Dispersal Potential Impacts Size Clines of Grasshoppers Across an Elevation Gradient

by

Richard Allman Levy

B.A. University of Colorado, 2010

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Master of Science
Department of Museum and Field Studies
2014

This thesis entitled:
Dispersal Potential Impacts Size Clines of Grasshoppers Across an Elevation Gradient
written by Richard A. Levy
has been approved for the Department of Museum and Field Studies

____
Dr. Dena Smith

____
Dr. César Nufio

____
Dr. Robert Guralnick

Date____________

The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline.

Levy, Richard Allman (M.S. Museum and Field Studies)

Dispersal Potential Impacts Size Clines of Grasshoppers Across an Elevation Gradient

Thesis directed by Dr. César Nufio and advised by Dr. Dena Smith

Body size is an important life history trait that determines reproductive success of insects through its effects on their physiology, ecology, and behavior. Changes in body size may have both a genetic component when local conditions select for optimal body size and an environmental component when it is influenced by temperature, food availability, and other environmental factors. Along elevational gradients a decrease in body size with an increase in elevation are commonly observed within insects when seasonality is present. No cline may also be observed when insects compensate for an abbreviated season by increasing their growth rate at high elevations or when environmental conditions fail to produce an effect. However, a key trait that has not been previously considered to influence the observed size cline of an insect is dispersal potential. Here, I hypothesize that an increase in dispersal potential, which suggests an increase in gene flow, impacts the type of size cline exhibited by grasshoppers across an elevation gradient. The impact of dispersal potential on two key life history traits associated with body size, reproductive potential and reproductive output, is also examined. I use two long winged species, *Melanoplus sanguinipes* and *Camnula pellucida*, to represent the high dispersal group and two short winged species, *Aeropedellus clavatus* and *Melanoplus boulderensis*, to represent the low dispersal group. My findings support the hypothesis that dispersal potential influences the body size-elevation relationship and reproductive potential of a grasshopper species across an elevation gradient. However, my findings fail to show dispersal potential impacts reproductive output. This study suggests dispersal potential may influence the evolution of body size of insect species and should be considered in future studies.

CONTENTS

CHAPTER

LIST OF TABLES:

TABLE

LIST OF FIGURES:

FIGURE

CHAPTER 1:

INTRODUCTION

Body size has been shown to greatly impact the biology of insects and their interactions with their environment. A larger body size may increase an insect's fecundity (Honek 1993), longevity (Hodin 2009) and starvation resistance (Slansky 1985) while potentially increasing their competitive advantage (Joern and Klucas1993, Denno et al. 1995), diet breath (Vincent 2006) and ability to secure mates (Shine 1989). However, a larger body size may also increase an insect's required development time (Chown and Gaston 2010) and metabolic demands (Fielding and DeFoliart 2008, Ashby 1998, Peters 1983), while increasing its reliance on an extended growing season and resource demand (Chown and Gaston 2010, Ikeda et al 2011). Because body size influences many aspects of an insect's biology, an insect's optimal size should reflect an adaptive trade-off between the costs and benefits of a given body size and local conditions (Kingsolver and Huey 2008, Blackenhorn 2000, Blackenhorn and Demont 2004). For example, although larger organisms have the potential for a higher reproductive success, a smaller body size may be favored under conditions where short growing seasons select for individuals with an accelerated development time (Blackenhorn 2000). While both environmental conditions and maternal effects may influence the expression of traits such as body size and rates of growth and development (Bernardo 1996, Cherrill 2002, Mousseau and Dingle 1991), life history theory predicts that the expression of these traits should also reflect genetic changes between populations as they adapt to their prevailing local conditions (Roff 1992).

As elevational and latitudinal gradients are associated with profound changes in season length, seasonal and daily temperatures, and levels of precipitation, these gradients provide an opportunity to understand how environmental conditions can influence the evolution of body size in organisms (Mayr 1956, Gaston 2000). In mammals and birds for example, colder climates associated with increases in elevation and latitude generally lead to increases in body size as these endotherms attempt to minimize heat loss by decreasing their surface-to-volume ratios. The increase in body size with latitude (which has been expanded to include elevational gradients) is known as Bergmann's rule (Bergmann 1848, Blackenhorn and Demont 2004, Atkinson 1994). In insects however, an increase in elevation or latitude is often associated with a decrease in body size, a pattern often referred to as the converse Bergmann's rule (Mousseau 1997, Mousseau and Roff 1989, Telfer and Hassal 1999, Dean 1982, Scott and Dingle 1990, Orr 1996, Berner and Blackenhorn 2006). In ectotherms, this decrease in body size along these spatial gradients is primarily attributed to a decrease in season length, although decreases in seasonal temperatures or resource have also been invoked as contributing factors (Chown and Klok 2003, Roff 1983, Nygren et al 2008, Stillwell et al 2007). That is, under conditions where seasonal or resource constraints are present, it is expected that populations will be selected to trade-off larger body sizes for early maturation (through accelerated development rates) to increase their reproductive success (Stearns and Koella 1986, Stearns 1992, Roff 1992, Pöykkö and Hyvärinen 2012).

Although a decline in insect body size with an increase in elevation or latitude is a pervasive pattern in the literature (Mousseau 1997, Mousseau and Roff 1989, Telfer and Hassal 1999, Dean 1982, Scott and Dingle 1990, Orr 1996, Berner and Blackenhorn 2006), insect size-clines along these spatial gradients may also follow Bergmann's rule, or may even fail to show a measurable size-cline relationship (Blackenhorn and Demont 2004; also see Shelomi 2012 for an examination of possible patterns). In the absence of seasonality, insect body size may increase with declines in temperature across spatial scales, as the simple result of an inverse relationship between developmental temperature and body size in ectotherms known as the temperature-size rule (Atkins 1994, Angilletta and Dunham 2003, Gosh et al. 2013). A positive size cline may also occur where seasonality is present but where populations along these gradients increase their growth or assimilation rates in a manner that leads to a relative increase in body size at higher elevations or latitudes (Chown and Klok 2003; Blackenhorn and Demont 2004). While the design of a study (gradient size, traits measured, and range of taxa examined) may contribute to whether a body size cline is detected (Shelomi 2012), the lack of a change in body size alongelevational and latitudinal gradient may suggest that climatic, seasonal or resource associated changes are not significant enough to influence changes in body size. Alternatively, a lack of a change in body size may result when individuals at higher elevations and latitudes evolve an increased intrinsic growth rate to compensate for a shorter developmental window (Berner and Blackenhorn 2006). While studies have shown that changes in body size and dispersal potential may influence community structure and the rate of evolution (Juliano 1983, Bie et al 2012), to my knowledge, the role that dispersal potential may play in determining the strength of a size-cline relationship has rarely been studied (see Cassel-Lundhagen et al 2010). Increases in gene-flow could, for example, influence the degree to which the body size of a given population reflects an adaptation to local conditions.

Across the Northern Front Range of the Rocky Mountains of Colorado there are 72 species of grasshoppers that vary in their diapause strategies, diet breadths, sizes, and the times of the season in which they are active (Alexander and Hilliard 1969). Congruent with many studies (Berner and Blackenhorn 2006, Telfer and Hassal 1999, Orr 1996, Scott and Dingle 1990, Dean 1982), grasshoppers in this region commonly display a converse Bergmann's rule body-size pattern where-by a reduction in body size is associated with an increase in elevation (Van Horn 1965, Ashby 1997, Buckley et al. 2013). In this study four grasshopper species (two long winged and two short winged) found along a high grassland prairie to alpine gradient were used to test the hypothesis that an increase in dispersal potential influence an insect species' size-cline relationship along elevational gradients. In this study I also examine the reproductive potential (number of ovarioles, number of functional ovarioles, and the proportion of ovarioles that are functioning) and output (clutch sizes and egg mass) of these four species to determine whether differences in dispersal potential influence the manner in which these traits are expressed.

