

A GENERALIST GRASSHOPPER SPECIES (MELANOPLUS FEMURRUBRUM) IS  
ADAPTED TO VARIABLE ENVIRONMENTS ALONG A LATITUDINAL GRADIENT

by

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## Abstract

Temperature and food quality vary across broad latitudinal gradients, greatly affecting performance by insect herbivores. The contribution of each varies latitudinally so that geographically distinct populations are challenged by differences in nutritional needs and energetic demands. While there has been extensive work studying diet selectivity and nutritional ecology of insect herbivores, few studies have focused on how insect herbivores adapt across such vast environmental gradients. The generalist-feeding grasshopper, *Melanoplus femurrubrum* (DeGreer), has a broad geographic range that extends across much of North America, making this species ideal for comparative investigations of intrinsic performance responses to extensive but predictable patterns of environmental variation. I compared responses by six populations collected from populations located from Texas to North Dakota (USA) using a common garden experimental design to investigate clinal responses in grasshopper performance. I examined responses in: (1) body size, (2) thermoregulation and adaptive coloration, (3) developmental and growth rates, (4) metabolic rates, (5) total consumption and rates, (6) diet ratio selection, and (7) digestive processing efficiencies across the latitudinal gradient. Grasshopper body size followed the Converse Bergmann's Rule with decreasing body size as latitude increased. Temperature influenced all other responses, but responses to diet were not always significant or directional. Latitudinal trends for development and growth rates were observed but mass-specific metabolic rates were similar for all populations. Total consumption was body size dependent but independent of diet type. Mass-specific consumption varied but no single directional trend was detected. There was a shift in carbohydrate-biased diet preference at low latitude toward protein-biased diet ratios at higher latitudes, suggesting adaptations to different energetic demands by these populations. However, post-ingestive (digestive) efficiencies demonstrated variable responses with northern populations observing highest efficiencies for some indices but not all. Overall, this research documents phenotypic plasticity to environmental variability to some degree for digestive efficiencies, but ecotypic responses in body size and diet preference among *M. femurrubrum* populations were observed.

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## List of Abbreviations

*M. femurrubrum* – *Melanoplus femurrubrum* (DeGreer)

ND – North Dakota

SD – South Dakota

NE – Nebraska

KS – Kansas

OK – Oklahoma

TX – Texas

NCDC – National Climatic Data Center

LTFR – Long Term Ecological Research

SMR – standard metabolic rate

HFL – hind femur length

p:c – protein:carbohydrate

RGR – Relative Growth Rate

AD – Approximate Digestibility

ECI – Efficiency of Conversion of Ingestion

ECD – Efficiency of Conversion of Digestion

NUE – Nitrogen Use Efficiency

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## Chapter 1 - Introduction

Insect herbivore performance is influenced by a variety of environmental variables including, but not limited to, temperature and food availability and quality. Thermal environments vary globally and are largely constructs of the physical properties (position in orbit, solar irradiation, atmospheric conditions, etc.) of their geographic location on Earth (Gates 1980). In this thesis I broadly examine the role of temperature as a driving environmental factor to understand insect performance in a geographic context (Yang and Joern 1994c, Addo-Bediako et al. 2000, Logan et al. 2002, Angilletta et al. 2003, Logan et al. 2007, Dixon et al. 2009, Miller et al. 2009). Insect physiology is driven by temperature (Chown and Nicolson 2004, Karasov and del Rio 2007) which varies latitudinally. Although daily temperature is variable, seasonal temperatures follow broader predictive patterns. A natural latitudinal/altitudinal gradient for temperature exists with decreasing average annual temperatures as latitude/altitude increases in the northern hemisphere. Traditional use of the term “season” (referencing: winter, spring, summer and autumn) spans a fixed range of days in the year and can be helpful to generalize climatic conditions at certain periods of the year. Growing season length, defined as the accumulation of temperatures above a certain minimum and calculated as degree-days, is an excellent reference of latitudinal variability, making seasons more directly comparable across broad geographic distances (Pruess 1983, Scriber 2002). Overall growing season length, like temperature, decreases with increasing latitude/altitude (Fig. 1.1) with fewer accumulated degree-days in northern versus southern locations (Fig. 1.2) in the northern hemisphere. A growing season is generally considered to be the portion of the year when conditions support new plant vegetative and reproductive growth, or more simplistically, the period of aboveground

plant biomass accumulation. Growing seasons are most commonly linked to agricultural/horticultural plants but can also be synonymously considered to describe the period of time ephemeral insects such as grasshoppers (Orthoptera: Acrididae) and other herbivores are present aboveground. 10°C is the approximate temperature at which warm-season plant (i.e. corn and sorghum) growth is considered physiologically constrained by thermal conditions. Insect egg development is also limited when conditions fall below similar base temperatures (Fisher 1994, Hao and Kang 2004), and 10°C has also been used to determine degree-days for evaluating ecological processes in adult grasshoppers (Kemp and Onsager 1986, Fisher 1994, Hao and Kang 2004, Brust et al. 2009).

Heterogeneity in plant nutritional quality and palatability exists at multiple spatial and temporal scales, ranging from within plant variation (Bernays and Chapman 1994) to directional variation along latitudinal gradients (Reich and Oleksyn 2004, Salgado and Pennings 2005). Such environmental differences are challenges to the ability of insect herbivores to fulfill specific nutritional and metabolic needs (Raubenheimer and Simpson 1996, Gouveia et al. 2000, Sterner and Elser 2002, Behmer et al. 2003, Behmer 2009) given their local environment. Nitrogen (N) is often a limiting nutrient for insects (Mattson 1980, Joern and Behmer 1998, Berner et al. 2005, Fielding and Defoliart 2007). Insects may require different diet ratios depending on activity (Nespolo et al. 2008), life stage and/or body size (Hahn 2005, Boswell et al. 2008, Whitman 2008), which can vary within communities, minimizing diet overlap (Behmer and Joern 2008) and can vary with environment and geographically (Barimo and Young 2002, Fielding and Defoliart 2008, Miller et al. 2009). Nutritional requirements may also result in adaptations to seasonal environmental constraints which may ultimately drive variation in developmental rates along environmental gradients (Tauber et al. 1986).

Body size influences almost all other physiological processes in animals (Yang and Joern 1994c, Gillooly et al. 2001, Angilletta et al. 2004, Brown et al. 2004, Sears and Angilletta 2004, Chown et al. 2007, Karasov and del Rio 2007, Whitman 2008, Ho et al. 2010). Insect body size also interacts with environmental factors such as food quality and temperature to influence performance, but may also be adaptive to local conditions. The pattern of body size has been studied extensively in a variety of taxa and shown to follow distinct latitudinal clines (vanVoorhies 1996, Mousseau 1997, Partridge and Coyne 1997, Arnett and Gotelli 1999a, b, Blackburn et al. 1999, Blanckenhorn and Demont 2004, Walters and Hassall 2006, Bidau and Marti 2007a, Ho et al. 2010) which may be constrained by season length (Roff 1980, Dingle 1986, Tauber et al. 1986, Kivelä et al. 2011).

I sampled six populations of the mixed feeding grasshopper, *Melanoplus femurrubrum* (DeGeer), from a broad geographic gradient spanning roughly 20° of latitude (2,000 km) along similar longitude (Fig. 1.3). I used a common garden experimental design to broadly examine variation in grasshopper performance in response to temperature and food quality among populations across this broad geographic gradient. Extensive studies of diet selectivity and nutritional ecology of insect herbivores exist (Scriber and Slansky 1981, Waldbauer and Friedman 1991, Bernays and Chapman 1994) including work on grasshoppers (Joern and Behmer 1997, Zanotto et al. 1997, Gouveia et al. 2000, Simpson and Raubenheimer 2000, Behmer et al. 2003, Raubenheimer and Simpson 2003, Behmer and Joern 2008, Fielding and Defoliart 2008, Jonas and Joern 2008, Miller et al. 2009), but few comparative studies of insect performance along geographic gradients exist (Dingle et al. 1990, Addo-Bediako et al. 2000, Arnett and Gotelli 2003, Fielding and Defoliart 2007, Terblanche et al. 2009), and few studies investigate multiple populations along gradual ecological continuums so that interpretations

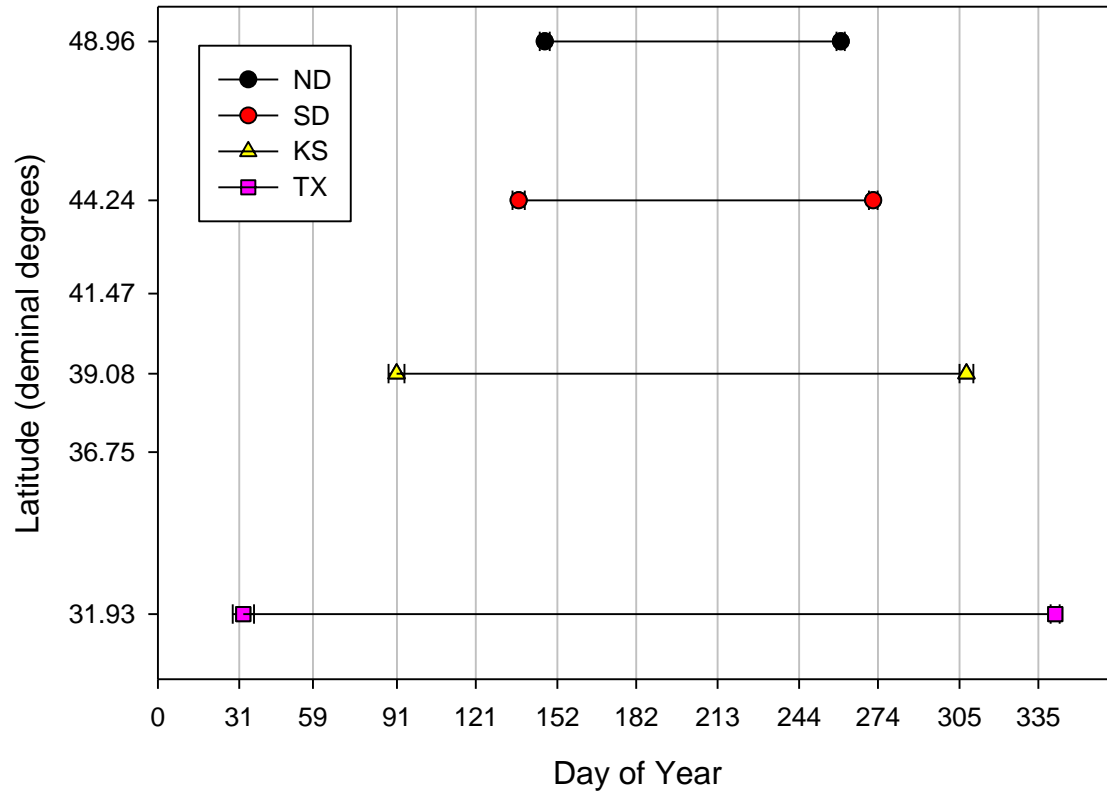
about responses are limited to higher or lower quantifications. My sample gradient encompassed a broader spectrum across a continuous ecological gradient than similar past studies.

Specifically I examined population responses to temperature and expected all populations to be influenced by increasing temperatures in similar ways because temperature drives insect performance. However, I anticipated local adaptation by populations would result in clinal responses. For example, I expected Northern populations to display shortest development times and highest growth rates because these populations would be adapted to short growing seasons with increasing development time and decreasing growth rate for populations at decreasing latitudes. Additionally, adaptive coloration may aid development and growth rates by enhancing thermoregulatory ability and populations may darken coloration with increasing latitude. I also expected grasshopper body size to follow the Converse Bergmann's Rule, consistent with many other Orthopteran species, likely resulting from seasonal constraints of temperature and growing season length. Because body size scales with metabolic rates and consumption, I also expected to find a clinal response with decreasing standard metabolic rates (SMR) as latitude increased. However, I expected that rates of consumption and processing (digestive) efficiencies would increase as latitude increased because populations in Northern latitudes are time-limited foragers. Additionally, because different environmental constraints are acting on these populations, I expected populations from warmer climates at low latitudes to select diets that were carbohydrate-biased while cooler climate, higher latitude populations would select diets that were protein-biased due to environmentally induced metabolic/nutritional demands. Likewise, I expected that Northern populations would be adapted to fewer accumulated degree days and may use increased processing efficiencies as a mechanism to optimize rapid development and growth.

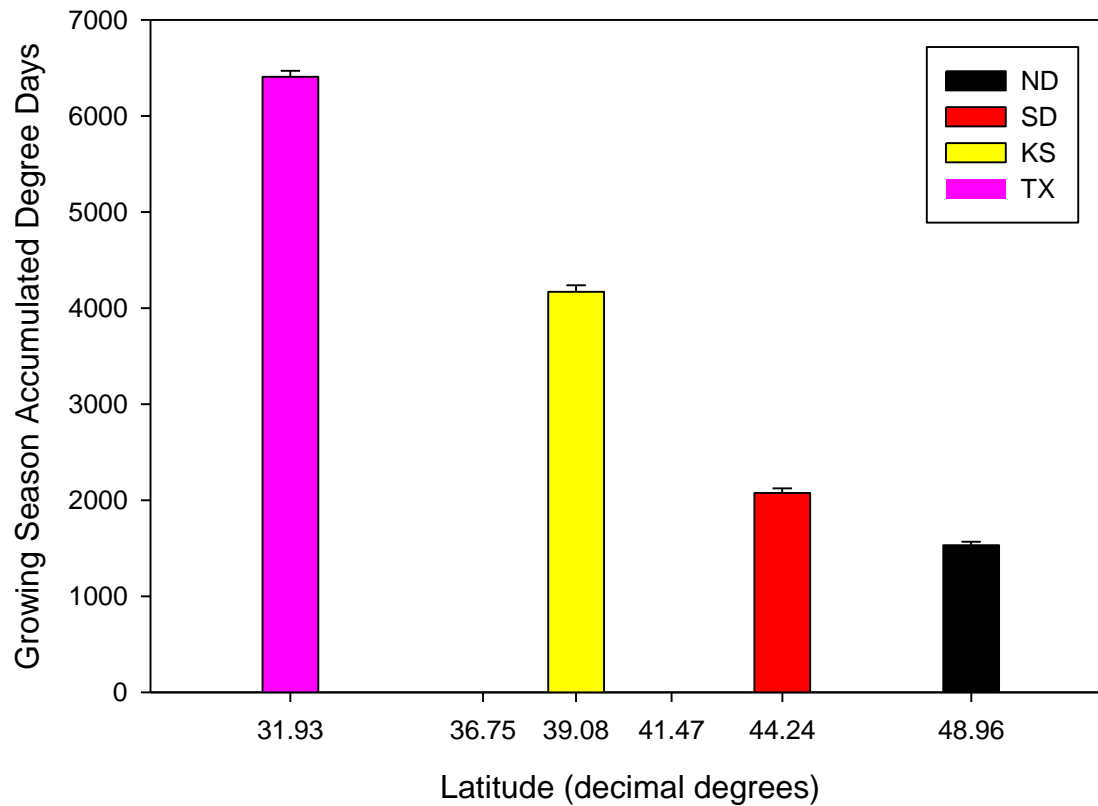
Chapter 2 documents general responses for body size, body coloration and metabolic rate. Body size followed the Converse Bergmann's Rule and scaled with whole organism standard metabolic rates, but mass-specific SMR was similar among populations. Diet quality did not consistently impact these responses. In Chapter 3, I examined intake and nutrient processing and assimilation. I found total intake to follow a clinal body size pattern with significant temperature and diet effects, but mass specific rates of consumption illustrate adaptation to seasonal constraints in northern populations which had significantly higher rates of consumption on a per gram basis. Diet preference also shifted with latitude. I used common nutritional indices to determine processing and assimilation difference among population and found no consistent latitudinal responses, although outcomes varied depending on the treatment. Finally, I conclude my thesis in Chapter 4 with a summary of all of my major findings.



