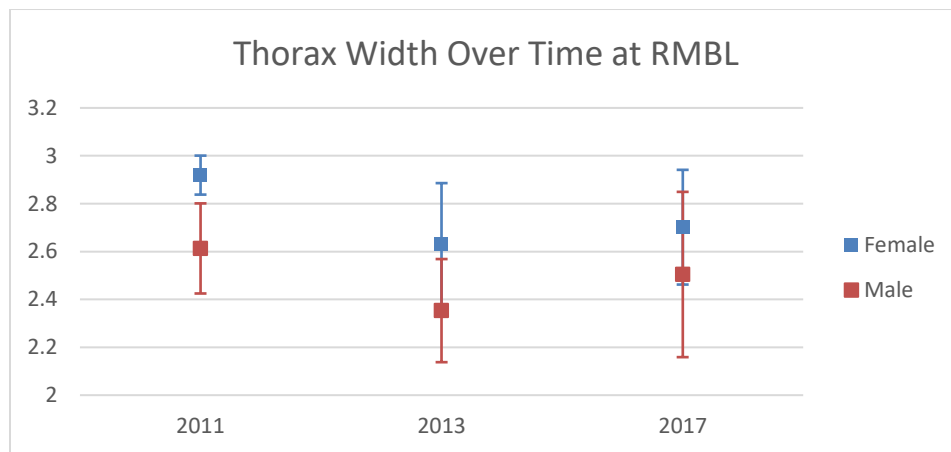


Figure 2.5 Average thorax width has decreased since 2011, but not steadily.

Aspect ratio was significantly different among sites ($F_{4,4} = 4.34$, $P = .003$). Statistically, only KC is distinguishable from any of the other populations (Table 2), and it is only different from BT and RMBL at $\alpha = .05$. RMBL, our isolated population is

remarkable here because it contains individuals with lower aspect ratios than any of the native sites, and has a wider range. (Figure 2.6)

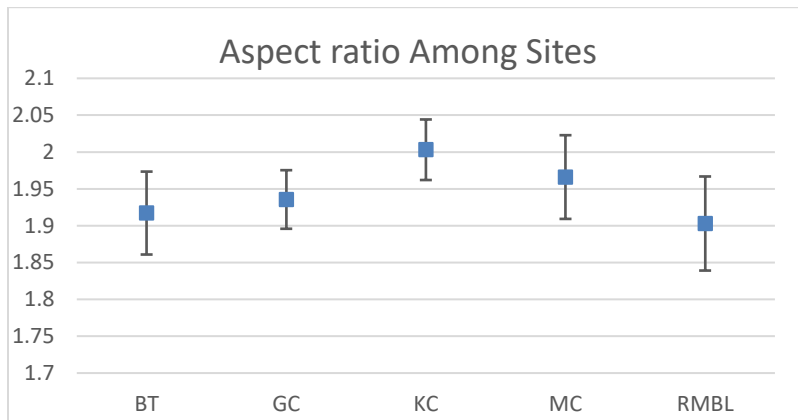


Figure 2.6 Aspect ratio is a rough measure of wing shape, with higher values associated with long-distance flight.

Again, change in aspect ratio among years at RMBL is nonsignificant for both sexes ($F_{1,67}=1.64$, $P=.205$) (Figure 2.7), though there is differentiation between the sexes (TukeyHSD, $P<.0001$). However there was a consistent reduction in wing length over time, though it was only significant for males (Figure 2.8).