Based on the literature associated with the life history patterns of insects that follow a converse Bergmann body-size pattern along elevational gradients, if dispersal potential influences the relative levels of gene flow among populations, then I make the following three predictions: 1) Due to the direction of prevailing winds, which carry long winged grasshoppers from lower to higher elevations in the Front Range (Alexander 1964), the body size clines along the gradient should be gradual for the long winged species and steep for the short winged species that are more locally adapted. 2) While the literature has repeatedly demonstrated that larger females within and between species tend to have more ovarioles (the reproductive units of the insect ovaries) (Bellinger and Pienkowski 1985, Chapman and Joern 1990, Honek 1993), studies designed to detect a relationship between grasshopper body size and ovariole numbers along spatial gradients have often failed to detect such a relationship (Dearn 1977,Leather 1988, Davidowitz 2008, Debano 2008). The lack of a predictable change in ovariole numbers along spatial gradients suggests to some a strong phylogenetic overprint in the number of ovarioles associated with some species (Chapman and Joern 1990, Bellinger and Pienkowski 1985) or that a variety of genetic and environmental factors can influence the relationship between body size and fecundity in a complex manner along gradients (Branson 2008, Taylor and Whitman 2010, Chapman and Joern 1990). However, because the number of ovarioles that function is determined by environmental conditions such as resource availability and stress (Stauffer and Whitman 1997, Branson 2008), I predict that due to an increase in gene flow from warmer (lower elevation) to cooler (higher elevation) environments, that a relative decline in the proportion of functioning ovarioles with elevation will be associated with long winged species, while the locally adapted short winged species will show no difference in the proportion of functioning ovarioles along the same gradient. 3) In regards to reproductive output, the number of eggs deposited per clutch may not be a life history trait that is independent of the proportion of functioning ovarioles (Bellinger and Pienkowski 195, Branson 2008) (prediction 2). In turn, one may even predict that clutch sizes will decrease in short winged species due to their expected reduction in body size with increases in elevation and may decrease in long winged species because they are less locally adapted to conditions at higher elevations. However, while changes in egg size may not be completely independent from changes in clutch sizes (Cherril 2002, Chown and Gaston 2010), given a general trend towards species of grasshoppers producing larger egg masses in environments with a shorter growing season (Dingle and Mousseau 1994), I predict that changes in egg masses will have a steep positive relationship with elevation in the more locally adapted short winged species than in long winged species.

CHAPTER 2:

METHODS

Field sites

The grasshopper species included in this study were collected from 5 sites located along a 2000 m elevational gradient within Boulder County, Colorado, USA. Running along the 40th parallel, this gradient covers a vertical range from 1577 to 3515 m and crosses five distinct life zones (Alexander and Hilliard 1969, Nufio et al 2010). As one moves from the high plains to the alpine life zones, there is an increase in total precipitation (Greenland et al 1985, Gochis et al 2004) and a decrease in the average daily temperature (McGuire et al 2012). This decrease in temperature and reduction in the season length with elevation leads to a decrease in the number of growing degree days available for growth and development during a season (Greenland et al 1985, McGuire et al. 2012).

Table 1. Location and description of surveyed sites along the elevational gradient.

Site	Elevation (m)	Latitude	Longitude	Life zone classification	Mean annual temp.(°C)	Mean season length (days)*
Red Fox	1577	40.061	-105.193	high plains	10.67	199.8
A1	2195	40.015	-105.377	lower montane	7.93	164.5
B1	2591	40.023	-105.430	upper montane	5.98	148.0
C1	3048	40.036	-105.547	sub alpine	1.79	100.5
D1	3515	40.059	-105.617	alpine	-3.50	84.4

* defined as the number of days between last winter freezing and first fall freezing temperatures.

Species

The four grasshopper species in this study were field collected over two years, 2012 and 2013, at each site beginning in early June and continuing until mid-August. The four species selected for this study are all univoltine, locally common, and each is found in at least four of the five sites surveyed along the elevational gradient. These species are also all egg-diapausers that overwinter within egg pods deposited into the soil.

The two grasshoppers chosen to represent species with low dispersal potential were *Aeropedellus clavatus* (Thomas) (Acrididae: Gomphocerinae) and *Melanoplus boulderensis* (Otte) (Acrididae: Melanoplinae). While female *A. clavatus* are brachypterous, males along this gradient have short wings that are used to produce courtship songs but that only support short distance flights close to the tops of vegetation, making them unlikely to be dispersed for great distances (Alexander 1964, *per. obs*). This species feeds predominantly on grasses and sedges and has an extensive range that extends from Alaska to Arizona and from lowland prairies to the alpine (Alexander and Hilliard 1964, Pfadt 1994). Both female and male *M. boulderensis* (part of the *Melanoplus dodgei* species complex; Otte 2012) are brachypterous forb feeders and while its species complex extends from southern Wyoming, Colorado to New Mexico, *M. boulderensis is* primarily restricted within foothills to alpine environments near the 40th parallel in Boulder County (Alexander and Hilliard 1969, Otte 2012). Along the gradient of study, *A. clavatus* is found at Red Fox, A1, B1, and D1, while *M. boulderensis* occurs at sites A1, B1, C1, and D1 (Table 1).

The two long winged grasshoppers chosen to represent species with a high dispersal potential were *Melanoplus sanguinipes* (Fabricious) (Acrididae: Melanoplinae) and *Camnula pellucida* (Scudder) (Acrididae: Oedipodinae). Both females and males of these species have long wings. *M. sanguinipes* is a polyphagous species (feeding on grasses and forbs) that can be found in grasslands throughout most of North America (Pfadt 1994) and within Colorado. This species is a resident at elevations near or below 3000 m (Alexander and Hilliard 1969). Within the Front Range of Colorado individual *M. sanguinipes* are frequently found as accidentals at higher elevations, suggesting that gene flow between populations is important. During a survey in 1959, 15% of 4,881 individuals that were collected over the season at sites above 3000 m were thought to have originated from lower elevations (Alexander 1964). *C. pellucida* is a grass and sedge specialist that inhabits grasslands throughout North America and is found at elevations below 3000 m in Colorado (Alexander and Hilliard 1969, Pfadt 1994). While this species has long wings and is known to disperse to new areas when food resources become depleted (Pfadt 1994), this species is not thought to be as frequently dispersed to higher elevations as *M. sanguinipes* within the Front Range of Colorado (Alexander 1964). Both *M. sanguinipes* and *C. pellucida* occur at Red Fox, A1, B1 and C1.

Field and egg collection techniques

Female and male grasshoppers of each species were field collected and brought to the lab. While males were frozen so that they could be measured at a different date (see below), females were placed individually into plastic rearing containers that contained 50 mm of damp sand as a medium for oviposition. These females were provided an *ad labium* amount of organic romaine lettuce and wheat grass that was replaced every other day. Although some species were considered forb or grass specialists, all species were observed to feed on both types of vegetation. The rearing containers were then placed into growth chambers set to a 14:10 day/night cycle with 27 and 22^{o}C being the set day and night associated temperatures, respectively. Light was provided via florescent tubes.

Females were removed from their rearing containers every two days and the containers were examined for the presence of an egg pod. If a female was found to have laid an egg pod, both the pod and female were placed together in a glass vial and stored in a freezer until they were measured (see below). If a female had not laid an egg pod, she was placed into a new rearing container with food and returned to the growth chamber. The placement of the rearing containers within the growth chamber was changed every two days and all species were placed within the same growth chamber, at times with all four species being processed simultaneously. On several occasions females were collected from the field and immediately frozen to increase the number of females that would be available for body size and ovariole counts along the gradient.

Body size measurements, reproductive potential and output

The body size of females and males was estimated by measuring femur length, a variable that is widely used to estimate body size in grasshoppers as well as other insects (e.g. Monk 1985, Wall and Begon 1987, de Souza Santos and Begon 1988, Danner and Joern 2004, Branson 2008). Femur length, rather than body mass or length, was chosen because female body mass and abdomen length change considerably during the different stages of egg development and after an oviposition event. Femur length was assessed by measuring the longest distance from end to end to the nearest one hundredth of a millimeter using digital calipers. Both right and left femurs were measured twice and a mean femur length was calculated by averaging the length of both femurs.

To determine whether fecundity associated traits differed between long and short winged species along the gradient, the total number of ovarioles and number of functional ovarioles was counted by dissecting females in a saline solution under a dissecting microscope. Ovarioles make up a series of tubes that form the ovaries and the number of functioning ovarioles reflects the number of ovarioles that are allocated to egg development (see Figure 1). These two variables reflect the potential reproduction of an individual as the number of ovarioles is the upper limit of the number of primary oocytes that can be developed at one time, while the number of functioning ovarioles reflects the realized limit of the number of primary oocytes are being developed at one time.

Figure 1. Ovarioles from *A. clavatus.* (A) indicates functioning ovariole; (B) indicates non-functioning ovariole.

The reproductive output of females of each species along the gradient was determined by counting the number of eggs associated with the clutches they deposited within the rearing containers. The per capita egg mass associated with each clutch was then determined by removing the eggs from each pod (along with any associated sand or other matrix), weighing all associated eggs together to the nearest ten-thousandth of a gram and dividing this total weight by the number of eggs within the pod.