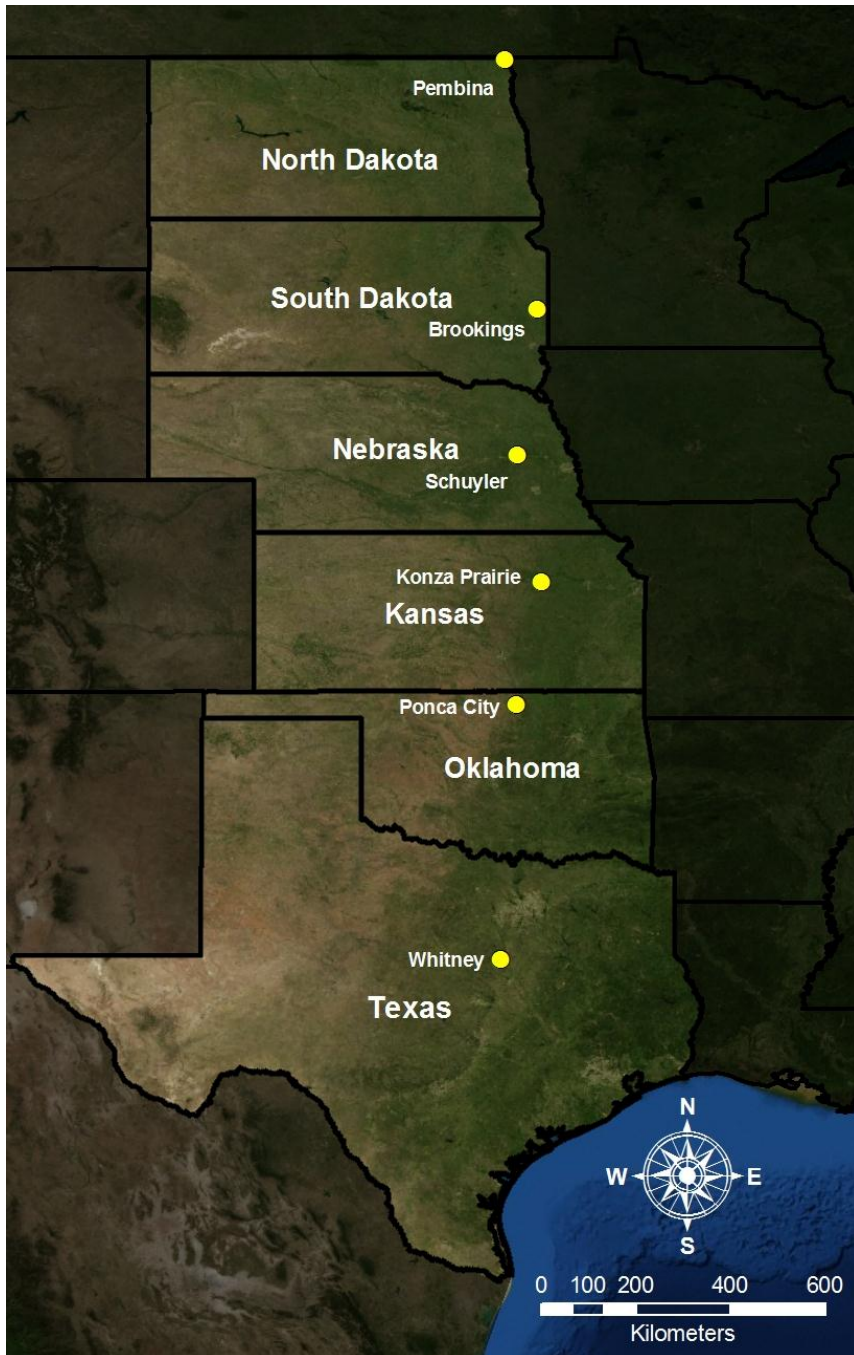
## Figures and Tables



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**Figure 1.2 Total growing season accumulated degree days calculated using daily mean temperatures and base 10°C for locations along a latitudinal gradient across the central US. Mean daily temperatures from NCDC (ND, SD, and TX) and LTER Konza Prairie Biological Station (KS; AWE01) weather datasets were used to calculate growing season length. Bars are arranged by latitude along the x-axis, increasing latitude from left to right (L to R: TX (pink); KS (yellow); SD (red); ND (black)).**



**Figure 1.3** Collection sites for grasshopper sample populations. Yellow dots represent collection sites per GPS coordinates. Location names are that of the closest city with the exception of the KS site which appears as the recognized National Science Foundation-Long Term Ecological Research biological station name.

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Collection Site	Collection Date	°N	°W	Weather Station ID	Distance (km)
Pembina, <i>Pembina</i> , North Dakota	August 18, 2008	48° 57.771''	97° 16.932''	326947	2.39
Brookings, <i>Brookings</i> , South Dakota	August 16, 2008	44° 14.313''	96° 40.010''	391076	13.20
Schuyler, <i>Colfax</i> , Nebraska	August 17, 2008	41° 28.023''	97° 03.106''	257640	2.26
Konza Prairie Biological Station; Manhattan, <i>Riley</i> , Kansas	August 9, 2009	39° 04.801''	96° 35.769''	<i>LTER Site Data</i>	2.77
Ponca City, <i>Kay</i> , Oklahoma	August 30, 2008	36° 45.165''	97° 03.951''	347196	4.30
Whitney, <i>Bosque</i> , Texas	September 7, 2009	31° 55.973''	97° 21.181''	419715	7.89

## **Chapter 2 - Converse Bergmann's Rule in the red-legged grasshopper (*Melanoplus femurrubrum*): Body size and performance variation along a latitudinal gradient**

### **Abstract**

Insect physiology is driven by temperature which can influence nutritional needs and energetic demands. Because temperature varies latitudinally, nutritional and energetic demands may also vary latitudinally. There has been extensive work studying diet selectivity and nutritional ecology of insect herbivores, especially grasshoppers, but few studies have focused on potential adaptive responses across vast environmental gradients. The generalist grasshopper *Melanoplus femurrubrum* (DeGreer) has a broad geographic range covering much of North America making this species ideal for a comparative investigation of performance in response to environmental variation. I conducted a common garden experiment using grasshoppers from six populations along a broad latitudinal gradient to examine grasshopper body size, development, growth and standard metabolic rates (SMR) in response to temperature and food quality manipulations. I expected clinal grasshopper responses to food quality and temperature. Specifically, I hypothesized that individuals from northern populations would be smaller overall but develop more rapidly as an adaptation to shorter and cooler growing seasons. I also hypothesized that grasshoppers from higher latitudes would have lower SMR than individuals from lower latitudes because insect metabolism is driven by temperature but also scales with body size. My results supported my hypothesis for clinal body size, development, and whole organism SMR, however, mass-specific SMR did not follow a clinal pattern. Comparing populations that occur across broad gradients can provide valuable insight into mechanisms

underlying species patterns and adaptability to different physical conditions, especially in the context of a changing environment.

## **Introduction**

Many species distributions span broad geographic gradients. However, individual populations often experience narrower ranges of environmental conditions than the species overall (Thompson 2005). One then expects adaptation to local environments to vary clinally along critical gradients, responding in a monotonic or step-fashion (Endler 1977). For example, temperature and growing season length vary predictably along latitudinal and altitudinal gradients, possibly affecting overall energy budgets or the ability to obtain nutrients by insect herbivores (Bailey and Mukerji 1977, Sterner and Elser 2002, Chown and Nicolson 2004, Fielding and Defoliart 2007, Karasov and del Rio 2007, De Block et al. 2008, Nespolo et al. 2008). Shorter season length and lower average and operational temperature profiles at northern sites can limit plant productivity, leading to overall limitations in resource availability for herbivores. Time available to forage and digest food by ectotherms at daily or seasonal scales can also be inhibited by physiologically sub-optimal, lower ambient temperatures (Yang and Joern 1994c, Logan et al. 2003) and the cost of maintaining body temperatures within operational ranges also increases for ectotherms at lower temperatures. All such factors influence the overall energy and nutrient budget of a consumer (Raubenheimer and Simpson 1994, Sterner and Elser 2002, Chown and Nicolson 2004). Because average growing season length is longer and average daily temperatures are higher at southern sites in the northern hemisphere, consumers generally have increased time available to obtain and process food than do their northern conspecifics as long as it does not get too hot (Roff 1980, Kivelä et al. 2011). Consumers at these locales also may require more energy intake to offset losses from increased

temperature-dependent metabolism. Finally, plant quality of significance to herbivores may also vary along latitudinal temperature gradients. The temperature-plant physiological hypothesis predicts lower C:N ratios at sites experiencing cooler conditions (Reich and Oleksyn 2004), where plants should be more palatable or digestible to herbivores (Salgado and Pennings 2005, Ho et al. 2010).

Latitudinal gradients present opportunities to evaluate how insect herbivores meet challenges of satisfying nutritional needs to be allocated to growth, reproduction, and maintenance under variable conditions of food quality and temperature (Addo-Bediako et al. 2000, Arnett and Gotelli 2003, De Block et al. 2008, Monaco et al. 2010). Although insects are mobile, most species do not travel great distances and are thus limited by local environments (Chapman and Joern 1990). As a result, significant latitudinal clines are observed in temperature tolerances (Addo-Bediako et al. 2000, Castaneda et al. 2005, Ragland and Kingsolver 2008) and body size (Masaki 1967, Roff 1980, Arnett and Gotelli 1999a, Blackburn et al. 1999, Blanckenhorn and Demont 2004, Bidau and Marti 2007b, Huizenga et al. 2008, Ho et al. 2010).

Body size is an important phenotypic trait of organisms (Whitman 2008, Chown and Gaston 2010), influencing all aspects of life including cell size, physiological processes (i.e., metabolism, developmental and growth rates) (Gillooly et al. 2001, Brown et al. 2004, Kingsolver and Huey 2008), and species interactions (Brooks and Dodson 1965, Brose 2010). Increases in body size with latitude (Bergmann's Rule) are well known for a variety of organisms, especially vertebrates, at multiple taxonomic levels (vanVoorhies 1996, Arnett and Gotelli 1999a, Blackburn et al. 1999, Ho et al. 2010). For insects and other ectotherms, the Converse Bergman's Rule, where body size declines with increased latitude, is also well recognized (Masaki 1967, Roff 1980, Mousseau 1997, Sears and Angilletta 2004, Bidau and

Marti 2007a, Ho et al. 2010). Such variety among unrelated taxa begs the question of what generalizations can be made about latitudinal clines in body size (Blanckenhorn and Demont 2004). With this in mind, answers may come from understanding how abiotic and biotic factors such as environmental temperature, habitat heterogeneity and resource availability can influence insect herbivore body size and life history responses. For example, non-optimal (lower or higher) environmental temperatures can limit an insect's time for foraging or digestion, thus limiting nutrient assimilation.

The importance of temperature and body size to insect growth, development and metabolic rates is well known (Harrison and Fewell 1995, Gillooly et al. 2001, Angilletta et al. 2003, Brown et al. 2004, Terblanche et al. 2007). The standard use of day-degree calculations for tracking temperature-dependent insect development focuses on physiological time as a better description of life cycle dynamics than using time alone (Pruess 1983, Tauber et al. 1986, Taylor and Karban 1986, Hao and Kang 2004, O'Neill and Rolston 2007, Brust et al. 2009). Completion of life cycles in insects such as grasshoppers is temperature dependent (Tauber et al. 1986, Capinera and Horton 1989, Scriber 2002). Grasshopper populations in northern portions of their geographic range typically deposit eggs in the soil during the growing season where they overwinter until hatching as nymphs the following growing season. However, extensive variation exists regionally where southern, warmer regions can exhibit multiple generations in a growing season while grasshoppers in extreme northern regions can require two growing seasons to complete the life cycle (Dingle et al. 1990). Completing the life cycle in one year at higher latitudes (and/or altitudes) where season length is restricted and the total temperature range is reduced requires that insects develop more quickly than their conspecifics at lower latitudes (or at lower elevation) through faster growth rates (Dingle et al. 1990, Dingle and Mousseau 1994)



and/or by maturing at a smaller body size (Dingle et al. 1990, Fielding and Defoliart 2007). Latitudinal variation in whole organism metabolism could explain latitudinal patterns as it generally scales with body mass (Brown et al. 2004, Hui and Jackson 2007, Karasov and del Rio 2007, del Rio 2008). Because body size and metabolic processes vary latitudinally, comparing populations that occur across broad gradients can provide valuable insights into mechanisms underlying species patterns and adaptability to different physical conditions, especially in the context of a changing environment (Bale et al. 2002, Elser et al. 2010). In addition, using a common garden experimental design allows detection of adaptive responses for body size and metabolic strategies along this environmental gradient.