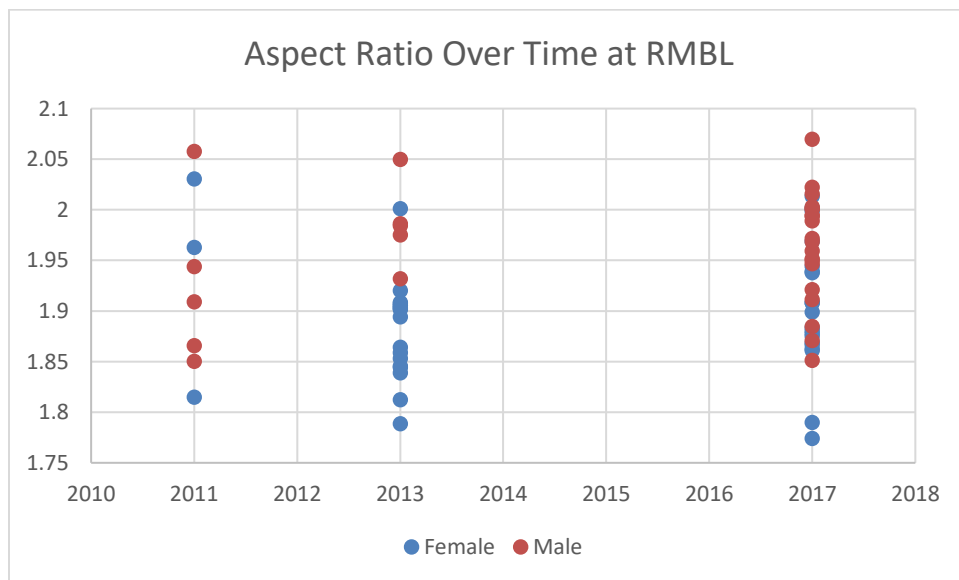


Figure 2.7 Minor change over time in aspect ratio, but not significant.

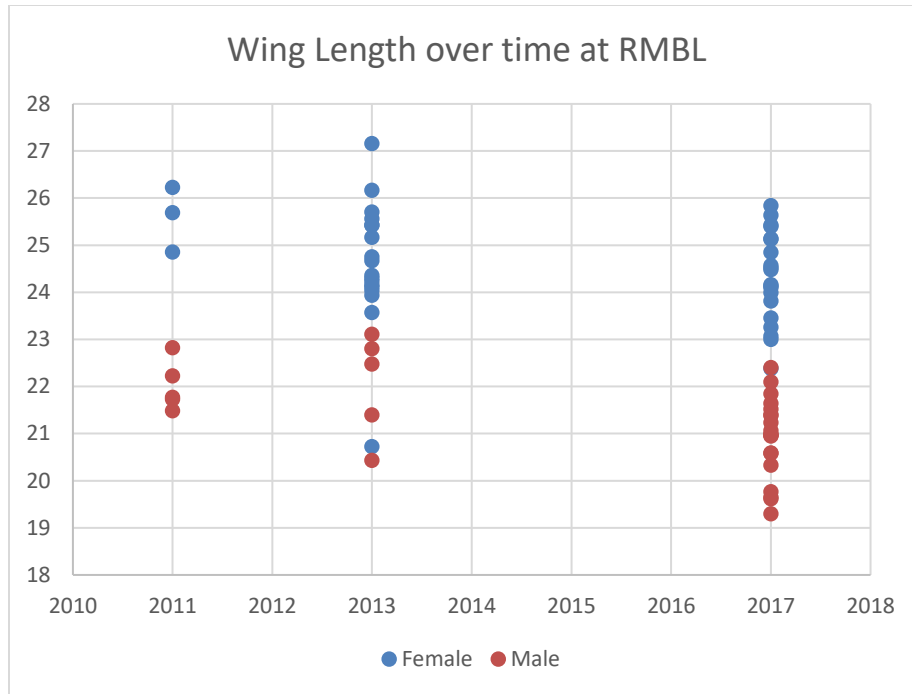


Figure 2.8 A slight decrease over time for both sexes, only significant in males

Wing loading is negatively correlated with longer-distance flight, so the populations with the smaller averages in Figure 4 are the ones which we would expect to have greater dispersal capability. The overall distribution was normal, with the same two pairs of sites as seen in the other traits measured (Table 2). We again used an ANOVA on wing loading, which showed significance for both site ($f=7.137$, $p=7.74E-05$), and wing wear ($F4=3.49$, $p=0.007$), and no evidence for an interaction between wing wear and site ($F8,58=.804$, $p=.6$) Wing loading decreases with wing wear, which is unsurprising. RMBL is most similar to BT and GC(Figure 2.9).