Statistical Analyses

For each species multiple linear regression analyses were used to determine the relationship between male and female body sizes (femur lengths) and elevation, independently. Multiple linear regressions with sex (male/ female), elevation and an interaction between the two were used to determine whether the rate of change in body size differed between males and females. For each species, General Linear Models (GLM) were used to determine whether there was a significant elevation and female size effect on the number of ovarioles, clutch sizes, and individual egg masses (dependent variables that were normally distributed). In regards to GLM models designed to examine factors that help explain clutch sizes, I also added the number of functioning ovarioles as a dependent variable to test whether this measure of reproductive potential helps to explain the clutch sizes (a measure of reproductive output). In regards to GLM models designed to examine factors that help explain clutch sizes, I also added the number of functioning ovarioles as a dependent variable to test whether this measure of reproductive potential helps to explain clutch sizes (a measure of reproductive output). For *A. clavatu*s, however, because the number of ovarioles associated with females along the gradient was relatively fixed, with most females having 6 ovarioles, a logistic regression was conducted to determine whether females had 6 ovarioles or not was associated with increases in elevation. Analysis of variance (ANOVA) and post-hoc Tukey's honest significance tests were also conducted to examine the degree to which mean body sizes, number of ovarioles, clutch sizes and individual egg masses of populations of each species differed from each other along the gradient. As the proportion of functional ovarioles was bi-modally distributed, for each species a multiple logistic regression was conducted to determine whether a female had a full complement of functional ovarioles (all ovarioles were functional) or not, was explained by elevation and female body size. In all statistical models, I tested for interaction effects and present only those that were significant. All statistical analyses were performed using JMP, Version 11 (SAS Institute 1989-2013).

CHAPTER 3:

RESULTS

Body Size

Consistent with the converse Bergmann's rule, the body size of both males and females of the two short winged grasshopper species, *A. clavatus* (Male: r^2= 0.62, P<0.0001, n= 100; Female: r^2= 0.76, P<0.0001, n= 120) and *M. boulderensis* (Male: r^2= 0.61, P<0.0001, n= 100; Female: r^2= 0.69, P<0.0001, n= 128), declined with elevation (Figure 2 A and B). However, whereas the average body size of females within each population declined along the gradient in *A. clavatus* ($F_{3,116}$ = 145.07, p< 0.0001), in *M. boulderensis* female body size decreased with elevation but did not differ between the two mid-elevation sites ($F_{3,124}$ = 129.86, p = <0.0001; Table 2). For both of the short winged species, there was a significant interaction between sex (male/ female) and elevation, that showed that the rates at which female body sizes declined with elevation were steeper than those experienced by males (Elevation x Sex; $F_{3,216}$ = 8.95, p=0.0031, $F_{3,224}$ = 31.27, p< 0.0001for *A. clavatus* and *M. boulderensis* respectively).

In the long winged species, no body size clines were detected along the elevational gradient for *C. pellucida* (Male: r^2= 0.006, P= 0.48, n= 86; Female: r^2= 0.008, P=0.34, n= 117) *and M. sanguinipes* (Male: r^2= 0.02, P= 0.19, n= 99; Female: r^2= 0.69, P= 0.3406, n= 121) (Figure 1 C and D). As expected, potential differences in body sizes along the gradient males and females did not differ in the degree to which they changed their body sizes with elevation ($F_{3,199}$ = 1.27, p< 0.26; $F_{3,212}$ = 2.17, p< 0.14 for *C. pellucida* and *M. sanguinipes,* respectively).

Figure 2. The relationship between body size (mean femur length + SE) and elevation for short winged, (A) M. boulderensis, (B) A. clavatus, and long winged, (C) C. pellucida, (D) M. sanguinipes, and species. Female = _____ , Male = _ _ _ _

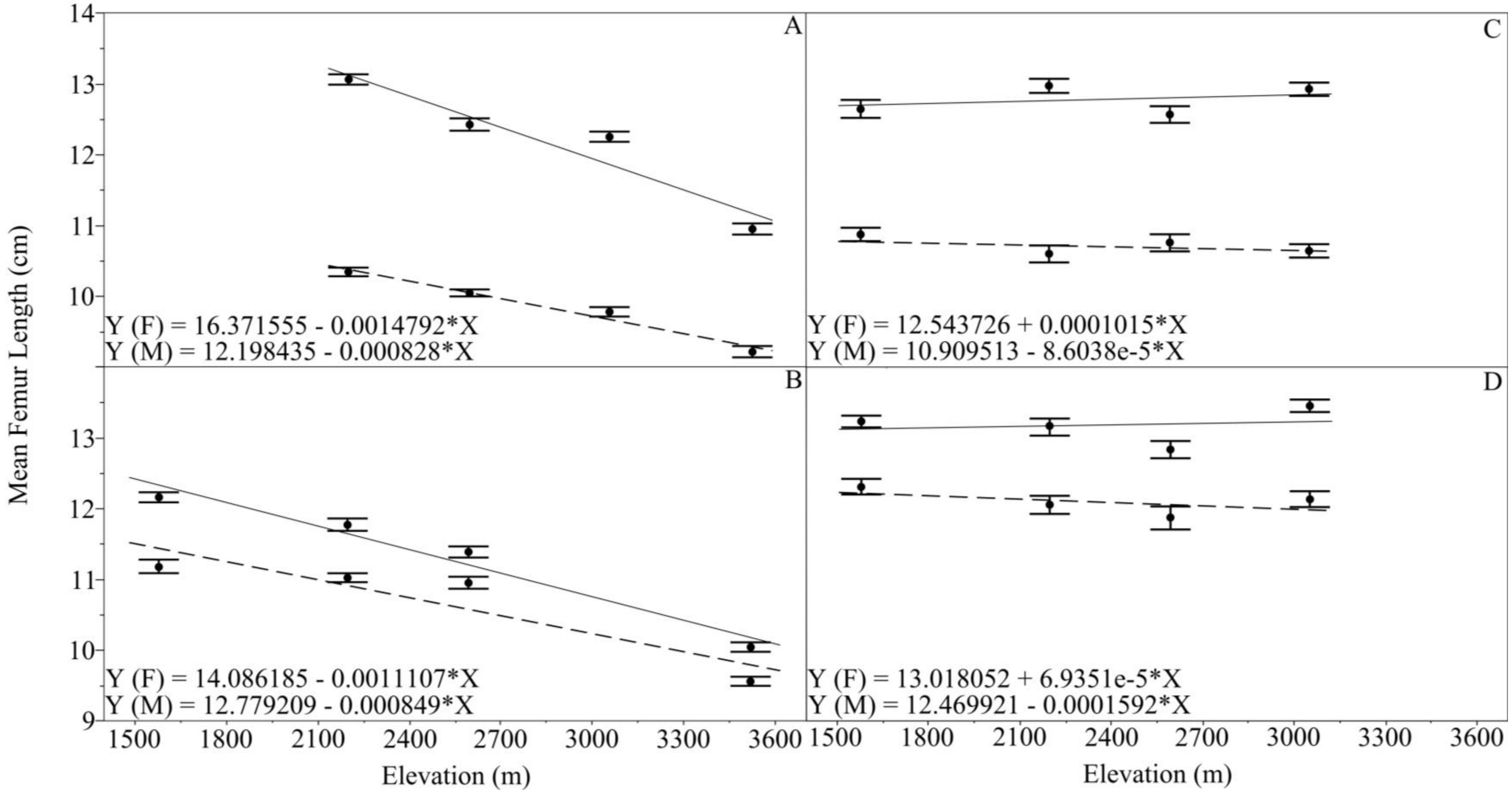

Table 2. Body size measurements of male and female grasshoppers of four species surveyed along the elevational gradient. Within each species and sex, differences in mean body sizes between populations are denoted by different letters (Tukey–Kramer test, P<0.05).