In this study, I evaluated body size, cuticular color and life history responses among six populations of the generalist-feeding grasshopper *Melanoplus femurrubrum* (DeGreer) along a broad latitudinal gradient (Texas to North Dakota). Lab-reared offspring were obtained from individual populations and used in a common garden experiment to assess variation in key grasshopper traits expected to affect developmental rates (body size, cuticle color, growth rate, and metabolic rate) in response to temperature and food quality manipulations. I tested the following hypotheses: (1) grasshopper body size follows a negative latitudinal size cline typical of many insects (Converse Bergmann's Rule) as expected if thermal and seasonal restrictions occur; (2) shorter season length in Northern latitudes results in smaller body size, as expected from tradeoffs between growth and development time (Roff 1980, Tauber et al. 1986); (3) individuals from the northern populations should exhibit faster development rates to meet challenges of shorter season length at higher latitudes (if they can complete the life cycle in a single season) (Tauber et al. 1986); (4) standard metabolic rates (SMR) of whole organisms should decrease with increased latitude or with higher food quality (lower C:N ratio) conditions

(Zanotto et al. 1997); and (5) cuticle color should be darker with increased latitude, facilitating body temperature regulation (relatively higher body temperatures compared to paler morphs) in cooler environments (Fielding and Defoliart 2005, Clusella Trullas et al. 2007).

## **Methods**

### ***Study Organism***

Grasshoppers (Orthoptera: Acrididae) are insect herbivores that are active aboveground during the growing season when food resources are abundant and thermal conditions are within operational physiological ranges. The red-legged grasshopper *M. femurrubrum* is a medium-sized (9.7-15.7 mm adult hind femur length, personal observation), generalist-feeding species that is distributed throughout most of North America (Bailey and Mukerji 1977, Vickery and McE. Kevan 1986, Thompson 1992, Capinera et al. 2004). Individuals generally overwinter as eggs throughout most of the geographic range, hatch in mid-spring, becoming adults from late-spring/early-summer, where they complete the life cycle as long as conditions are suitable (Bailey and Mukerji 1977). This species is univoltine in the northern portion of its distribution, sometimes requiring two growing seasons to complete one life cycle at the most northern range limit. Southern populations with longer, warmer growing seasons (Dingle et al. 1990, Dingle and Mousseau 1994). This species is common along roadsides and disturbed and recovering areas such as old field habitats (Beckerman 2002). *Melanoplus femurrubrum* can be considered an economically important crop pest, although populations only infrequently reach sufficient densities to affect total crop yields (Vickery and McE. Kevan 1986).

### ***Collection sites***

Grasshoppers were collected using sweep nets from six locations at similar longitude across the Central Plains of the United States, spanning roughly 2,000 kilometers and roughly 20° of latitude from Texas northward to North Dakota (Fig 1.3, Table 1.1). All collection sites were near well-travelled roadsides. Three sites were directly adjacent to agricultural fields (ND, SD, NE), one site was a prairie restoration (SD) and one was a grazed natural prairie (KS) site. Two sites were tracts of land that were fallow; one site was a vacant lot (OK) and one was public recreation or hunting land (TX).

Climate profiles constructed using National Climatic Data Center (NCDC) datasets for TX, SD and ND as shown in Figure 2.1. Konza Prairie Biological Station climate data (LTER dataset AWE01) was used for the KS location (Table 1.1). Only incomplete NCDC datasets were available for OK and NE locations so climate profiles were not constructed for these two locations.

### ***Field Studies – Body temperature, size and color***

Body Temperature and Thermoregulation. Adult *M. femurrubrum* were collected in the field using sweep nets in July–August, 2009 and 2010. Body temperatures were measured using an instant-read, handheld Microprocessor Thermometer (Model: HH21A, 0.1/1°C Resolution, +/- 0.1% rdg + 0.6°C) equipped with a Type K-J-T thermocouple and hypodermic probe (Type K, all equipment: Omega Engineering, Inc., Stamford, CT, USA). Body temperatures were measured immediately after capture by inserting the hypodermic probe in a posterior direction through the animal's neck membrane adjacent to the post occipital suture approximately 3 mm beneath the pronotum. Individuals were handled gently between the thumb and forefinger for no more than a few seconds to minimize altering the body temperatures; insect body temperatures can rapidly

change depending on posture in the canopy or behavioral activity (Kemp 1986). Ambient air temperature was also recorded at the capture location of each individual; the probe was held at the surface where the insect was collected but shaded from direct sunlight to measure the ambient surface air temperature. Body temperature of thirty individuals (targeted 1:1 sex ratio) and associated microhabitat temperatures were measured at each site. Individuals were then stored in a freezer as representative field controls in uniquely numbered vials for additional analyses and to serve as a field comparison to experimental grasshopper results (see Results - Body Size, Pronotum Color). To get a sense of thermoregulatory capabilities, ambient environmental temperature and grasshopper internal body temperatures were fit to a logistic function model developed by Kemp (1986) based on grasshopper body and environment air temperatures collected in a manner similar to this study. Profiles of body temperature over the full range of ambient air temperatures at each site to characterize thermoregulation were not constructed as they were not always encountered during field collections. However, fitting values of body temperature for all sites over a restricted range of ambient air temperatures to a general, existing model was used to see if population-level shifts were obvious. While admittedly a weak test of thermoregulatory capabilities by individuals, the approach provided some sense of the general nature of behavioral control of body temperature among populations along the latitudinal gradient.

Body Size. Hind femur lengths (HFL) were measured as a proxy of overall body size (Colombo 1997, Thompson 1999, Bidau and Marti 2008) (hereafter HFL and body size are used interchangeably). The left hind femur from each adult was detached from the body and measured using a microscope equipped with an ocular micrometer. Measurements were recorded to the nearest micrometer and converted to millimeter units. The HFL index predicts body

weight quite well (Fig. 2.2; experiment: ♀, dry mass = 9.5280 (HFL) – 59.4544,  $r^2 = 0.71$ ,  $P = <0.0001$ ; ♂, dry mass = 9.1554 (HFL) – 51.9737,  $r^2 = 0.69$ ,  $P = <0.0001$ ; field: ♀, dry mass = 24.5927 (HFL) – 214.7687,  $r^2 = 0.44$ ,  $P = <0.0001$ ; ♂, dry mass = 17.5473 (HFL) – 128.8668,  $r^2 = 0.66$ ,  $P = <0.0001$ ).

Pronotum Color. Pronotum color for all individuals (field and experiment) was quantified with a spectrometer at the USDA-ARS Center for Grain & Animal Health Research (Manhattan, KS). Pronotums were removed from adult grasshopper bodies and carefully flattened between two glass microscope slides. Prepared samples were then stored in a jar with desiccant for a minimum of two weeks to allow them to dry flat and reach constant moisture content. Full spectra (350-2500 nm) absorbance data were collected from dry pronotums and characterized using RS<sup>3</sup> software (version 3.1.11) and an ASD QualitySpecPro Benchtop Spectrometer (software and equipment: Analytical Spectral Devices, Boulder, CO, USA) with a 3 mm diameter bifurcated fiber-optic probe 2 mm from the specimen resting on a spectralon. Spectral files were converted to CIE L\*A\*B\* visible color (360-830 nm) values using GRAMS / AI Spectroscopy software (version 8.0, Thermo Electron Corporation). CIE-L\*A\*B\* color uses three components to define the color space occupied to represent overall pigment. The first component, CIE-L\*, is a measure of lightness ranging from 0, absolute black/dark, to 100, absolute white/bright. Red (+) / green (-) color is quantified in CIE-A\* while CIE-B\* is a quantification of yellow (+) / blue (-). I used variation in L\* to determine overall changes in color light/darkness.

### ***Grasshopper rearing***

Adult *M. femurrubrum* collected from the six study sites (Table 1.1) were maintained in the laboratory at Kansas State University (Manhattan, KS, USA) in separate populations to

collect eggs for experiments (methods - Behmer and Joern 2008). Caged populations were kept at ca. 30°C using incandescent light bulbs positioned next to the cage (14:10, L:D) with ample water and food (organic romaine lettuce leaves, wheat seedlings and a mixture of wheat bran and Brewer's yeast). Small containers filled with autoclaved sand treated with distilled water and a 0.05% methylparaben anti-fungal solution were provided for oviposition. Egg cups were removed from colony cages and stored at room temperature for approximately 4-6 weeks before placing them into a refrigerator (ca. 4-5°C) for a minimum of 16 weeks to break diapause. Egg cups were then placed in empty sterilized rearing cages at room temperature in front of a light source (as described above), kept moist with distilled water, and monitored daily for hatching. First-generation nymphs from each location were maintained in colony conditions through the completion of the fourth instar.

### *Feeding trials*

Immediately upon molting to fifth instar, feeding trials were conducted in three temperature controlled chambers (1 Conviron (Canada) and 2 Percival (Percival Scientific, Inc., IA, US) chambers) set to 14:10 L:D conditions and constant day/night temperatures of 25, 31 or 37°C. These temperatures were selected because they bracket (approximately) the typical minimum and maximum body temperatures that these populations encounter in the field during the growing season. These temperatures are also commonly used in other whole organism physiological studies (Zanotto et al. 1997, Gouveia et al. 2000, Miller et al. 2009, Terblanche and Chown 2010). Grasshoppers were individually weighed within < 12 hours of molt and placed in isolated clear plastic containers (18.5x13x9cm) with a 4cm screen vent on the lid (Fig. 2.3). Contact paper applied to cage sides prevented individuals from receiving any visual cues from outside their cages, which may influence feeding behavior. Each cage housed a metal

perch, a 40 mL water cup with cotton wick, and one 4cm petri dish containing a randomly assigned food source (artificial or natural, control diet). Dry artificial diets differing in protein (p) and carbohydrate (c) concentrations known to support grasshopper development and survival (Simpson and Raubenheimer 1993, Behmer and Joern 2008) were used. Two sets of diet were used in these trials (Table 2.1). Diets with combined protein and carbohydrate ratios of 42% of total diet (ACE; p14c28 =A, p21c21=C, p28c14=E) were used to rear grasshoppers from ND, SD, KS and TX populations. Diets with combined protein and carbohydrate ratios of 35% of total diet (BCD; p14c21=B, p21c14=D) and a 42% balanced diet (C) were used to rear grasshoppers from NE and OK populations. Diet combinations were chosen to bracket variability that may be encountered in the field, but these nutrient ratios were still sufficiently balanced to support survival (Slansky and Feeny 1977, Simpson and Raubenheimer 1993). Each individual was fed *ad libitum*, but not excessively, one of four designated artificial diets (Schmidt and Reese 1986). Artificial diets were weighed at the start of each feeding trial and replaced with a fresh, pre-weighed dish containing diet (of the same type) every third day until an individual molted or died. Grasshopper cages were monitored daily for the duration of the instar until the end of the feeding trial. Grasshoppers were stored frozen.

### ***Respirometry***

Respirometry measurements were taken for all individuals in the feeding trials on the third day of feeding. Food was removed for at least 3 hours prior to respirometry trials to standardize the effects of digestion on resting or standard metabolic rate (SMR) measurements. Flow-through respirometry measuring carbon dioxide (CO<sub>2</sub>) production was used to estimate metabolism (Lighton 2008, Terblanche and Chown 2010). Air was drawn into the system with a PP-2 Dual Pump (v 2.0, Sable Systems International, Las Vegas, NV, USA) and then scrubbed

of water and CO<sub>2</sub> as it passed through a DrieRite/Ascarite filter tube. Flow was adjusted and maintained at 100 mL min<sup>-1</sup> using a 2 Channel Mass Flow Controller (v 1.0, Sable Syst. Int.) in conjunction with a Side Track Flow Meter (Sierra Instruments). Grasshoppers were held in individual 12 mL glass chamber tubes housed in a PTC-1 Peltier Effect temperature control Portable chamber (Sable Syst. Int.) connected to a Spirometer Multiplexer (v 2.0, Sable Syst. Int.), allowing up to seven individuals to be analyzed at one time with one chamber empty to measure baseline CO<sub>2</sub> concentration. The temperature control chamber was adjusted to the corresponding feeding trial temperature treatment (25, 31, 37 °C) prior to each respirometry recording. Each individual within a single chamber was analyzed for a total of 15 continuous minutes. Oxygen was scrubbed from the air using a magnesium perchlorate filter tube positioned just before it was analyzed for carbon dioxide content using a CA-10a CO<sub>2</sub> Analyzer (Sable Syst. Int.). The mean volume of CO<sub>2</sub> (VCO<sub>2</sub>; mL min<sup>-1</sup>) values for each individual were obtained using ExpeData acquisition and analysis software (build 1.1.20, Sable Syst. Int.).

### *Statistical analysis*

Field and experiment grasshoppers were analyzed separately in all analyses. Body size responses along the gradient were analyzed using analysis of variance (ANOVA) with population (field and experiment), temperature and diet (experiment only) as a fixed effects and sex as a main effect. All treatments were pooled by population in the common garden experiments. Tukey HSD was used to determine population size similarities. Statistical analysis of spectra was conducted using ANOVA with population and temperature (experiment only; all diets pooled) as fixed effect(s). Development, relative growth rate (RGR), and SMR data were limited to laboratory feeding trial experiment grasshoppers only. Development and growth rate were analyzed using ANOVA with population, temperature and diet as fixed effects. The mean



VCO<sub>2</sub> consumed was analyzed using ANOVA (whole organism SMR) and ANCOVA (mass-specific SMR) with population and temperature as fixed effects and sex and initial body mass as covariates. All statistical analysis was performed using SAS (v9.2, Cary, NC).