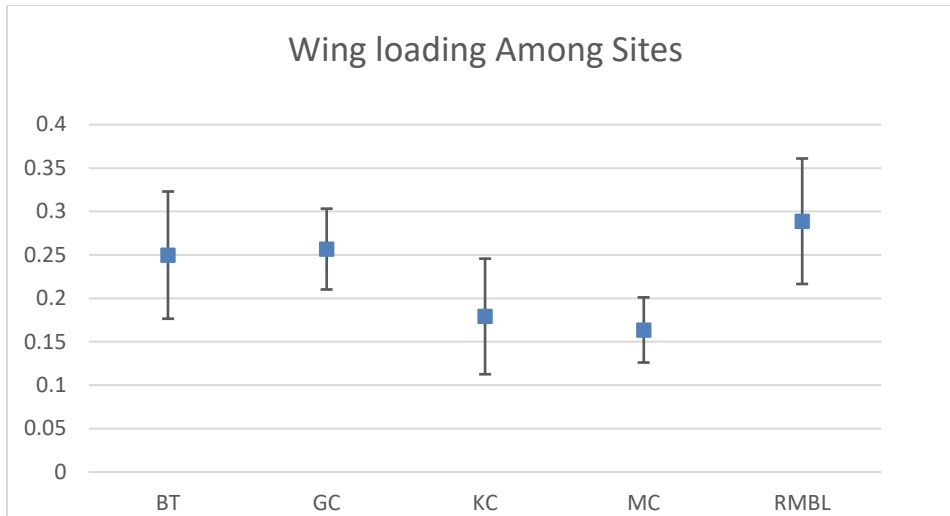


Figure 2.9 Wing loading is a measure of how much lift must be generated per square unit of wing area. Higher values indicate greater effort required to stay airborne

Centroid position was nonsignificant among sites ($F_{4,63}=1.37$, $p=.253$), and no sites were statistically distinguishable through Tukey's HSD.

Discussion

The analysis of the native populations showed that the dispersal traits measured covaried, and the native populations sorted into two morphologically low dispersal populations (GC and BT), and two high dispersal populations (MC and KC). In the comparisons between the native sites and RMBL, RMBL always fell at the lower end of the spectrum defined by the native populations, slightly but nonsignificantly further toward low dispersal characteristics. This matches our original predictions, and is most noticeable in aspect ratio, where RMBL contains individuals lower on the scale than any in the native range and has a wider range in aspect ratio than the other populations. The presence of individuals with unusually low aspect ratio at RMBL may be an indication of adaptation to isolation. That RMBL has a wider range in aspect ratio is itself entirely

unexpected. Given that the population has been subject to multiple bottlenecks in its past, genetic variability is lower here than elsewhere (McCoy et al 2014), which would lead one to expect less phenotypic variation within the site. The question becomes how plastic is this trait, and what other factors might be influencing it.

RMBL is unusual in another respect, having narrower thoraces on average. This was not predicted, but it supports the idea that allocation to the thorax size should decrease with isolation. RMBL has a similar relative thorax size to GC and BT, the two morphologically ‘low’ dispersal populations, which, while not statistically significant is slightly lower in RMBL. The thorax width however is significantly smaller in RMBL, and it is the only population in which this difference occurs. As seen in Figure 3, all four populations from the native range have similar average thorax widths, though the ranges vary.