		Femur Length		
Species	Site	Sample Size	Mean	± S.E.
M. boulderensis				
Male	A1	25	13.35 a	0.06
	B1	25	10.15 b	0.05
	C1	25	9.78 c	0.07
	D1	25	9.22 d	0.08
Female	A1	32	13.06 a	0.07
	B1	32	12.43 b	0.09
	C1	32	12.25 b	0.07
	D1	32	10.95 c	0.08
A. clavatus				
Male	RF	25	11.19 a	0.10
	A1	25	11.03 a	0.06
	B1	25	10.96 a	0.08
	D1	25	9.56 b	0.07
Female	RF	30	12.16 a	0.07
	A1	30	11.77 b	0.09
	B1	30	11.39 c	0.08
	D1	30	10.05 d	0.07
M. sanguinipes				
Male	RF	25	12.31 a	0.13
	A1	25	12.06 a	0.14
	B1	24	11.87 a	0.13
	C1	25	12.14 a	0.13
Female	RF	31	13.23 a	0.08
	A1	30	13.15 ab	0.12
	B1	30	12.85 b	0.11
	C1	30	13.45 a	0.09
C. pellucida				
Male	RF	11	10.88 a	0.16
	A1	25	10.6 a	0.11
	B1	25	10.76 a	0.11
	C1	25	10.65 a	0.12
Female	RF	27	12.65 ab	0.13
	A1	30	12.98 a	0.10
	B1	30	12.57 b	0.12
	C1	30	12.93 ab	0.09

Reproductive Potential

The reproductive potential of female grasshoppers is thought to be defined by the number of ovarioles and more precisely by the number of functional ovarioles because these structures place an upper limit on the rates of egg production (Stauffer and Whitman 1997, Branson 2008, Taylor and Whitman 2010)). In *M. boulderensis* the number of ovarioles associated with females was a function of elevation and not female size (Table 3 and 4) such that females at higher elevation had more ovarioles on average than females at lower elevations, regardless of their size. In *A. clavatu*s the number of ovarioles associated with females along the gradient was relatively fixed with 75 out of 80 females having 6 ovarioles and each site, except B1, containing at least one or two individuals with fewer than 6 ovarioles. Whether a female had 6 ovarioles or not was not associated with changes in elevation (x^2= 1.26, P=0.26). In both *M. sanguinipes* and *C. pellucida*, the two winged species, the number of ovarioles associated with females was not explained by changes in elevation or female size (Table 3 and 4).

In *M. boulderensis,* whether or not all ovarioles became functional was explained by an interaction between body size and elevation, such that at lower elevations increases in body size had a stronger effect on whether a complete set of ovarioles became functional than at higher elevations. Finally, in the two winged species, *M. sanguinipes* and *C. pellucida* the probability that all ovarioles on a female were functional declined with elevation and was not related to females size (Table 5).

Table 3. The number of ovarioles as a measure of female size and elevation for the short winged (*M. boulderensis* and A. *clavatus*) and long winged (*M. sangunipes* and *C. pellucida*) species. No interaction effects were detected.

		Number of ovarioles		
Species	Variable	d.f.	F	*P*
M. boulderensis	Mean femur length	1	0.01	0.91
	Elevation	1	10.35	0.002
A. clavatus	Mean femur length	1	1.74	0.19
	Elevation	1	2.52	0.12
M. sanguinipes	Mean femur length	1	0.23	0.63
	Elevation	1	0.20	0.65
C. pellucida	Mean femur length	1	0.12	0.73
	Elevation	1	0.11	0.74

Table 4. Reproductive potential as measured by the number of ovarioles and proportion of functioning ovarioles. Differences in mean number of ovarioles are denoted by different letters (Tukey–Kramer test, P<0.05).

		Number of ovarioles			Functional ovarioles	
Species	Site	Sample size	Mean	± S.E.	Proportion	± S.E.
M. boulderensis	A1	25	14.72 c	0.24	0.93	0.36
	B1	27	16.04 b	0.34	0.86	0.55
	C1	26	16.38 ab	0.31	0.86	0.64
	D1	25	17.48 a	0.49	0.93	0.64
A. clavatus	RF	20	5.90 a	0.10	0.68	0.10
	A1	20	5.75 a	0.14	0.75	0.20
	B1	20	6.00 a	0.00	0.66	0.15
	D1	20	5.95 a	0.05	0.67	0.05
M. sanguinipes	RF	24	25.79 ab	0.58	0.97	0.55
	A1	24	27.00 a	0.44	0.92	0.58
	B1	24	24.92 b	0.58	0.94	0.60
	C1	25	26.00 ab	0.40	0.88	0.51
C. pellucida	RF	18*	26.11 a	0.59	0.98	0.53
	A1	20	25.05 a	0.56	0.93	0.50
	B1	20	25.20 a	0.57	0.93	0.66
	C1	20	26.50 a	0.52	0.92	0.69

*Sample size for proportion functioning ovarioles at RF was 17.

Table 5. Results of multiple logistic regressions to determine whether the proportion of functional ovarioles was dependent on elevation or mean femur length. Only significant interaction effects are reported.

Species	Variable	d.f.	Parameter estimate	± S.E.	Wald x^2	*P*
A. clavatus	Elevation	1	4.23E-03	3.00E-03	2.31	0.09
	femur length	1	-3.44	2.51	1.89	0.08
	Elevation*femur length	1	-1.17E-02	7.75E-03	2.64	0.0029
M. boulderensis	Elevation	1	-8.42E-04	7.52E-04	1.25	0.0377
	femur length	1	-1.04	4.99E-01	4.32	0.26
	Elevation*femur length	1	1.28E-03	6.51E-04	3.91	0.048
C. pellucida	Elevation	1	2.73E-03	7.02E-04	21.42	<0.0001
	femur length	1	1.01	6.06E-01	3.15	0.07
M. sanguinipes	Elevation	1	1.68E-03	4.88E-04	13.8	0.0002
	femur length	1	-6.14E-01	4.50E-01	1.94	0.16

Reproductive Output

Within the short winged group, two distinct patterns were observed. When comparing the number of eggs deposited during an oviposition event in populations of *M. boulderensis* (Table 6) it appeared that only sites A1 and C1 differed from each other such that A1 had the lowest average clutch size and C1 had the highest. A GLM, however, showed a strong positive effect of elevation on clutch sizes when controlling for female size, which also contributed positively to increases in clutch size (Table 7). The number of functional ovarioles also explained clutch size when elevation and mean femur length were taken into account (Table 8). Furthermore, in *M. boulderensis* clutch size was explained by a three way interaction between body size, functional ovariole number, and elevation (Table 8). The positive effect of body size on clutch size is most pronounced when females have more, relative to fewer, functioning ovarioles. In turn, this proportional increase in clutch size in larger females, relative to smaller females, that have a higher number of functioning ovarioles is further magnified by an increase in elevation (Table 8). Although in *A. clavatus* clutch size did not appear to vary significantly across the gradient (Table 6), there was a significant elevation effect when female body size was accounted for (Table 7). That is, females of similar size laid smaller clutches at higher elevations.

Within the high dispersal species, the clutch size of neither *M. sanguinipes* nor *C. pellucida* was explained by elevation, although larger females laid more eggs per clutch (Table 7). While the mean clutch sizes of *M. sanguinipes* were similar at each site along the gradient, in *C. pellucida* the largest clutch sizes were detected at the lowest (Red Fox) and highest elevations (C1) (Table 6).

In respect to egg mass, in *M. boulderensis* there was a negative relationship between per capita mean egg mass and elevation (Table 6). While egg mass was not explained by elevation or female sizes in *A. clavatus* there was an interaction between female size and elevation, such that at lower elevations, larger females laid eggs with lower mass than smaller females while at higher elevations larger females laid eggs with higher mass than smaller females (Table 7). In *M. sanguinipes* egg mass was associated with elevation, but not female body size (Table 7). A relationship between egg mass and elevation was also found in *C. pellucida*, however this positive relationship is attributed to eggs having a greater mass above the high plains (Table 6, 7).

Table 6. Reproductive output as measured by the number of eggs per clutch and per capita egg mass.

		Clutch Size			Egg Mass		
Species	Site	Sample Size	Mean	± S.E.	Sample Size	Mean (mg)	± S.E. (mg E-04)
M. boulderensis	A1	23	13.52 b	0.26	24	7.24 a	1.30
	B1	22	14.14 ab	0.62	24	6.23 b	1.50
	C1	23	15.83 a	0.51	24	5.49 c	1.30
	D1	24	15.25 ab	0.57	24	4.08 d	1.40
A. clavatus	RF	20	4.2 a	0.25	20	3.80 b	2.70
	A1	20	3.4 a	0.15	20	4.82 a	1.70
	B1	20	3.4 a	0.20	20	4.34 ab	2.10
	D1	20	3.9 a	0.26	20	4.44 ab	9.00
M. sanguinipes	RF	23	20.91 a	0.91	24	2.04 c	7.00
	A1	25	21.13 a	0.78	24	2.13 bc	6.00
	B1	24	20.28 a	0.65	24	2.48 a	5.00
	C1	23	21.83 a	0.90	25	2.29 ab	6.00
C. pellucida	RF	13	21.62 ab	1.19	14	3.33 b	1.40
	A1	20	19.50 b	0.77	20	4.42 a	1.10
	B1	20	17.95 b	0.95	20	4.14 a	1.80
	C1	20	23.30 a	0.89	20	4.41 a	1.90

Table 7. Clutch size and egg mass as a measure of female size and elevation for the short winged (*M. boulderensis* and A. *clavatus*) and long winged (*M. sangunipes* and *C. pellucida*) species. Only significant interaction effects are reported.