## Results

### *Body Size*

Broadly speaking, grasshopper body size (HFL) decreased with increasing latitude following the Converse Bergmann's Rule clinal body size pattern in all field-collected and common garden experiment individuals, regardless of treatment (Fig. 2.4). Size dimorphism between sexes is common in insect species and was seen in *M. femurrubrum*. Overall, female HFLs were about 31% larger than males in the field and about 8% larger than males in the lab experiments across the sampled populations (Table 2.2). Body size for both sexes varied among populations in the field (♀:  $F_{5,77} = 64.17$ ,  $P = < 0.0001$ ; ♂:  $F_{5,89} = 101.77$ ,  $P = < 0.0001$ ) and in the experiment (♀:  $F_{5,227} = 90.84$ ,  $P = < 0.0001$ ; ♂:  $F_{5,234} = 123.75$ ,  $P = < 0.0001$ ). There were no significant treatment effects of temperature or diet on grasshopper body size in the common garden experiment so all treatments were pooled by population for these analyses. Grasshoppers from North Dakota, the northern-most population, were smallest in size overall. In field-caught samples, grasshoppers became progressively larger as latitude decreased. In the lab-reared common garden populations, body size (HFL) for TX, OK, KS and NE populations are all statistically similar; there appears to be a plateau in body size until there is a significant decline in body size for the SD and then ND grasshopper populations.

## ***Body color***

Body color, in terms of light/darkness ( $L^*$ ) varied among individuals from field collected populations ( $F_{5,177} = 14.62$ ,  $P = < 0.0001$ ) with darkest individuals originating from highest latitudes, as predicted (Fig. 2.5 panel A).  $L^*$  values increased latitudinally with significant population ( $F_{5,415} = 25.46$ ,  $P = < 0.0001$ ), temperature ( $F_{2,415} = 227.48$ ,  $P = < 0.0001$ ), population\*temperature ( $F_{10,415} = 3.16$ ,  $P = 0.0006$ ) and marginally significant temperature\*diet ( $F_{10,415} = 2.35$ ,  $P = 0.0537$ ) treatment effects in the laboratory feeding trials as well (Fig. 2.5 panels D, G, J). Green/red-ness ( $A^*$ ) increased (became more red) with increasing latitude in the field ( $F_{5,177} = 10.32$ ,  $P = < 0.0001$ ) and had significant population ( $F_{5,415} = 28.23$ ,  $P = < 0.0001$ ), temperature ( $F_{2,415} = 13.77$ ,  $P = < 0.0001$ ), diet ( $F_{2,415} = 3.45$ ,  $P = 0.0328$ ), population\*temperature ( $F_{10,415} = 2.30$ ,  $P = 0.0122$ ), and temperature\*diet ( $F_{4,415} = 2.82$ ,  $P = 0.0247$ ) treatment effects in the laboratory feeding trials. There was one increasing trend among field sampled grasshoppers (Fig 2.5 B) but there were two separate visible latitudinal trends increasing between TX-OK-KS and NE-SD-ND toward more red space across all temperature treatments (Fig 2.5 E, H, K). Yellow/blue-ness ( $B^*$ ) increased (became more blue) with increasing latitude in the field ( $F_{5,177} = 10.77$ ,  $P = < 0.0001$ ) and had significant population ( $F_{5,417} = 2.95$ ,  $P = 0.0126$ ), temperature ( $F_{2,417} = 24.63$ ,  $P = < 0.0001$ ), population\*temperature ( $F_{10,417} = 2.86$ ,  $P = 0.0019$ ), and temperature\*diet ( $F_{4,417} = 2.39$ ,  $P = 0.0506$ ) treatment effects in the laboratory feeding trials. An increasing  $B^*$  with increasing latitude trend existed for field sampled grasshoppers (Fig. 2.5 C) and a similar pattern of two separate increasing trends (as seen for  $A^*$ ) existed between TX-OK-KS and NE-SD-ND for  $B^*$  (Fig 2.5 L), but this pattern only under the highest temperature treatment (37°C) while no patterns emerge under 25 or 31°C feeding trial individuals (Fig. 2.5 F, I).

### ***Field thermoregulation***

Grasshoppers from all sites were able to regulate body temperatures in a similar fashion to achieve values as high as 16°C above ambient air temperatures. There were no sex related differences in body temperature detected with in any of the populations sampled in this study, nor were there any significant patterns in preferred body temperatures or the amount of variation within body temperature to ambient air temperature patterns across the latitudinal gradient (Fig. 2.6;  $r^2 = 0.7853$ ,  $p < 0.0001$ ).

### ***Performance - development, growth and metabolism***

Developmental Rates. Development rate was temperature dependent ( $F_{2,418}=606.09$ ,  $P = <0.0001$ ) and varied by population ( $F_{5,418}=44.66$ ,  $P = <0.0001$ ) with a population\*temperature interaction ( $F_{10,418}=2.49$ ,  $P = 0.0065$ ). Sex was also a significant main effect ( $F_{1,418}=6.05$ ,  $P = 0.0143$ ) for development time, data were plotted with sexes pooled as patterns were similar across all temperatures (Fig 2.7).

Relative Growth Rates. Relative growth rate (RGR) increased with temperature ( $F_{2,227} = 310.61$ ,  $P = <0.0001$ ) across all populations ( $F_{3,227}= 37.18$ ,  $P = <0.0001$ ) with a significant population\*temperature interaction ( $F_{6,227} = 5.89$ ,  $P = < 0.0001$ ; Fig. 2.8 A); reaction norms are shown in figure 2.8 B. North and South Dakota populations and NE, KS, OK, and TX populations responded similarly when reared at the lowest (25°C) temperature treatment. At moderate 31°C temperatures, RGR increased with latitude among populations. At the highest temperature treatment (37°C), populations at the extreme ends of the gradient achieved faster developmental rates overall than populations from the central locations along the gradient.

Whole-Individual SMR. Diets were pooled within population and temperature treatments because no effects of diet or subsequent interactions on whole organism or mass-specific SMR

were detected. Standard metabolic rates increased with temperature across all treatments ( $F_{2,265}=202.89$ ,  $P = <0.0001$ ). There was also an increase in SMR with decreased latitude ( $F_{3,265}=14.51$ ,  $P = <0.0001$ ) and no significant interactions between temperature treatments and populations (Fig 2.9 A, 2.10 A).

Mass-specific SMR. There was no significant effect of population on mass-specific SMR ( $F_{3,265} = 1.58$ ,  $P = 0.1953$ ), but mass-specific SMR increased as temperature increased ( $F_{2,265} = 148.69$ ,  $P = <0.0001$ ). Again there was no significant effect of diet quality (Fig. 2.9 B, 2.10 B) on this response.

## Discussion

Latitudinal gradients and associated clines of phenotypic traits provide key insights into adaptive challenges facing organisms. Seasonal temperature along with resource availability and quality are main factors impacting insect herbivore behavior and physiological processes in the field. Here, I used a common garden experiment to test whether grasshoppers from different populations along a latitudinal gradient had similar performance in response to manipulations of temperature and food quality. Patterns in grasshopper body size followed the Converse Bergmann's Rule, but body size among populations did not differ significantly among temperature or diet treatments. No latitudinal patterns were detected in behavioral thermoregulatory ability across populations, although there was a darkening trend for pronotum color with increasing latitude suggesting a potential adaptation to cooler climates in higher latitudes. Clinal patterns were found for development and whole organism SMRs, traits inherently linked to body size. However, growth rate and mass specific SMR exhibited variable latitudinal responses among treatments.

## ***Body Size***

Populations of *M. femurrubrum* along a mid-continental latitudinal gradient in North America conform to the Converse Bergman's Rule like many other orthopteran species globally (Mousseau 1997, Blanckenhorn and Demont 2004, Bidau and Marti 2008), where body size for field-collected phenotypes decreased monotonically as one goes north. Common garden experiments using first generation offspring showed a slightly different picture of this cline, although the trend is similar. As hypothesized, body size decreased with latitude in both the laboratory and field-caught grasshoppers. Shorter season lengths likely promote smaller body size because completion of the life cycle must fit within the available time frame for which environmental conditions are favorable (but see Grant et al. 1993), and smaller individuals require less time and nutritional resources to develop (Roff 1980, Grant et al. 1993, Arnett and Gotelli 1999b, Fielding and Defoliart 2007, De Block et al. 2008). As the length of the growing season increases, there is potential for multiple generations per season leading to overall decline in body size as the complete life cycle for each generation must fit within a single growing season, similar to time pressures experienced by northern populations (Roff 1980, Kivelä et al. 2011) which might help explain why females from TX populations were slightly smaller than their OK neighbors in our lab based trials although we did not see significant deviations in the trend in size in our analyses of field caught animals.

We observed a plateau in overall body size in common garden experiments with increasing body size as one moved south from North Dakota, and then leveled off midway along the gradient with NE, KS, OK, and TX populations achieving similar sizes (Fig. 2.4). The differences in body size between field-caught individuals and those reared under common-garden conditions increase as one moves from the northern populations toward Texas. Although

more specific genetic data are required to tease apart differences in responses among populations to common environmental conditions, it does appear that intermediate and southern populations show more phenotypic plasticity. Based on Tukey similarity groupings for field caught grasshoppers (Fig 2.4 A), KS and OK populations do not differ in size, TX population males are significantly larger, and TX females group with KS females. This pattern suggests that a switch-point for body-size ecotypes along this gradient exists between the NE and SD populations.

### ***Effects of Food Quality and Temperature***

While temperature can be a major factor influencing insect development and performance (Sibly and Atkinson 1994, Yang and Joern 1994c, Harrison and Fewell 1995, vanVoorhies 1996, Gillooly et al. 2001, Clarke 2003, Angilletta et al. 2004, Fielding 2004, Clarke 2006, Walters and Hassall 2006, Terblanche et al. 2007, Kingsolver and Huey 2008, Ragland and Kingsolver 2008, Dixon et al. 2009, Irlich et al. 2009, Miller et al. 2009), food quantity and quality have proven to be important as well (Yang and Joern 1994c, Miller et al. 2009). Insects can be highly nitrogen limited (Mattson 1980, Behmer and Joern 1993, Joern and Behmer 1998, Sterner and Elser 2002, Berner et al. 2005), but food quality did not significantly influence grasshopper performance in this common garden study for the diet combinations used. Although performance in some grasshopper species varies over the range of nutritional levels used (Zanotto et al. 1993, Fielding and Defoliart 2007), individuals can compensate for such differences to maintain relatively constant levels of performance (Zanotto et al. 1993, Bernays and Chapman 1994, Zanotto et al. 1997, Miller et al. 2009). Because grasshoppers can encounter highly variable plant quality at multiple scales (among seasons, years and available host species) (Bernays and Chapman 1994), mechanisms to compensate for suboptimal diets should exist (Yang and Joern 1994a, Zanotto et al. 1996, Joern and Behmer 1997, Zanotto et al. 1997, Gouveia et al. 2000). Given that *M.*

*femurrubrum* is a mixed-feeder, this species may be highly adapted to environmental variability in forage quality to meet their stoichiometric needs. The skewed diet choices (p14c28 and p28c14) may not have been sufficiently imbalanced from nutritional needs to prevent compensation, and differences may not have been sufficient in that all diets provided adequate amounts of protein (ca. 2 and 4% respectively). If so, grasshoppers could compensate for moderately insufficient nutrient availability and not exhibit decreased performance.

As expected, insect growth rates increased with temperature across all populations. Insect feeding rates (Lactin and Johnson 1995), digestive and developmental rates are strongly temperature dependent (Yang and Joern 1994c, Woods and Kingsolver 1999, Terblanche et al. 2007, Irlich et al. 2009). As long as upper suitable limits are not exceeded, higher ambient temperature generally allows longer durations of potential feeding time throughout the day, but is accompanied by increased energy expenditures. At 25 °C individuals from all populations appear limited by thermal conditions and developed at similar rates regardless of source population (Fig. 2.8). The lowest temperature treatment used is about 10-13 °C below the estimated optimal temperature of most grasshoppers, but still within the range that allows individuals to achieve optimal body temperatures through thermoregulation. As temperatures increased, growth rates among populations became more variable. At 37 °C, the highest temperature treatment in this study, there was a “U” shaped pattern across all populations, suggesting a possible trade-off between time needed to complete the life cycle and location. In general, ectotherms from northern populations must capitalize on favorable conditions during the short growing season to reach reproductive age and deposit their eggs before temperatures drop (Conover and Present 1990, Dingle and Mousseau 1994). Because northern populations must adapt to limited season length, perhaps by compensating with increased nutritional efficiency,

reduced body size seems to be a reasonable trade-off (Fielding and Defoliart 2007).

Grasshoppers are income breeders, and egg production comes from resources gathered as adults (Branson 2004). Shorter time to complete the life cycle can be accompanied by an overall decreased body size in insects, while leaving time to secure resources and reproduce (Taylor and Karban 1986).

Grasshopper SMR also increased with temperature across all populations in the common garden experiment as expected. Whole organism SMR was greatest at lower latitudes and decreased as one moved north, reflecting differences in body size. This pattern was not maintained for mass-specific SMR, and individuals for all populations exhibited similar mass-specific SMR. This indicates that populations along this latitudinal temperature gradient have not evolved an alternate strategy in respect to basic metabolic processes. Because size in *M. femurrubrum* follows the Converse Bergmann's Rule, the strong latitudinal cline for whole organism SMR appears driven by body size and all populations along the latitudinal gradient operate with the same basic SMR responses. A temperature response is still present but the latitudinal response of mass-specific SMR is lost and differences in energy needs and metabolism largely reflects body size differences.

Behavioral and physiological mechanisms facilitate thermoregulation, giving insects the ability to elevate body temperatures above suboptimal ambient air temperatures (Kemp 1986, Lactin and Johnson 1997, O'Neill and Rolston 2007, Angilletta 2009). Field data from this study were fit to the curve developed by Kemp (1986), which regressed ambient air temperature and internal body temperatures for grasshoppers in the field in Montana for one population over the full range of available temperatures. Body temperatures were observed to be greater than ambient temperatures in all populations studied here, suggesting that all individuals sampled



were able to alter internal body temperatures behaviorally and were not simply matching ambient conditions (Kemp 1986, O'Neill and Rolston 2007, Angilletta 2009). High variability in temperature differences between air and grasshopper body temperatures existed, but the distribution of mean body temperatures and mean ambient air temperatures followed a similar pattern spanning the entire sampled gradient. In drawing conclusions, however, one must remember that this study was not designed with the specific intent to test the relative abilities among grasshopper populations to thermoregulate across a latitudinal gradient *per se* over the complete range of operational temperatures. However, it was clear that all populations could modify internal body temperatures in a similar fashion as they all fit relevant portions of the same standard model of thermoregulation (Fig. 2.6). No population level advantage or clinal pattern in thermoregulatory ability by behavioral means was detected. One must also remember that field sampling was conducted within limited thermal conditions in the field, and all populations were not compared for the full range of ambient temperature conditions. I conclude that there were no adaptive differences in thermoregulation capabilities among populations that would modify our understanding of the role of temperature to the body size cline in *M. femurrubrum*.