The most surprising result found is the lack of a significant correlation between endurance time and temperature. *Euphydryas gillettii* performs best at a body temperature between 30-40c (Boggs unpub), and begins to fly at approximately 24c (Boggs unpub). The highest air temperature we recorded during endurance testing was 30.9C and most were between 24-28C. Absent solar radiation, air temperature and body temperature will be closely coupled, and we took steps to eliminate radiation as a factor. As most of our testing was performed below optimal temperatures, it may be that individual variation in ability to function outside of optimal conditions drowned out differences between sites. Niitepõld et al (2009) showed that heterozygotes for PGI in *Melitaea cinxia* were able to operate at lower temperatures than the homozygotes. Given

that *M. cinxia* is a fairly close relative to *E. gillettii*, this may be an interesting avenue for future research using the genetic samples collected in the field.

The two pairings that emerged in the native range are interesting in that they remained remarkably consistent. The MC-KC pair always showed morphological measures that were correlated with long-distance flight while BC-BT tended to be in the less dispersive category. This suggests that there may be divergent selection between these populations, and that within these populations the traits measured are being selected in similar directions. One possible explanation for the pairings is in the habitat quality. Both KC and MC had low density of hostplants as compared to GC and BT. Hostplants in KC and MC were more likely to be overshadowed by neighboring growth which may make them more difficult to locate (Pers. obs.). That, and the lower density of hosts would plausibly select for stronger fliers. GC and BT were both much more open and had more abundant *L. involunrata*. Of the two of them, I would categorize BT as the higher-quality habitat. While both of them were considerably better than MC and KC, BT had several dense clusters of *Lonicera* which received full sunlight for most of the day. GC may have had more hostplants available, but they were spaced further apart and there were more sources of shade present there. It should be pointed out that we were not expecting habitat quality to play a role, so we did not attempt to quantify it in the field. The most that can be said without further study is that our observations of the habitat are consistent with the pattern of variation in dispersal morphologies.

Similar patterns of variation in dispersal ability are seen in *Pararge aegeria*. In 1998, Berwaerts, Van Dyck Van Dongen, and Matthysen, published a study comparing the morphology of male *P. aegeria*, originating from landscapes of differing degrees of

fragmentation. They examined color patterning on the wings and measured relative thorax mass, and found a positive correlation between degree of fragmentation and the relative thorax size. This could be another explanation for the pattern we saw in the four native sites. Both of the sites that fell at the high-dispersal end of the spectrum not only seemed to be of poorer quality, but they also were smaller. When measuring connectivity, patch size is often used in the calculations, with smaller patches having less connectivity. Taking this as a guideline, our two sites with a high relative thorax size would have less connectivity, and the two with low thorax size would have high connectivity, which fits the pattern found in *P. ageria*.

A final interesting result lies in how similar RMBL was to GC. The progenitors of the population at RMBL were collected from a site only 1.2 kilometers from the site designated GC in our study. This original site went extinct by 2010 (boggs, unpub), probably due to the habitat drying. We found no current resident butterflies at that original collection site, despite the presence of suitable hostplants. The two locations are close enough that gene flow almost certainly occurred if they were occupied concurrently. If we make the assumption that the current GC and the original were similar, that leads to the conclusion that the founding members of RMBL started at the low end of the dispersal spectrum. This may help explain why RMBL was able to persist at very low population sizes. Since it was already comprised of individuals with less-dispersive traits, they tended to remain in their natal patch. We did collect genetic samples from all individuals captured, so an interesting future project would be seeing how far GC and RMBL have diverged.

The results of our comparison of RMBL to the native sites serve as a confirmation of the idea that dispersal capabilities will decrease in isolated populations. It was always on the low-dispersion end of the distribution, and by all morphological measures was slightly, though not significantly further out on that extreme than the GC, its cousin population. This implies some ongoing change at the population level that may become significant given enough time. Overall, extended isolation seems to have a depressive effect on dispersal capabilities. If more populations experience extreme fragmentation, this trend may contribute to the creation of an extinction vortex, as the reduced dispersal cuts down on gene flow and decreases recolonization rates. We can draw some encouragement from this population, as it has persisted in a single habitat patch for forty years. And, despite this decrease in dispersal capability, it has managed to generate a daughter colony, albeit a small one and fairly close by.

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APPENDIX A

MAP OF COLLECTION SITES