		Clutch size			Egg mass		
Species	Variable	df	F	*P*	df	F	*P*
M. boulderensis	Mean femur length	1	28.87	<0.0001	1	0.21	0.65
	Elevation	1	40.53	<0.0001	1	64.56	<0.0001
	Mean femur length*elevation	1	0.36	0.55	1	0.53	0.47
A. clavatus	Mean femur length	1	4.84	0.031	1	0.2	0.66
	Elevation	1	7.56	0.0075	1	0.96	0.33
	Mean femur length*elevation	--	--	---	1	8.06	0.0058
M. sanguinipes	Mean femur length	1	15.15	0.0002	1	0.34	0.56
	Elevation	1	1.4	0.24	1	16.3	0.0001
	Mean femur length*elevation	--	--	---	--	--	---
C. pellucida	Mean femur length	1	17.52	<0.0001	1	2.93	0.09
	Elevation	1	0.15	0.7	1	11.34	0.0012
	Mean femur length*elevation	--	--	---	--	--	---

Table 8. Clutch size as a measure of female size, elevation, and number of functional ovarioles in the short winged species *M. boulderensis*.

M. boulderensis	Clutch Size		
Variable	df	F	P
Mean femur length	1	30.56	<0.0001
Elevation	1	35.96	<0.0001
Number functional ovarioles	1	0.58	0.45
Number functional ovarioles*elevation	1	7.65	0.0078
Number functional ovarioles*mean femur length	1	2.23	0.14
Elevation*Mean femur length	1	0.37	0.55
Mean femur length*elevation*number functional ovarioles	1	4.52	0.0381

CHAPTER 4:

DISCUSSION

Elevational gradients provide an opportunity to understand how environmental factors influence the evolution of body size. Studies along these gradients have shown that seasonality, temperature, precipitation, and resource availability can impact the size of an insect, and that combined with other factors such as changes in growth and development rates, can impact the type of size cline that is observed (Chown and Klowk 2003, Atkinson 1994, Stillwell and Fox 2009, Blackenhorn and Demont 2004). In this study I address the hypothesis that dispersal potential can influence the ability of organisms to develop body size clines along elevational, and potentially along other types of gradients. Consistent with this dispersal hypothesis, I found that males and females of short winged species showed a strong decline in body size, while males and females of long winged species, with potentially higher dispersal rates, failed to show a size cline with increases in elevation. Reproductive potential, as measured by the proportion of ovarioles that are functional, decreased with elevation in both long winged species but remained stable in both short winged species. However, while I predicted that there would be a stronger relationship between egg mass and elevation in short winged relative to long winged species, I found that egg mass increased with elevation in both long winged species, and that it either decreased or showed no relationship with elevation in the two short winged species.

Impact of dispersal potential on body size

Univoltine insects with a generation time that requires a significant proportion of the available season length are expected to follow the converse Bergmann’s rule across elevational gradients (Chown and Gaston 1999, Chown and Klok 2003). In this study, consistent with the hypothesis that dispersal potential (a proxy for gene flow rates between populations) can influence body size clines in insects, I found that males and females of both species from the low dispersal group displayed a converse Bergmann size cline, while the long winged species failed to show such a size-cline relationship along the gradient. While this dichotomy exists, further examination revealed complexities. For example, the relationship between body size and elevation differed between the two short winged species, a result of differences in body size between the extreme elevations in *A. clavatus*. Size cline relationships also differed between males and females within the short winged species, while this was not the case in either long winged species (Figure 2, Table 2).

Still, contrary to my prediction, that an increase in dispersal potential would only weaken the relationship between body size and elevation, my results suggest that a potential increase in gene flow can negate the expected decrease in size with elevation. This counter-gradient variation may be the result of genetic differences in growth or assimilation rates that counteract environmental effects (a shortened season length in this case) in such a way as to reduce the expected differences in body sizes between populations (Levinton and Monahan 1983, Blackenhorn and Demont 2004). While this may offer an alternative explanation for why long winged species lack a size cline across this gradient, the decrease in reproductive potential with elevation discussed below suggests that either this compensation would be associated with a cost (similar adult body sizes being reached at the expense of a decreased reproductive potential) or that no compensation occurs (development and assimilation rates are similar across populations) and that long winged species are insufficiently adapted to higher elevations. To further differentiate between the dispersal and counter-gradient explanations for why body size did not change with elevation, additional study is necessary. For example, rearing experiments could be used to determine whether growth rates increase at the higher elevations, whether there is a reproductive cost associated with this compensation, or whether growth rates are rather uniform along the gradient but a grasshopper's potential reproduction declines when exposed to the conditions associated with a shorter season length. Prevalence of gene flow across the gradient as well as genetic differences among populations through DNA analysis is also necessary to quantify the rate of gene flow among populations.

Impact of dispersal potential on reproductive potential

In principle, the number of ovarioles within an individual acts as an upper limit on the number of eggs that can be deposited in a single clutch (Chapman and Joern 1990). Ovariole number is an evolved trait that may represent the probability of reproduction under given environmental conditions (Chapman and Joern 1990, Stauffer and Whitman 1997), may be a function of adult body size (Honek 1993, Bellinger and Pienkowski 1985), may be the result of phylogenetic history (McKitrick 1993), or a combination of these factors.

In this study, female body size did not explain variation in ovariole number for any of the four species (Table 4). Such a disassociation between ovariole number and female body size was unexpected due to the strong intraspecific correlation documented in a review on the subject (Honek 1993); however relationships may be found less often in field studies examining size and potential fecundity across environmental gradients due to the many factors at play (Davidowitz 2008, Leather 1988, Honek 1993). In *M. boulderensis* the number of ovarioles increased with elevation (Table 3) but was not related to female size (Table 4). These findings are similar to those from a study of three Australian grasshoppers in which ovariole number increased with elevation but were typically not correlated with body size (Dearn 1977). A greater number of ovarioles at high elevation, where season length is short and less predictable, suggests that individuals able to develop more eggs simultaneously (and thus oviposit more at once) may be favored over individuals that produce the same or greater number of eggs over a longer period. There was little variation in ovariole number in *A. clavatus* across the elevation gradient, and this variation was not explained by female body size or elevation, suggesting that this trait is highly conserved. Within the long winged species, ovariole number did not vary with elevation or body size, which may be the result of increased gene flow across the gradient, or of phylogenetic conservation (Tables 3 and 4).

While ovariole number serves as an upper limit on the number of eggs laid per clutch, the number of functional ovarioles may more adequately reflect an organism's realized reproductive potential because it signifies the number of ovarioles that are actually producing eggs. The proportion of ovarioles that will become functional may be determined primarily by the body size of an adult grasshopper or the conditions experienced, such as resource availability or quality, temperature, and stress due to population density (Laws and Belovsky 2010, Branson 2008, Belovsky and Slade 1995, Stauffer and Whitman 1997, Branson 2004, Moehrlin and Juliano 1998, Hatle et al. 2004). The degree to which a population is locally adapted to such conditions may also influence the proportion of ovarioles that will become functional. This second prediction, that dispersal potential influences local adaptation and therefore the proportion of functioning ovarioles across an elevational gradient, was also supported. I found that within the long winged group the proportion of functional ovarioles decreases with elevations, while there was no decrease in the short winged group (Table 5). The decrease in proportion of function ovarioles observed in the long winged species, but not in the short winged species, suggests that the increased dispersal potential has limited the ability of long winged populations to adapt to local conditions higher up along the gradient. Research to test how dispersal potential impacts reproductive potential should be continued, such as common garden experiments that examine the proportion of functional ovarioles of individuals of long winged species form high elevation populations reared under the same conditions as those from low elevation populations. The translocation of locally adapted short winged species to either higher or lower elevations than their native population may also provide insight on how functional ovarioles are impacted by environmental conditions. Additionally, the impact of dispersal potential on rates of oosorption, the process in which eggs are resorbed under conditions of inadequate resource availability or other sources of stress, could also be used to determine how populations allocate resources to reproduction under environmental conditions both inside and outside of the range wherein they are locally adapted (Stauffer and Whitman 1997, Branson 2008).