Among possible adaptive responses to variable environments, cuticle color in insects influences mate attraction, crypsis (Tauber et al. 1986, Cease et al. 2010) and thermoregulation (Fielding and Defoliart 2005, Clusella Trullas et al. 2007). Insects can use adaptive coloration (melanism) to decrease solar reflectance in cooler environments (Forsman 1999, Clusella Trullas et al. 2007, Alho et al. 2010, Bear et al. 2010, Frentiu and Chenoweth 2010). Darkened cuticle color modulates individual energy balance by influencing absorption of radiant solar energy in environments where the ambient temperature alone may be otherwise sub-optimal

(Wigglesworth 1957, Joern 1981, Dingle et al. 1990, Fielding and Defoliart 2005, Clusella Trullas et al. 2007). Insect survival is often dependent on maintaining effective body temperatures in operational ranges because of the critical role body temperature plays in regulating and maintaining biological processes (Kemp 1986, Clusella Trullas et al. 2007). Our spectral analysis data were consistent with the hypothesis that these grasshopper populations could combat cooler temperature profiles by darkening their cuticle color and thus using potential solar radiation more efficiently in northern populations. All physical (structural and/or chemical) properties of the cuticle were assumed to be similar and were not analyzed otherwise. Although, Lactin and Johnson (1996) used black and white image analysis to determine cuticle reflectance at different observed postures and showed no significant contribution of body orientation to energy interception ( $p > 0.7$ ), Gunn (1942) suggested that many factors, including surface structural components of the cuticle may aid in raising or lowering body temperature.

## Conclusions

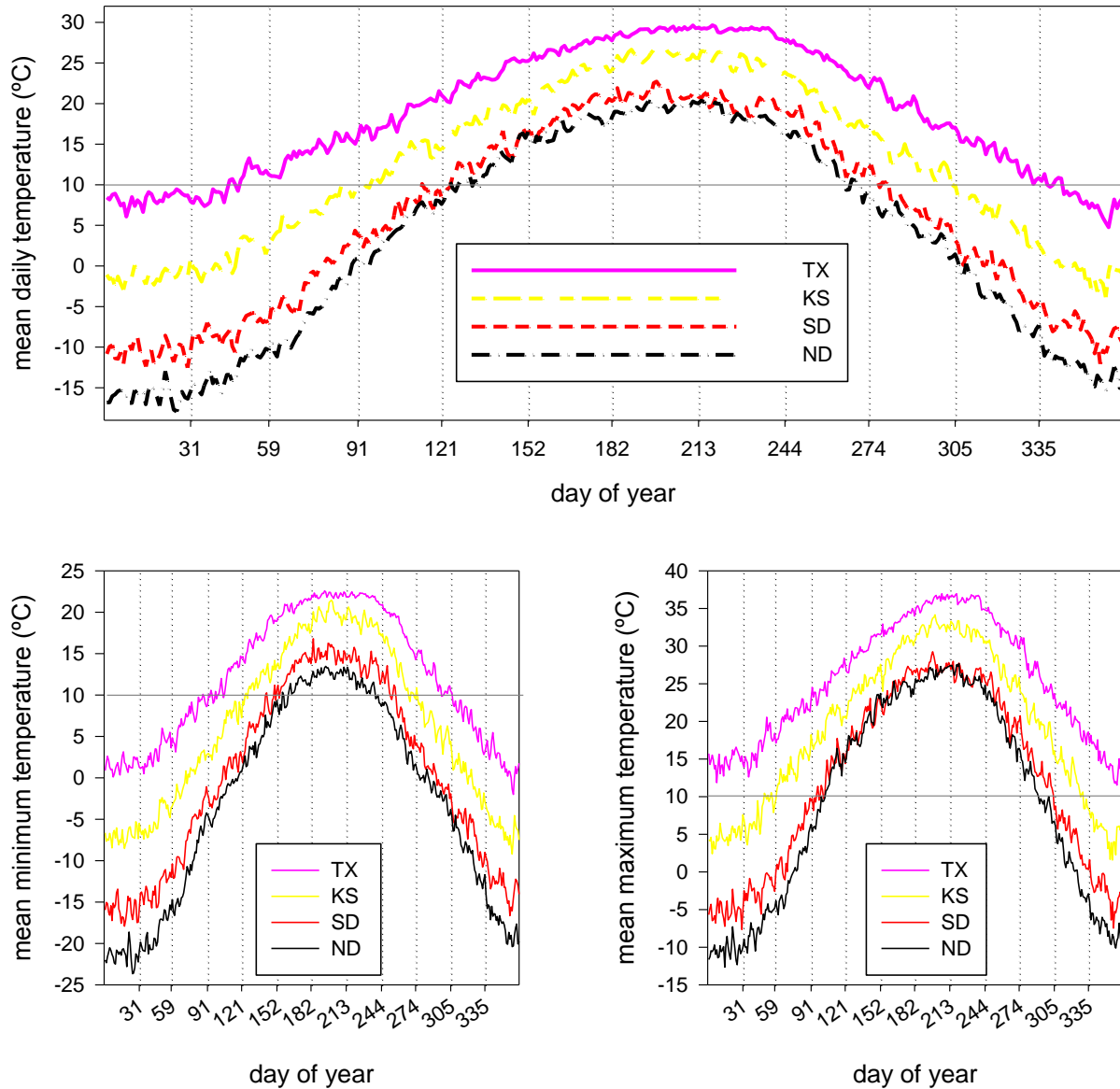
Overall, a clear latitudinal pattern in body size was observed for *M. femurrubrum* following the Converse Bergmann's Rule, and results from common garden trials suggest that ecotypic variation for grasshopper body size exists. Diet quality was not a significant contributing factor for grasshopper performance, although individuals from these grasshopper populations may be able to use compensatory post-ingestive processing capabilities that may be responsible for the observed phenotypic clines. Differences in physiological mechanisms measured here that might influence resulting body sizes among individuals from different populations varied along the latitudinal gradient. Faster growth rates resulted as temperature treatments increased but populations at the extreme ends of the gradient developed faster in all temperature treatments. Similarly, SMR is largely a mass dependent relationship, and when

mass-specific SMR was considered, latitudinal patterns suggested that all populations were essentially achieving similar temperature-dependent metabolic activity on a per gram basis. These results emphasize the importance of insect body size and also the inextricable role of temperature driving performance in *M. femurrubrum* along this latitudinal gradient.

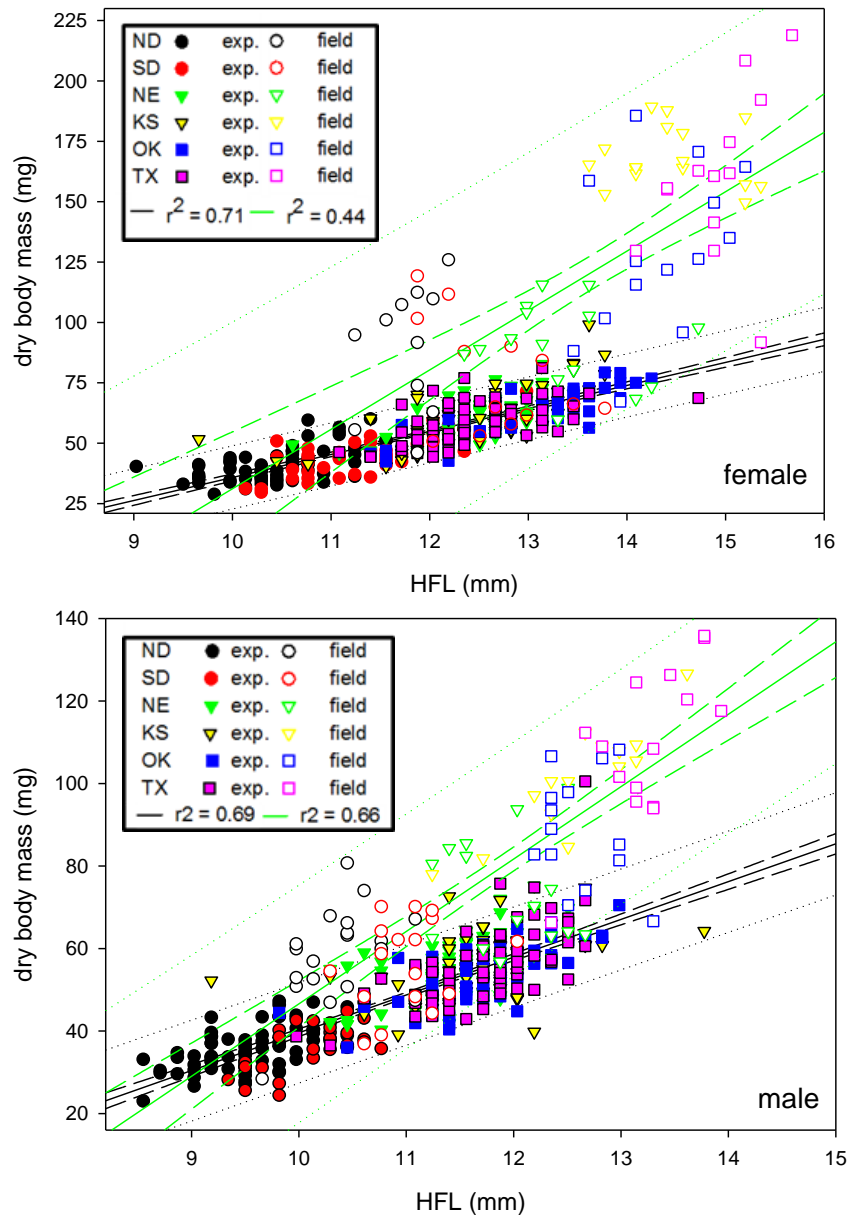
### **Acknowledgements**

I thank Dr. J. Nippert for laboratory space, D. Vu and Drs. R. Welti and K. Schrick for growth chamber sharing, T. Grace for discussions about spectral analysis and E. Maghirang and Dr. F. Dowell at the USDA-ARS Center for Grain & Animal Health Research for allowing me to invade their lab and use their equipment for spectral analysis, and C. Shannon for statistical consulting. This work was funded in-part by the Division of Biology, NSF LTER, and the Institute for Grassland Studies and NSF DEB-0456522. This is publication XX-XXX-X from the Kansas Agricultural Experiment Station.

## Figures and Tables

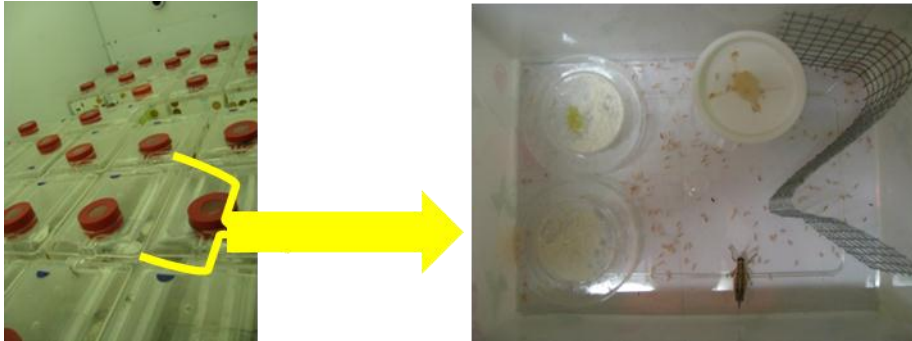


**Figure 2.1 Annual thermal profile (daily mean °C) for grasshopper sample population collection sites. The x-axis represents the days of the year in Julian calendar days with tick marks denoting the end of each month. Horizontal solid gray line at 10°C represents the value used to calculate degree days for agricultural monoculture crops and grasshoppers. These reference lines emphasize the season length differences experienced across latitude. From top to bottom: State – line color combinations are as follow; TX – solid black; OK – dotted red; KS – short dash green; NE – short dash-dot-dot yellow; SD – medium dash blue; ND – short dash-dot pink.**

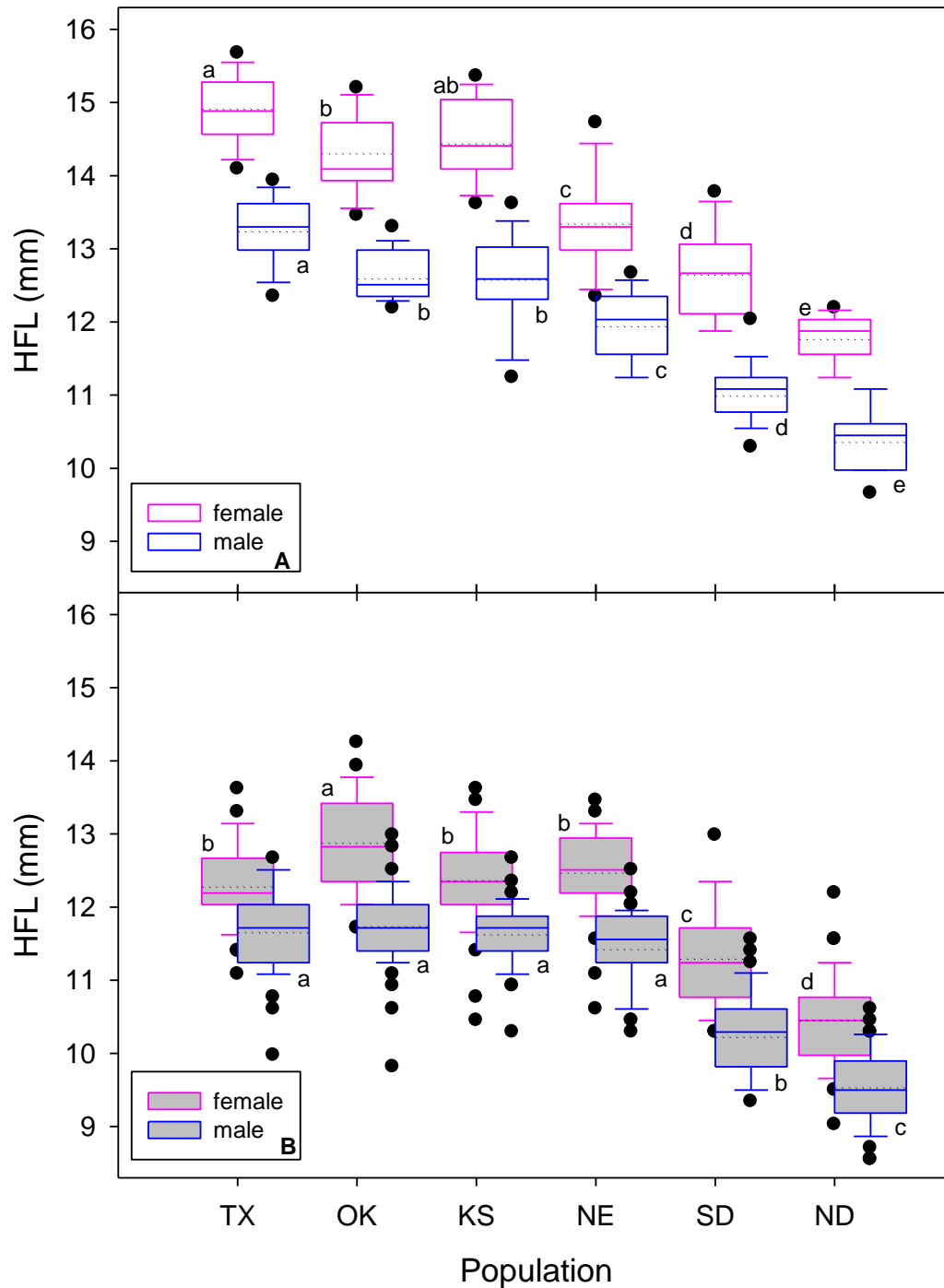


**Figure 2.2 Adult grasshopper hind femur length (HFL) plotted against dry body mass for females (top) and males (bottom) collected from the field (open symbols) and the laboratory feeding experiments (filled symbols). HFL is used as a proxy for overall body size in adult grasshoppers because of its demonstrated strong correlation with dry body mass. In the laboratory experiments 70% ( $\text{♀ } r^2 = 0.71$ ;  $\text{♂ } r^2 = 0.69$ ) of the variability in body mass as predicted by HFL in adults grasshoppers could be explained. Among field**