Impact of dispersal potential on reproductive output

The clutch size, along with the mass of each egg, represents the realized reproductive output of an individual. Clutch size is not completely independent of functional ovariole number, as the number of primary oocytes that can develop simultaneously is limited to the number of ovarioles that are capable of producing an egg under given conditions (Stauffer and Whitman 1997, Chapman and Joern 1990). However, due to the possibility of oosorption, the reabsorption of an oocyte due to deficient resources or stressful conditions, clutch size offers a better measurement for actual reproductive output. In *M. boulderensis* clutch size was explained by an interaction between body size, functional ovariole number, and elevation (Table 7). Larger females with more functional ovarioles laid proportionally larger clutches, a pattern that was magnified with increases in elevation. Larger clutches associated with females with more functional ovarioles is intuitive, as more eggs can be developed at once, resulting in larger clutches. The increase in clutch size in larger females at higher elevations may be the result of selection for more eggs deposited in a single oviposition due to the abbreviated season. A 1977 study of three Australian brachypterous grasshoppers (Dearn) found that at high elevations, with shorter seasons, females only had a 30% chance of laying their first clutch of eggs before the season ended. While this study was unable to measure clutch size, it reported that ovariole number increased with elevation, suggesting larger potential clutch sizes at high elevations. In my study, egg size decreased with elevation in *M. boulderensis* while clutch size increased. This suggests a reproductive tradeoff is occurring, such that the benefits of larger eggs at higher elevations (discussed below) do not outweigh the benefits of large clutches in an unpredictable, seasonal environment in which the probability of laying multiple clutches is reduced.

In *A. clavatus*, clutch size had limited variability and was not explained by elevation, clutch size, or the number of functional ovarioles. Like previously discussed reproductive traits in this species, clutch size may be constrained by its phylogenetic history.

The clutch sizes of neither long winged species, *M. sanguinipes* and *C. pellucida*, were explained by elevation but was a function of female body size (Table 7). This suggests that larger females are able to lay larger clutches regardless of the elevation. Due to the described relationship between clutch size and the number of functional ovarioles, clutch size should not exceed the number of ovarioles that are functional. Curiously, however, both species from the long winged group maintained similar clutch sizes across the gradient while their associated number of functional ovarioles decreased with elevation. If these species are not locally adapted to the higher elevations, females at high elevations may be storing eggs within their calyx (Renucci et al 1990) and delaying oviposition until they developed a similar number of eggs.

Oviposition is energetically expensive and exposes the female to the threat of predation, which makes laying large clutches beneficial. However, delayed oviposition until a given number of eggs have been developed, in environments with short and unpredictable season length may lead to a diminished overall fitness, as fewer total eggs may be laid despite the preserved large body size at high elevations. Delay of oviposition can be mediated by an individual, for example a study investigating oviposition behavior of the butterfly *Battus philenor* it was suggested that abdominal stretch receptors may serve a function in detecting egg load (Odendaal & Rausher 1990). They observed that egg load as detected by such internal mechanisms may mediate insect oviposition behavior, providing evidence that a female may retain eggs until a critical mass has been reached. Additional studies that measure the total reproductive output such as eggs per season must be conducted to determine if long winged populations at high elevations actually have reduced fitness compared with lower elevation populations.

Larger eggs produce larger first instar grasshopper nymphs which require less time to attain adult size (Chown and Gaston 2010, Stauffer and Whitman 1997). Therefore, under time constrained conditions, such as short season length at high elevations, it is expected for egg mass to be greater. Larger egg size and thus larger hatchlings also have improved survivorship (Fox and Czesak 2000). My third prediction, that egg mass should increase with elevation in the short winged group, but will have no relationship with elevation in the long winged group was not supported. Egg mass increased with elevation in both long winged species. The underlying mechanism causing egg mass to increase with elevation is unknown, however it may be explained by phenotypic plasticity. Egg size has been shown to be plastic (Chown and Gaston 2010) with egg size inversely related to temperature when rearing in a laboratory (Ernsting and Isaaks 1997) and inversely related photoperiod length (Cherrill 2002). In this case, larger egg size of long winged species at higher elevations in my study may be a response to the local conditions experienced, producing larger offspring with higher fitness under cooler temperature regimes and shorter season lengths, and thus a possible example of adaptive plasticity (Cherrill 2002, Capinera 1979, Dingle & Mosseau 1994, Monk 1958, Sibly & Monk 1987). However, if populations of long winged species at higher elevations are adapted to the warmer and longer season conditions found at low elevations, they may be overcompensating by producing eggs larger than necessary, coming at a reproductive cost, as fewer eggs can be laid due to limiting resources. Further study is necessary to measure how egg size of long winged species across my elevation gradient may respond to temperature or day length over the course of a season, or how many eggs are produced per generation at different elevations to determine if overcompensation occurs.

Variability of life history adaptation

This study demonstrates that the expression of life history characteristics can vary in a predictable fashion with environmental factors found across an elevation gradient, however I also show that an increased dispersal potential can reduce or even nullify such a relationship. Interrelatedness between variables, such as body size and potential or realized fecundity, proposes that cascade effects may be the consequence of changes in a single or few traits. Yet the adaptive strategies that are selected for within a population may defy notions of strict interdependencies between traits. For instance, the observed lack of a relationship between the number of ovarioles and body size in each species examined in this study, especially in the case of *M. boulderensis* in which ovariole number is predominately explained by elevation, contradicts the well-established principle that larger body sizes result in increased fecundity. The ability of life history traits to vary independently or dependently under the pressure of both genetic and environmental constraints illustrates the complex nature of adaptation and the opportunity for additional factors, such as dispersal potential, to influence which patterns are observed.

Conclusions

This study finds support for the hypothesis that dispersal potential influences body size clines across an elevation gradient, suggesting that our attempts to discern why certain trends are observed across spatial or temporal gradients may be narrow. This however is not a criticism, as general principles, such as Bergmann’s rule and its converse, are important and helpful to comprehending how organisms may adapt to their environments. However, once applied to natural systems, these principles may be overridden by specific elements.

As body size holds major implications associated with key life history traits, especially those related to fecundity and fitness, and environmental gradients are commonly used to study how both body size and such life history traits evolve, it is important to consider dispersal potential or other possibly influential characteristics when examining the underlying mechanisms acting on the expression of such traits. A better knowledge of how factors such as dispersal potential impact life history trends across spatial or temporal scales will aid in the advancement of our understanding of how organisms adapt to their conditions; an advancement that becomes ever the more crucial in respect to global climate forecasts.

ACKNOWLEDGMENTS:

I would like to thank Dr. César Nufio for teaching me more about science than I could ever imagine, for encouraging me to pursue a career in entomology, for challenging me as much as he does, for having confidence in me, and for being a true role model and friend. I thank Dr. Dena Smith for her outstanding advice, our many discussions, her continual support, and for all that she has taught me. I also thank Dr. Deane Bowers for the use of her laboratory space and resources, Dr. Robert Guralnick for our lively discussions, Virginia Scott for allowing me to take up so much freezer space over the course of this project, and Dr. Talia Karim for her advice and insect impersonations. I thank Shawn Cohen for her unyielding love and support for me to pursue my dream to become a scientist, my mother and father for everything, and my little sister Annie. I would also like to thank Danielle Forte, Deb Wagner, Evan Anderson, Erin Leckey, Lindsay Walker, and Aly Seeberger for their support and friendship. Finally I would like to thank Kelsey Scheckel, Eric Lord, and Paul Henning for their outstanding contributions to this study in which I could not have done without, the Undergraduate Research Opportunity Program for funding those just mentioned, the National Science Foundation (DEB-1543813) and the William Burt Fund for supporting this thesis, and the University of Colorado Museum of Natural History.

BIBLIOGRAPHY:

Alexander, G. 1951. The occurrence of Orthoptera at high altitudes, with special reference to Colorado Arcididae. -Ecology 32: 104–112.

Alexander, G. 1964. Occurrence of grasshoppers as accidentals in the Rocky Mountains of Northern Colorado. -Ecology 45: 77–86.

Alexander, G., and Hilliard, J.R. 1964. Life History of *Aeropedellus clavatus,* Orthoptera: Acrididae in the Alpine Tundra of Colorado. -Annals of the Entomological Society of America 57: 310–317.

Alexander, G., and Hilliard, J.R. 1969. Altitudinal and seasonal distribution of Orthoptera in the Rocky Mountains of Northern Colorado. -Ecological Monographs 39: 385–432.

Angilletta, M.J., Jr, and Dunham, A.E. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. –The American Naturalist 162: 332–342.

Ashby, P.D. 1997. Conservation of mass-specific metabolic rate among high- and low-elevation populations of the acridid grasshopper *Xanthippus corallipes*. -Physiological Zoology 70: 701–711.

Ashby, P.D. 1998. The Effect of Standard Metabolic Rate on Egg Production in the Acridid Grasshopper, *Xanthippus corallipes*. -American Zoologist 38: 561–567.

Atkinson, D. 1994. Temperature and organism size-a biological law for ectotherms? -In: Advances in Ecological Research, Academic Press 25: 1-58

Atkinson, D. 1996. Ectotherm life-history responses to developmental temperature. –In: Animals and Temperature, Cambridge University Press,.

Atkinson, D., and Begon, M. 1987a. Ecological correlates and heritability of reproductive variation in two co-occurring grasshopper species. -Ecological Entomology 12: 129–138.

Atkinson, D., and Begon, M. 1987b. Reproductive variation and adult size in two co-occurring grasshopper species. -Ecological Entomology 12: 119–127.

Ayres, M.P., and Scriber, J.M. 1994. Local adaptation to regional climates in *Papilio canadensis* Lepidoptera: Papilionidae. -Ecological Monographs 64: 465–482.

Ballinger, R.E. 1983. Life history variation. -In: Lizard Ecology: Studies of a Model Organism, R.B. Huey, and E.R. Pianka, eds. Cambridge, Mass.: Harvard University Press, pp. 241–260.

Bellinger, R.G., and Pienkowski, R.L. 1985. Interspecific variation in ovariole number in Melanopline grasshoppers Orthoptera: Acrididae. -Annals of the Entomological Society of America 78: 127–130.

Belovsky, G.E., and Slade, J.B. 1995. Dynamics of two Montana grasshopper populations: relationships among weather, food abundance and intraspecific competition. -Oecologia 101: 383–396.

Bergmann, C. 1848. Über die verhältnisse der wärmeökonomie der thiere zu ihrer größe.

Bernardo, J. 1996. Maternal Effects in Animal Ecology. -American Zoologist 36: 83–105.

Berner, D., and Blanckenhorn, W.U. 2006. Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. -Journal of Animal Ecology 75: 130–139.

Berner, D., et al. 2004. Grasshopper populations across 2000 m of altitude: is there life history adaptation? -Ecography 27: 733–740.

Bidau, C.J., and Martí, D.A. 2007. Clinal variation of body size in *Dichroplus pratensis* Orthoptera: Acrididae: inversion of Bergmann's and Rensch's rules. -Annals of the Entomological Society of America 100: 850–860.

Bie, T., et al. 2012. Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. -Ecology letters 15: 740-747.

Blanckenhorn, W.U. 2000. The evolution of body size: what keeps organisms small? -The

Quarterly Review of Biology 75: 385–407.

Blanckenhorn, W.U., and Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? -Integrative and Comparative Biology 44: 413–424.

Branson, D.H. 2003. Reproduction and survival in *Melanoplus sanguinipes* Orthoptera: Acrididae in response to resource availability and population density: the role of exploitative competition. -The Canadian Entomologist 135: 415–426.

Branson, D.H. 2004. Relative importance of nymphal and adult resource availability for reproductive allocation in *Melanoplus sanguinipes*, Orthoptera: acrididae. -Journal of Orthoptera Research 13: 239–245.

Branson, D.H. 2008. Influence of individual body size on reproductive traits in Melanopline grasshoppers Orthoptera: Acrididae. -Journal of Orthoptera Research 17: 259–263.

Buckley, L.B., et al. 2013. Phenotypic clines, energy balances and ecological responses to climate change. –Journal of Animal Ecology 83:41-50 .

Capinera, J.L. 1979. Qualitative variation in plants and insects: effect of propagule size on ecological plasticity. -The American Naturalist 114: 350–361.

Cassel-Lundhagen, et al. 2010. Limited gene flow may enhance adaptation to local optima in isolated populations of the Roesel's bush cricket (*Metrioptera roeselii*). –Journal of Evolutionary Biology 24: 381-390.

Chapman, R.F., and Joern, A. 1990. Biology of grasshoppers. -New York: Wiley.

Cherrill, A. 2002. Relationships between oviposition date, hatch date, and offspring size in the grasshopper Chorthippus brunneus. -Ecological Entomology 27: 521–528.

Chown, S.L., and Gaston, K.J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. -Biological Reviews 74: 87–120.

Chown, S.L., and Gaston, K.J. 2010. Body size variation in insects: a macroecological perspective. -Biological Reviews 85: 139–169.

Chown, S.L., and Klok, C.J. 2003. Altitudinal body size clines: latitudinal effects associated with changing seasonality. -Ecography 26: 445–455.

Danner, B.J., and Joern, A. 2004. Development, growth, and egg production of *Ageneotettix deorum*, Orthoptera: Acrididae in response to spider predation risk and elevated resource quality. -Ecological Entomology 29: 1–11.

Davidowitz, G. 2008. Population and environmental effects on the size-fecundity relationship in a common grasshopper across an aridity gradient. -Journal of Orthoptera Research 17: 265-271.

de Souza Santos, P.D.S., and Begon, M. 1987. Survival costs of reproduction in grasshoppers. -Functional Ecology 1: 215.

Dean, J.M. 1982. Latitudinal variation in voltinism and life history traits in the lesser migratory grasshopper. -University of Iowa.

Dearn, J.M. 1977. Variable life history characteristics along an altitudinal gradient in three species of Australian grasshopper. -Oecologia 28: 67–85.

DeBano, S.J. 2008. Morphometric condition as a measure of energetic content and reproductive potential in *Dactylotum variegatum*. -Journal of Orthoptera Research 17: 293–300.

Denno, R.F., et al. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. -Annual Review of Entomology 40: 297–331.

Dingle, H., and Mousseau, T.A. 1994. Geographic variation in embryonic development time and stage of diapause in a grasshopper. -Oecologia 97: 179–185.

Dingle, H., et al. 1990. Altitudinal variation in life cycle syndromes of California populations of the grasshopper, *Melanoplus sanguinipes* F. -Oecologia 84: 199–206.

Ernsting, G., and Isaaks, J. a. 1997. Effects of temperature and season on egg size, hatchling size and adult size in *Notiophilus biguttatus*. -Ecological Entomology 22: 32–40.

Fielding, D.J., and DeFoliart, L.S. 2008. Relationship of metabolic rate to body size in Orthoptera. -Journal of Orthoptera Research 17: 301–306.

Fox, C.W., and Czesak, M.E. 2000. Evolutionary ecology of progeny size in arthropods. –Annual Review of Entomology 45: 341–369.

Gaston, K.J. 2000. Global patterns in biodiversity. -Nature 405: 220–227.

Gochis, D.J., et al. 2004. Analysis of 2002 and 2003 warm-season precipitation from the North American monsoon experiment event rain gauge network. -Monthly Weather Review 132: 2938–2953.

Grant, A., et al. 1993. An alternative theory of grasshopper life cycles. -Oikos 66: 263–268.

Greenland, D., et al. 1985. The Bioclimates of the Colorado Front Range. -Mountain Research and Development 5: 251–262.

Groeters, F.R., and Shaw, D.D. 1996. Evidence for association of chromosomal form and development time from complex clines and geographic races in the grasshopper *Caledia captiva*, Orthoptera: Acridadae. -Biological Journal of the Linnean Society 59: 243–259.

Hadley, N.F., and Massion, D.D. 1985. Oxygen consumption, water loss and cuticular lipids of high and low elevation populations of the grasshopper *Aeropedellus clavatus*, Orthoptera: Acrididae. -Comparative Biochemistry and Physiology Part A: Physiology 80: 307–311.

Hatle, J.D., et al. 2003. Plasticity and canalization in the control of reproduction in the lubber grasshopper. -Integrative and Comparative Biology 43: 635–645.

Hillebrand, H., and Azovsky, A.I. 2001. Body size determines the strength of the latitudinal diversity gradient. -Ecography 24: 251–256.

Hodin, J. 2009. She shapes events as they come: plasticity in female insect reproduction. –In: Phenotypic Plasticity of Insects, D. Whitman, and T. Ananthakrishnan, eds. -Science Publishers.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. -Oikos 66: 483–492.