populations, 44% (females) and 66% (males) of the variation in body size was explained by HFL.

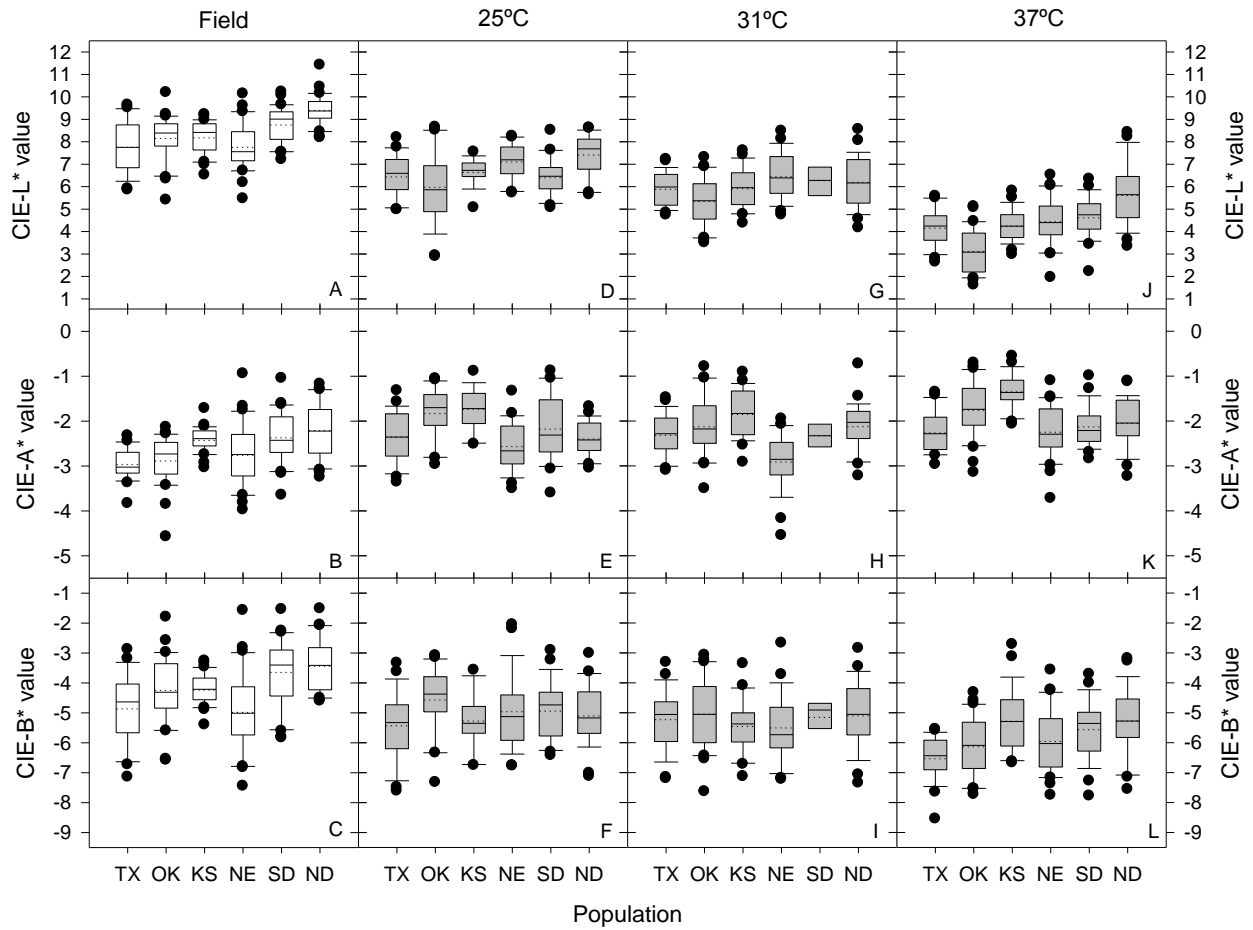


**Figure 2.3 Individual grasshopper cage used for feeding trials housed in temperature controlled chambers. Each cage contained converted petri dish diet dishes, a water cup and a perch.**



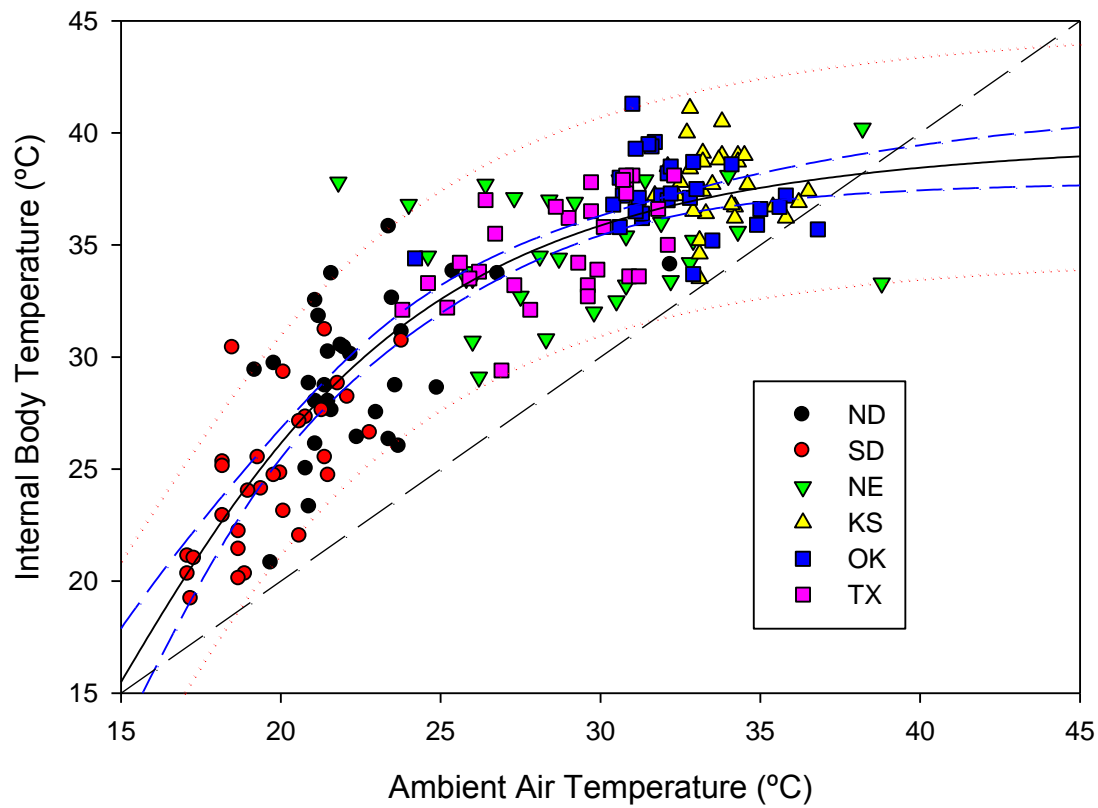
**Figure 2.4 Grasshopper body size as hind femur length (HFL; in millimeters) for all individuals from the field (A, open boxes) and feeding experiments (B, shaded boxes). In all boxes: dotted line represents the population mean while the solid line represents the median. Female HFL boxes are outlined in pink (top, A&B) while male HFL boxes are**

outlined in blue (bottom, A&B) for each population. Fisher HSD groupings among populations are denoted by letters at the upper left (female) or lower right (male) of boxes.

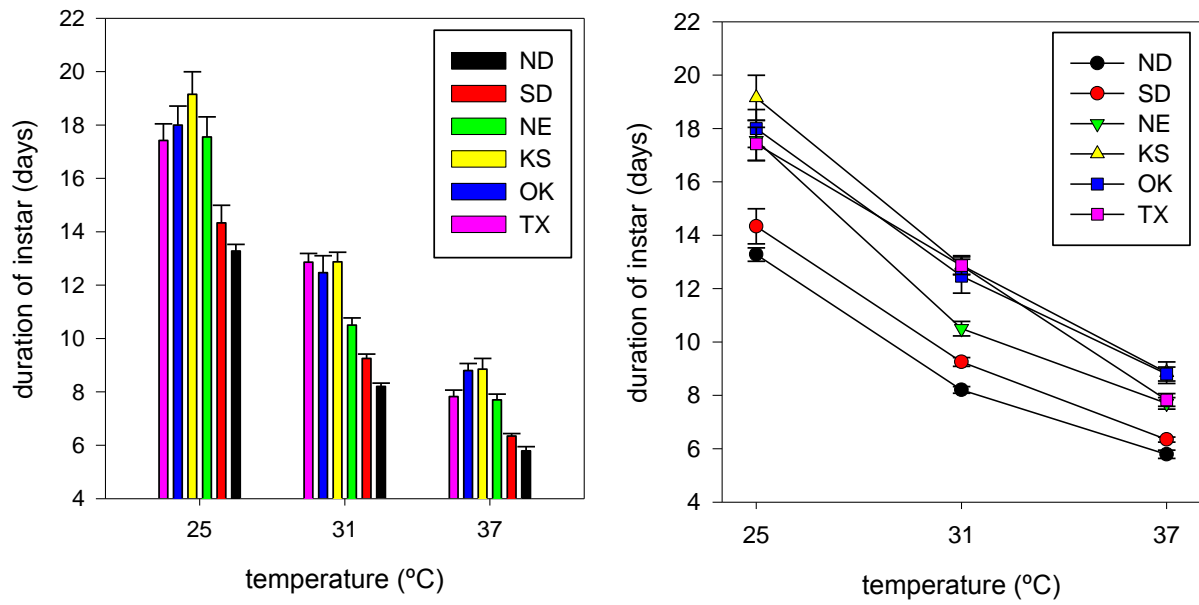


**Figure 2.5 Grasshopper body color. Pronotums from the field (unfilled boxes; panels A, B, C) and all feeding trials (filled boxes; 25°C: D, E, F; 31°C: G, H, I; 37°C: J, K, L) were quantified to CIE-L\*A\*B\* color space values using a spectrophotometer. Differences in hue (L\*) were used to represent a measure of darkness (panels A, D, G, J); 0 (absolute black) to 100 (absolute white).**