Ikeda, H., et al. 2012. Altitudinal life-cycle and body-size variation in ground beetles of the genus Carabus, subgenus Ohomopterus in relation to the temperature conditions and prey earthworms. -Pedobiologia 55: 67–73.

Joern, A., and Behmer, S.T. 1997. Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum*, Orthoptera: Acrididae. -Oecologia 112: 201–208.

Joern, A., and Klucas, G. 1993. Intra- and interspecific competition in adults of two abundant grasshoppers, Orthoptera: Acrididae from a sandhills grassland. -Environmental Entomology 22: 352–361.

Juliano, S. A. 1983. Body size, dispersal ability, and range size in North American species of Brachinus (Coleoptera: Carabidae). -The Coleopterists' Bulletin: 232-238.

Kingsolver, J., and Huey, R.B. 2008. Size, temperature, and fitness: three rules. -Evolutionary Ecology Research 10: 251–268.

Lactin, D.J., and Johnson, D.L. 1996. Behavioural optimization of body temperature by nymphal grasshoppers *Melanoplus sanguinipes*, Orthoptera: Acrididae in temperature gradients established using incandescent bulbs. -Journal of Thermal Biology 21: 231–238.

Laws, A.N., and Belovsky, G.E. 2010. How will species respond to climate change? Examining the effects of temperature and population density on an herbivorous insect. -Environmental Entomology 39: 312–319.

Leather, S.R. 1988. Size, reproductive potential and fecundity in insects: things aren’t as simple as they seem. -Oikos 51: 386.

Levinton, J.S., and Monahan, R.K. 1983. The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. II. Intraspecific comparisons between subspecies of *Ophryotrocha puerilisi*, Polychaeta: Dorvilleidae. -Biological Bulletin 165: 699.

Mayr, E. 1956. Geographical character gradients and climatic adaptation. -Evolution 10: 105–108.

McAnelly, M.L., and Rankin, M.A. 1986. Migration in the grasshopper *Melanoplus sanguinipes* Fab.. II. Interactions between flight and reproduction. -Biological Bulletin 170: 378–392.

McGuire, et al. 2012. Elevation-dependent temperature trends in the Rocky Mountain Front Range: Changes over a 56- and 20-Year Record. -PLoS ONE 7: e44370.

McKitrick, M.C. 1993. Phylogenetic constraint in evolutionary theory: has it any explanatory power? -Annual Review of Ecology and Systematics 24: 307–330.

Moehrlin, G.S., and Juliano, S.A. 1998. Plasticity of insect reproduction: testing models of flexible and fixed development in response to different growth rates. -Oecologia 115: 492–500.

Monk, K.A. 1985. Effect of habitat on the life history strategies of some British grasshoppers. -The Journal of Animal Ecology 54: 163.

Mousseau, T.A. 1997. Ectotherms follow the converse to Bergmann’s rule. -Evolution 51: 630–632.

Mousseau, T.A., and Dingle, H. 1991. Maternal effects in insect life histories. -Annual Review of Entomology 36: 511–534.

Mousseau, T.A., and Roff, D.A. 1989. Adaptation to seasonality in a cricket: Patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. -Evolution 43: 1483–1496.

Nijhout, H.F. 1984. Abdominal stretch reception in *Dipetalogaster maximus*, Hemiptera: Reduviidae. -Journal of Insect Physiology 30: 629–633.

Nufio, C.R., et al. 2010. Grasshopper community response to climatic change: Variation along an elevational gradient. -PLoS ONE 5: e12977.

Nygren, G.H., et al. 2008. Latitudinal body size clines in the butterfly *Polyommatus icarus* are shaped by gene-environment interactions. –Journal of Insect Science 8:47.

Odendaal, F.J., and Rausher, M.D. 1990. Egg load influences search intensity, host selectivity, and clutch size inBattus philenor butterflies. –Journal of Insect Behavior 3: 183–193.

Orr, M.R. 1996. Life-history adaptation and reproductive isolation in a grasshopper Hybrid zone. -Evolution 50: 704–716.

Otte, D. 2012. Eighty New Melanoplus species from the United States (Acrididae: Melanoplinae). -Transactions of the American Entomological Society 138: 73-167.

Peters, R.H. 1986. The Ecological implications of body size- Cambridge University Press.

Pfadt, R. 1994. Grasshopper species fact sheets.

Pöykkö, H., and Hyvärinen, M. 2012. To grow fast or to grow big? Time-limited larvae of *Eilema depressum* speed up their growth and reduce number of instars. -Entomologia Experimentalis Et Applicata 142: 145–152.

Renucci, M., et al. 1990. Ovaries and regulation of juvenile hormone titer in *Acheta domesticus* L. Orthoptera. –General and Comparative Endocrinology 78: 137–149.

Roff, D. 1980. Optimizing development time in a seasonal environment: The “Ups and Downs” of clinal variation. -Oecologia 45: 202–208.

Roff, D.A. 1983. Phenological adaptation in a seasonal environment: a theoretical perspective. -In: Diapause and Life Cycle Strategies in Insects, Springer London.

Roff, D.A. 1992. Evolution of life histories: Theory and Analysis- Springer.

Rourke, B.C. 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. -Journal of Experimental Biology 203: 2699–2712.

Scott, S.M., and Dingle, H. 1990. Developmental programmes and adaptive syndromes in insect life-cycles. –In: Insect Life Cycles, F. Gilbert, ed. Springer London, pp. 69–85.

Shelomi, M. 2012. Where are we now? Bergmann’s rule sensu lato in insects. -The American Naturalist 180: 511–519.

Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. –The Quarterly Review of Biology 64: 419–461.

Sibly, R., and Monk, K. 1987. A theory of grasshopper life cycles. -Oikos 48: 186–194.

Slansky, F., and Scriber, J.M. 1985. Comprehensive insect physiology, biochemistry and pharmacology: Regulation -digestion, nutrition, excretion V. 4.

Smith, C.C., and Fretwell, S.D. 1974. The Optimal balance between size and number of offspring. -The American Naturalist 108: 499–506.

Stauffer, T., and Whitman, D. 1997. Grasshopper oviposition. –In: The bionomics of grasshoppers, katydids and their kin. Gangwere, ed. -Wallingford Oxon OX10 8DE, England, UK: CAB International.

Stearns, S.C. 1976. Life-history tactics: a review of the ideas. –The Quarterly Review of Biology 51: 3–47.

Stearns, S.C. 1992. The evolution of life histories -Oxford; New York: Oxford University Press.

Stearns, S.C., and Koella, J.C. 1986. The evolution of phenotypic plasticity in life-history traits: Predictions of reaction norms for age and size at maturity. -Evolution 40: 893.

Steigenga, M.J., and Fischer, K. 2007. Within- and between-generation effects of temperature on life-history traits in a butterfly. -Journal of Thermal Biology 32: 396–405.

Stillwell, R.C., and Fox, C.W. 2005. Complex patterns of phenotypic plasticity: interactive effects of temperature during rearing and oviposition. -Ecology 86: 924–934.

Stillwell, R.C., and Fox, C.W. 2007. Environmental effects on sexual size dimorphism of a seed-feeding beetle. -Oecologia 153: 273–280.

Stillwell, R.C., and Fox, C.W. 2009. Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity. -Oikos 118: 703–712.

Tatar, M., et al. 1997. Altitudinal variation for senescence in *Melanoplus* grasshoppers. -Oecologia 111: 357–364.

Taylor, B.J., and Whitman, D.W. 2010. A test of three hypotheses for ovariole number determination in the grasshopper *Romalea microptera*. -Physiological Entomology 35: 214–221.

Telfer, M.G., and Hassall, M. 1999. Ecotypic differentiation in the grasshopper *Chorthippus brunneus*: life history varies in relation to climate. -Oecologia 121: 245–254.

Van Horn, D. 1965. Variations in size and phallic morphology among populations of *Melanoplus dodgei* (Thomas) in the Colorado Front Range Orthoptera: Acrididae. -Transactions of the American Entomological Society 91: 95–119.

Vincent, S.E. 2006. Sex-based divergence in head shape and diet in the Eastern lubber grasshopper *Romalea microptera*. -Zoology 109: 331–338.

Wall, R., and Begon, M. 1987. Population density, phenotype and reproductive output in the grasshopper *Chorthippus brunneus*. -Ecological Entomology 12: 331–339.

Walters, R.J., and Hassall, M. 2006. The temperature-size rule in ectotherms: may a general explanation exist after all? -The American Naturalist 167: 510–523.

Woods, H.A. 1999. Egg-mass size and cell size: effects of temperature on oxygen distribution. -American Zoologist 39: 244–252.