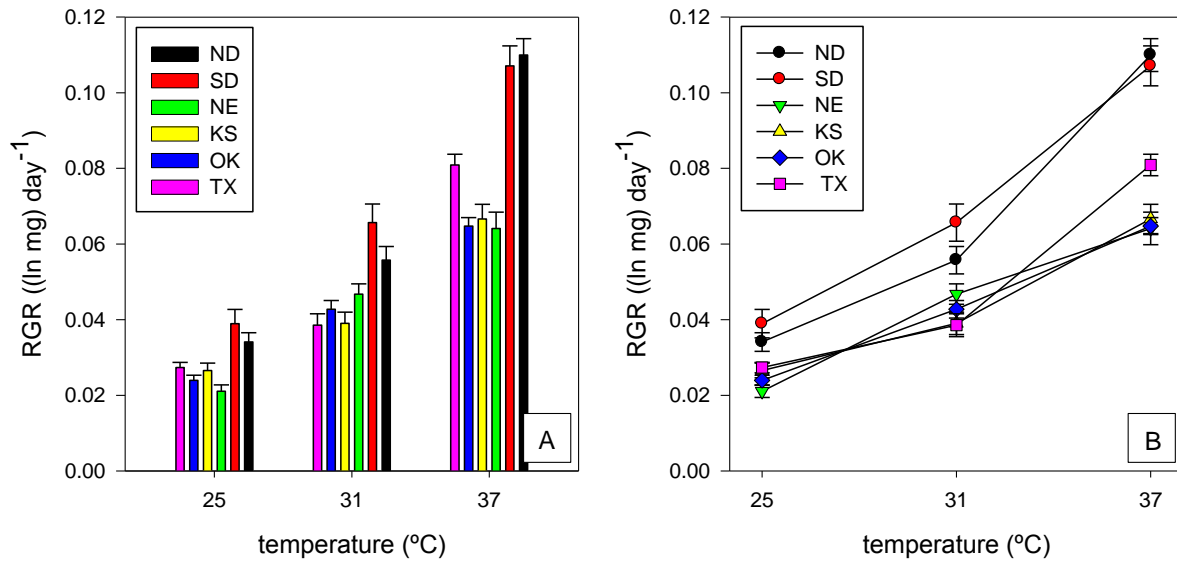




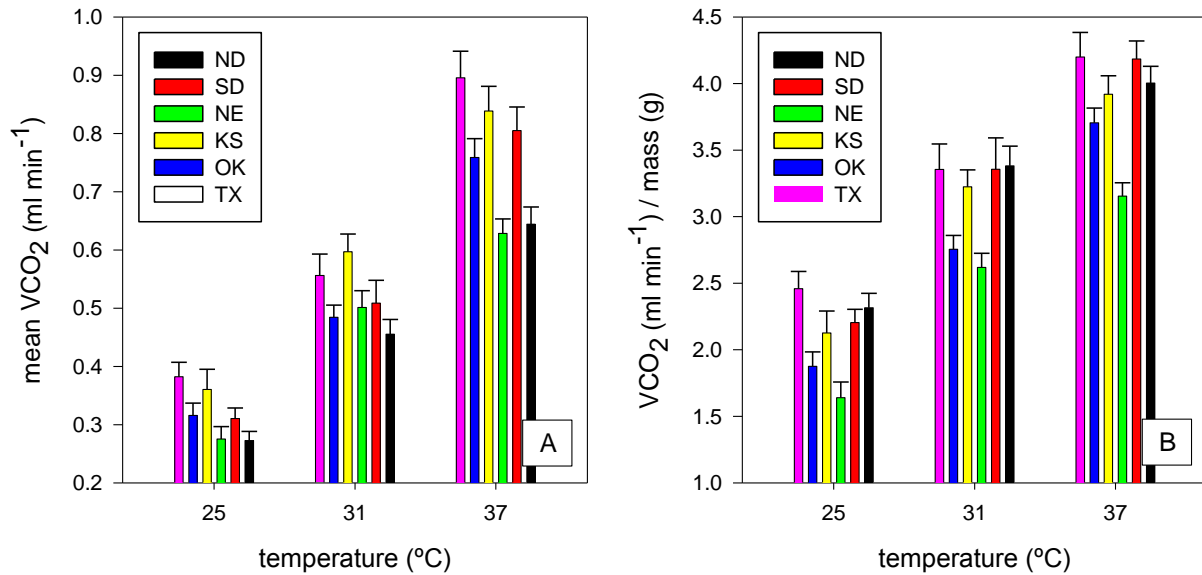
**Figure 2.6 Grasshopper internal body temperature across a latitudinal gradient. Data were fit with a logistic function using ambient air temperature as a predictor of internal body temperature ( $r^2 = 0.7853$ ,  $p < 0.0001$ ) (see Kemp 1986). The long dashed black line through the origin serves as a linearity reference. The solid black line extending through the data points is the best fit line bracketed by the 95% confidence interval band (dashed blue line) followed by the 95% predictor band (dotted red line).**



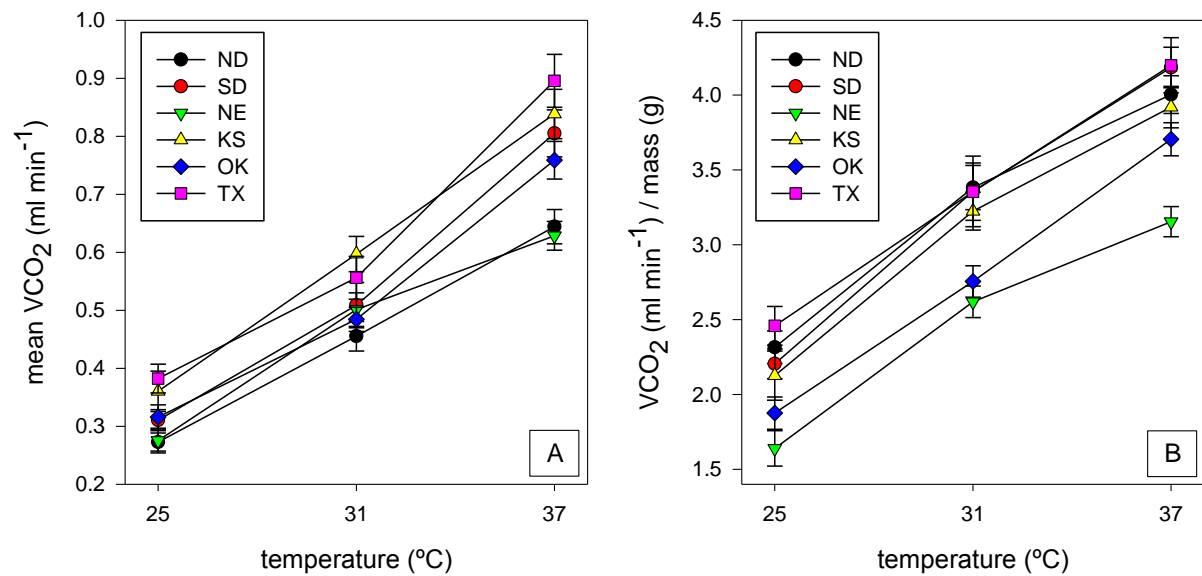
**Figure 2.7 Mean developmental rate for individuals from laboratory feeding trials. Development rate is defined as the duration (in days) of the fifth juvenile instar. Values are displayed as mean  $\pm$  S.E. in panel A and in the reaction norms displayed in panel B.**



**Figure 2.8 Mean relative growth rate (RGR) for individuals from laboratory feeding trials. Relative growth rate is calculated as  $\ln(\text{dry mass gain (mg)})$  over the duration of development time (days). Values are displayed as mean  $\pm$  S.E. in bars in panel A and in the reaction norms displayed in panel B.**



**Figure 2.9 Mean standard metabolic rate for all laboratory fed grasshoppers. Values are displayed as mean  $\pm$  S.E. Whole organism standard metabolic rate is displayed in panel A. Mass specific standard metabolic rate is displayed in panel B.**



**Figure 2.10 Reaction norms for mean standard metabolic rate for all laboratory fed grasshoppers. Values are displayed as mean  $\pm$  S.E. Whole organism standard metabolic rate is displayed in panel A. Mass specific standard metabolic rate is displayed in panel B.**

**Table 2.1 Feeding trial artificial diet treatment combinations. Each grasshopper was given a chemically defined cellulose based diet that varied in protein : carbohydrate (p%c%) concentration. There were four diet treatments per temperature treatment; single, non-choice diet (s) or a choice between two imbalanced but complimentary diets (c indicates combination). Populations in bold (ND, KS, TX) represent like single and choice diet combinations (ACE). SD grasshoppers were given the (ACE) non-choice but (BD) choice diet treatments. NE and OK populations were fed only the narrower breadth of diet options (BCD) for single and non-choice treatments.**

<i>Population</i>		<i>protein : carbohydrate</i>				
Site	latitude	p14c28 (A)	p14c21 (B)	p21c21 (C )	p21c14 (D)	p28c14 (E)
<b>ND</b>	<b>48.9629</b>	s, c		s		s, c
SD	44.2386	s	c	s	c	s
NE	41.4671		s, c	s	s, c	
<b>KS</b>	<b>39.0800</b>	s, c		s		s, c
OK	36.7528		s, c	s	s, c	
<b>TX</b>	<b>31.9329</b>	s, c		s		s, c

**Table 2.2 Average grasshopper body size for field and laboratory experiment populations. Hind femur length (HFL) is measured to the nearest millimeter (S.E.) and dry mass is measured to the nearest milligram (S.E.). Female body size was significantly larger than male body size across all populations. The percent by which females are larger than males is denoted after each trait category.**

Population		HFL (mm)		(%)	dry mass (mg)		(%)
		♀	♂	♀ > ♂	♀	♂	♀ > ♂
field	ND	11.77 (0.09)	10.37 (0.09)	11.95	89.18 (7.83)	58.53 (2.64)	34.37
	SD	12.65 (0.16)	11.00 (0.10)	13.08	77.95 (6.31)	56.45 (2.60)	27.58
	NE	13.35 (0.17)	11.95 (0.12)	10.51	90.74 (4.35)	73.79 (3.36)	18.68
	KS	14.45 (0.14)	12.59 (0.16)	12.88	168.43 (3.11)	100.71 (3.51)	40.21
	OK	14.31 (0.14)	12.60 (0.09)	11.95	128.67 (8.48)	87.17 (3.65)	32.25
	TX	14.92 (0.11)	13.24 (0.11)	11.21	160.20 (8.80)	109.36 (4.76)	31.74
	<b>average</b>	<b>13.66 (0.13)</b>	<b>11.87 (0.11)</b>	<b>11.93</b>	<b>121.27 (4.70)</b>	<b>79.33 (2.48)</b>	<b>34.58</b>
experiment	ND	10.48 (0.08)	9.54 (0.06)	8.97	41.15 (0.96)	34.96 (0.75)	15.04
	SD	11.29 (0.11)	10.20 (0.09)	9.71	44.84 (1.39)	37.52 (1.28)	16.32
	NE	12.47 (0.07)	11.42 (0.06)	8.41	60.71 (1.09)	54.64 (1.00)	10.00
	KS	12.39 (0.11)	11.63 (0.09)	6.12	59.98 (1.63)	55.80 (1.13)	6.97
	OK	12.86 (0.10)	11.68 (0.07)	9.16	61.00 (1.31)	53.22 (0.84)	12.75
	TX	12.34 (0.08)	11.65 (0.07)	5.57	57.25 (1.13)	54.95 (1.03)	4.02
	<b>average</b>	<b>11.97 (0.06)</b>	<b>11.10 (0.06)</b>	<b>7.27</b>	<b>54.31 (0.69)</b>	<b>49.37 (0.62)</b>	<b>9.10</b>

# **Chapter 3 - Shifting food preference and variation in processing efficiency by the red-legged grasshopper (*Melanoplus femurrubrum*) along a latitudinal gradient**

## **Abstract**

Insect herbivores are challenged to meet dietary needs in heterogeneous environments. Temperature and food quality are two factors that vary predictably along latitude resulting in insect populations that are adapted to specific habitat qualities. Herbivores can alter feeding strategy, selection preferences, post-ingestive processing, or some combination of these mechanisms to compensate for variation presented in their local environment. I conducted a common garden experiment using the generalist grasshopper, *Melanoplus femurrubrum* (DeGreer), from six populations along a broad latitudinal gradient to examine feeding and post-ingestive efficiency variation in response to temperature and food quality manipulations. I expected to find a difference in diet selection preferences based on population adaptations to environmental constraints resulting from latitudinal location. Specifically, I predicted southern populations to prefer carbohydrate-biased diets while northern populations would select protein-biased diets due to energetic and nutrient-limitations, driven by temperature. When restricted to diets that did not match preferred ratios, I expected to find compensatory feeding adjustments and I also anticipated northern populations to experience greatest digestive processing efficiencies, overall, due to season length constraints. My hypothesis for feeding preference was supported by a directional shift in diet preference from carbohydrate to protein-biased diet preference in southern to northern populations. Grasshoppers also modified consumption when restricted to a diet that did not match the self selected ratio. Grasshopper digestive efficiencies

had variable responses, however, with Northern populations achieving greatest efficiencies inconsistently. Overall, *M. femurrubrum* demonstrated variation in post-ingestive efficiencies but strong geographic diet selection preferences were observed.

## **Introduction**

Insect herbivores must satisfy metabolic and dietary needs in response to new challenges encountered as environments vary. For example, heterogeneous nutritional landscapes in natural plant communities can require insects to alter foraging and digestive responses to meet dietary needs (Bernays and Chapman 1994, Chown and Nicolson 2004, Karasov and del Rio 2007). Individuals may compensate by increasing consumption when resources are sub-optimal (Yang and Joern 1994b, Woods and Kingsolver 1999), or they may balance diets to reach optimal combinations of nutrients needed for development and growth by selecting different combinations of plants depending on nutritional content and needs (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993, Behmer et al. 2001, Raubenheimer and Simpson 2003, 2004, Berner et al. 2005, Lee 2007, Fielding and Defoliart 2008, Jonas and Joern 2008). Alternately, insects may mediate limiting environments by modifying physiological capabilities such as through increasing investment in internal structures (e.g., gut length) or by increased digestive processing efficiencies (Yang and Joern 1994a, Yang and Joern 1994c, Woods and Kingsolver 1999, Fielding and Defoliart 2007, Mitra and Flynn 2007).

All organisms face stoichiometric constraints that must be met to support survival, development and reproduction (Sternner and Elser 2002). While ‘ecological stoichiometry’ emphasizes elemental limitation and ‘nutritional ecology’ focuses on resource limitation in the form of biological molecules such as protein or carbohydrates (Elser et al. 2000, Sternner and Elser 2002, Raubenheimer and Simpson 2004, Moe et al. 2005, Raubenheimer et al. 2009), the



general conclusions are similar. Individuals are often challenged by the limited availability of key dietary components within their environments: a limited nutrient (generally nitrogen (N) or phosphorus (P)) or a source of energy (indexed as C from carbohydrate lipids and protein). Insect nutritional ecology evaluates consumer performance responses (development, growth, resource processing and efficiency) when faced with varying concentrations of these components in their diet, and much research has focused specifically on protein (p) and carbohydrate (c) (Scriber and Slansky 1981, Simpson and Raubenheimer 1993, Raubenheimer and Simpson 1994, Zanotto et al. 1994, Chambers et al. 1995, Simpson and Raubenheimer 2000, 2001, Raubenheimer and Simpson 2003, 2004, Behmer 2009). Protein and amino acids contribute to new biomass during development and maintaining tissue throughout the organism's lifetime (Mattson 1980, Chapman 1998, Karasov and del Rio 2007), and can also be used as an energy source under carbohydrate-limited conditions (Slansky and Feeny 1977, Scriber and Slansky 1981, Zanotto et al. 1993, Yang and Joern 1994b).

Successful nutrient acquisition and allocation underlies variable life history responses observed by organisms experiencing different environments (Tauber et al. 1986, Dingle et al. 1990, Harrison and Fewell 1995, Boggs 2009). Variation in environmental and seasonal constraints such as temperature and food of variable quality and quantity influence dietary preference (Waldbauer and Friedman 1991, Ayres and Scriber 1994, Goranson et al. 2004, Fielding and Defoliart 2007), consumption patterns and performance of insect herbivores at the local level (Yang and Joern 1994b, Chambers et al. 1995, Atkinson and Sibly 1997, Arnett and Gotelli 1999b, Berner et al. 2005, Fielding and Defoliart 2007). Given the fluctuating nature of dietary needs (Joern and Behmer 1997, 1998, Abrams and Schmitz 1999, Kingsolver and Srygley 2000, Branson 2004, Nespolo et al. 2008), one must consider possible post-ingestive and

behavioral mechanisms that allow organisms to maintain stoichiometric homeostasis in variable environments as they allocate resources to life history demands (Yang and Joern 1994c, Kingsolver and Srygley 2000, Logan et al. 2004, Fleming and Bateman 2007, Lee 2007, Melvin et al. 2007). Dietary needs can also vary according to developmental stage (Chapman 1990, 1998, Simpson et al. 2002), body size (Yang and Joern 1994a, Boswell et al. 2008), state of predation risk and/or competition for resources (Beckerman et al. 1997, Schmitz et al. 1997, Beckerman 2002, Branson 2010), or in response to parasitism/pathogens (Virtanen and Neuvonen 1999, Behmer and Joern 2008, Thompson and Redak 2008, Behmer 2009, Crawford et al. 2010, Cotter et al. 2011). Using a broad ranging grasshopper species and a common-garden experimental design, I was able to identify ecotypic responses in overall body size and whole organism standard metabolic rates, which varied by population along a latitudinal gradient (Chapter 2). Highly variable metabolic rates by individuals were observed that may help individuals mitigate the effects of variability in food quality encountered in natural environments although no mass-specific metabolic rates were observed among populations. This chapter investigates the possible role of variable food acquisition and digestion to moderate variable nutritional needs under a range of environments.

Organisms may adjust feeding to combat environmental variability by modifying amounts ingested or by selecting diets that differ in protein and carbohydrate (p:c, respectively). The geometric approach developed by Raubenheimer and Simpson (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993, Raubenheimer and Simpson 1994, 1996, Simpson and Raubenheimer 2000, Raubenheimer and Simpson 2003, 2004) effectively compares two diet components (p:c) in two dimensional space to document consumer performance. Defined laboratory diets can restrict the feeding organism to a fixed ratio of p:c in which only the amount

of intake can be adjusted. Outcomes can be plotted as fixed-ratio-isolines or “rails” within the plots; each rail corresponds to a fixed p:c ratio. The organism’s preferred ingestion target to balance nutrients (the intake target ratio) can be determined using defined diets combined with choice experiments that permit the organism to feed freely on two imbalanced but complementary diets. Choice trials thus allow the organism to indicate the preferred p:c ratio based on actual amounts of alternate diets that are eaten. The intake target that is selected by the consumer over a set time span (e.g., one complete developmental stage) represents the balance of energy and nutrients that is assumed to optimize growth and development (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993, Zanotto et al. 1994, Chambers et al. 1995, Zanotto et al. 1997, Behmer et al. 2002, Simpson et al. 2002, Clissold et al. 2009). Generally, grasshoppers and other taxa modulate intake in a relatively balanced fashion in terms of protein and carbohydrate (Behmer and Joern 1993, Simpson and Raubenheimer 1993, Behmer et al. 2001, Raubenheimer and Simpson 2003, Behmer and Joern 2008, Behmer 2009). Also, grasshoppers often employ what is known as the rule of compromise (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993, Behmer 2009), which leads to selection of a relatively balanced intake target by feeding on the available imbalanced but complementary diets in a way that minimizes over-feeding solely on any one component.

Post-ingestive mechanisms associated with digestion and nutrient assimilation may also counteract the effects of environmental heterogeneity. While animals in temperature-limited environments may be restricted by the amount of time that there is to forage or digest food (Yang and Joern 1994c, Harrison and Fewell 1995, Mitra and Flynn 2007), organisms may counter such time constraints by optimizing feeding and processing efficiency (Fielding and Defoliart 2007). Heterogeneity in plant quality (Bernays and Chapman 1994), which may vary latitudinally

(Reich and Oleksyn 2004, Salgado and Pennings 2005), can also intensify the need for adaptive post-ingestive efficiencies (Fielding and Defoliart 2008). However, few studies have investigated these nutritional processing efficiencies across multiple environments over broad geographic scales. Such investigation will enhance understanding of potential adaptive processing mechanisms that broad ranging species employ to combat latitudinal environmental heterogeneity.

In this chapter I focus on resource acquisition and post-ingestive processing mechanisms that the red-legged grasshopper, *Melanoplus femurrubrum* (DeGreer), uses to regulate growth and development. Grasshopper body size decreases with increasing latitude (Bidau and Marti 2007a, Fielding and Defoliart 2007, see also this thesis Chapter 2) and total consumption typically depends on body size (Yang and Joern 1994a, Hahn 2005, Fielding and Defoliart 2007, Boswell et al. 2008). While larger individuals consume more resources on average, cumulative consumption over a fixed developmental stage will decrease with latitude as individuals become smaller. Here, one expects individuals from populations at higher latitudes to increase consumption rates to support rapid development to compensate for the reduced duration of the season. Differences in nutritional intake target ratios are expected to vary according to latitude for two reasons. First, rapid development and smaller body size might limit the overall quantity of resources that an individual can process, so that individuals from higher latitude might be more sensitive to resource quality and thus more selective in diet choices, especially protein. Second, overall plant quality might be expected to decrease with increasing environmental temperatures so that individuals from more southern sites will encounter lower quality food. Plant development is also sensitive to general temperature patterns and growing season length; plant quality has been documented to increase with latitude (Barbehenn et al. 2004, Reich and

Oleksyn 2004). Northern populations may be locally adapted to require higher quality resources (e.g., higher protein content) while southern populations may be better able to process poorer quality resources (Barbehenn et al. 2004). Latitudinal variation in nutritional processing efficiency is expected in either case (Conover and Present 1990, Bale et al. 2002, Fielding and Defoliart 2007). Specifically, I predict an increase in nutritional efficiency with latitude. Again, rapid development should benefit and perhaps result from increased processing efficiency. Given that all organisms used in this study have specific energetic and nutritional needs, we used artificial diets in feeding experiments to determine: (1) if there are differences in the amount and rate of resources ingested, (2) if nutrient intake target/requirements differed, and (3) if grasshopper efficiencies differed among populations across latitude.

## **Methods**

### ***Study Organism***

Grasshoppers (Orthoptera: Acrididae) are insect herbivores that are active aboveground during the growing season when food resources are abundant and thermal conditions are within operational physiological ranges. The red-legged grasshopper *M. femurrubrum* is a medium-sized (9.7-15.7 mm adult hind femur length, unpublished data this study), generalist-feeding species that is distributed throughout most of North America (Bailey and Mukerji 1977, Vickery and McE. Kevan 1986, Thompson 1992, Capinera et al. 2004). Individuals generally overwinter as eggs throughout most of the geographic range, hatch in mid-spring, becoming adults from late-spring/early-summer, where they complete the life cycle as long as conditions are suitable (Bailey and Mukerji 1977). This species is univoltine in the northern portion of its distribution, sometimes requires two growing seasons to complete one life cycle at the most northern range

limit, and can have multiple generations in warmer, southern populations with longer, warmer growing season (Dingle et al. 1990, Dingle and Mousseau 1994). This species is common along roadsides and disturbed and recovering areas such as old field habitats (Beckerman 2002).

*Melanoplus femurrubrum* can be considered an economically important crop pest, although populations only infrequently reach sufficient densities to affect total crop yields (Vickery and McE. Kevan 1986).

### ***Collection sites***

Grasshoppers were collected using sweep nets from six locations at similar longitude ( $97.00 \pm 0.13^\circ\text{W}$ ; mean  $\pm$  SE) across the Central Plains of the United States, spanning roughly 2,000 kilometers and  $18^\circ$  of latitude from Texas northward to North Dakota (Fig 1.3, Table 1.1). All collection sites were near well-travelled roadsides. Three sites were directly adjacent to agricultural fields (ND, SD, NE), one site was a prairie restoration (SD) and one was a grazed natural prairie (KS) site. Two sites were tracts of land that were fallow; one site was a vacant lot (OK) and one was public recreation or hunting land (TX).

Climate profiles constructed using National Climatic Data Center (NCDC) datasets for TX, SD and ND as shown in Figure 2.2. Konza Prairie Biological Station climate data (LTER dataset AWE01) was used for the KS location (Table 1.1). Only incomplete NCDC datasets were available for OK and NE locations so climate profiles were not constructed for these two locations.

### ***Grasshopper rearing***

Adult *M. femurrubrum* collected from the six study sites (Table 1.1) were maintained in the laboratory at Kansas State University (Manhattan, KS, USA) in separate populations to

collect eggs for experiments (methods - Behmer and Joern 2008). Caged populations were kept at ca. 30°C using incandescent light bulbs positioned next to the cage (14:10, L:D) with ample water and food (organic romaine lettuce leaves, wheat seedlings and a mixture of wheat bran and Brewer's yeast). Small containers (egg cups) filled with autoclaved sand treated with distilled water and a 0.05% methylparaben anti-fungal solution were provided for oviposition. Egg cups were removed from colony cages and stored at room temperature for approximately 4-6 weeks before placing them into a refrigerator (ca. 4-5°C) for a minimum of 16 weeks to break diapause. Egg cups were then placed in empty sterilized rearing cages at room temperature in front of a light source (as described above), kept moist with distilled water, and monitored daily for hatching. First-generation nymphs from each location were maintained in colony conditions through the completion of the fourth instar.

### *Feeding trials*

Immediately upon molting to fifth instar, feeding trials were conducted in three temperature controlled chambers (1 Conviron (Canada) and 2 Percival (Percival Scientific, Inc., IA, US) chambers) set to 14:10 L:D conditions and constant day/night temperatures of 25, 31 or 37°C. These temperatures were selected because they bracket (approximately) the typical minimum and maximum body temperatures that these populations encounter in the field during the growing season. These temperatures are also commonly used in other whole organism physiological studies (Zanotto et al. 1997, Gouveia et al. 2000, Miller et al. 2009, Terblanche and Chown 2010). Grasshoppers were individually weighed within < 12 hours of molt and placed in isolated clear plastic containers (18.5x13x9cm) with a 4 cm screen vent on the lid (Fig. 2.3). Opaque contact paper applied to cage sides prevented individuals from receiving any visual cues from outside their cages, which may influence feeding behavior. Each cage housed a metal

perch, a 40 mL water cup with cotton wick, and one 4cm petri dish containing a randomly assigned food source (artificial or natural, control diet). Dry artificial diets differing in protein (p) and carbohydrate (c) concentrations known to support grasshopper development and survival (Simpson and Raubenheimer 1993, Behmer and Joern 2008) were used. Two sets of diet were used in these trials (Table 2.1). Diets with combined protein and carbohydrate ratios of 42% of total diet (ACE; p14c28 =A, p21c21=C, p28c14=E) were used to rear grasshoppers from ND, SD, KS and TX populations. Diets with combined protein and carbohydrate ratios of 35% of total diet (BCD; p14c21=B, p21c14=D) and a 42% balanced diet (C) were used to rear grasshoppers from NE and OK populations. Diet combinations were chosen to bracket variability that may be encountered in the field, but these nutrient ratios were still sufficiently balanced to support survival (Slansky and Feeny 1977, Simpson and Raubenheimer 1993). Each individual was fed *ad libitum*, but not excessively, one of four designated artificial diets (Schmidt and Reese 1986). To maintain an accurate measure, artificial diets were weighed at the start of each feeding trial and replaced with a fresh, pre-weighed dish containing diet (of the same type) every third day until an individual molted or died. Grasshopper cages were monitored daily for the duration of the instar until the end of the feeding trial. Grasshoppers were stored frozen.

### ***Elemental analysis of grasshopper tissue***

All frozen grasshoppers were lyophilized to constant dry mass before body tissue was ground into a fine powder with a mortar and pestle using liquid nitrogen. All individual crops were checked for food content, which was removed prior to grinding. Frass was ground into fine powder using a ball grinder. Aliquots of grasshopper tissue and frass samples were weighed (~1.5 mg), packed into pressed tin capsules (Costech Analytical Technologies, Inc., Valencia,



CA) and analyzed for carbon and nitrogen content using a NC Soil Analyzer (FlashEA 1112 Series, ThermoScientific, USA).

### ***Nutritional Indices***

Nutritional indices have been developed (Waldbauer 1968, Slansky and Feeny 1977) to estimate assimilation and diet processing efficiency. I determined feeding efficiency for individuals using laboratory feeding trial data to calculate approximate digestibility (AD), efficiency of conversion of ingestion (ECI), efficiency of conversion of digestion (ECD), and nitrogen use efficiency (NUE; Table 3.1).

### ***Statistical Analysis***

I used analysis of variance (ANOVA) to analyze overall consumption (total amount consumed, mg) and rate of consumption (mg/day) with population, temperature and diet as fixed effects. Analysis of covariance (ANCOVA) was used to analyze mass specific consumption (main effect: consumption; covariate: initial mass), rate of consumption (m.e.: consumption; cov.: duration of instar) and all ratio-based measures of digestive efficiency, including assimilation of digestion (AD; m.e.: assimilation; cov.: consumption), efficiency of conversion of digestion (ECD; m.e.: dry mass gain; cov.: assimilation), and efficiency of conversion of ingestion (ECI; m.e.: dry mass gain; cov.: consumption) (Raubenheimer and Simpson 1992, Horton and Redak 1993). Paired t-tests were used to determine differences in diet consumption for choice diet treatments. Necessary transformations were performed to satisfy normality requirements. All statistical analysis was performed using SAS (v9.2, Cary, NC).

Statistical analyses were performed for all data from laboratory feeding trials, however, analysis was separated based on diet treatments which were inconsistent due to experimenter error. Separate analysis was performed for the four populations (ND, SD, KS, TX) that used

diets A, C, and E in single non-choice feeding trials and three populations (ND, KS, TX) that used diets A and E in paired choice diets. The same analyses were run separately for the two populations (NE and OK) that used diets B, C, and D in single non-choice diets and for the three populations (SD, NE, OK) with choice combination of diets B and D (see Table 2.1). I will report results for populations fed ACE diet combinations in this thesis chapter.

## **Results**

### ***Consumption***

Whole organism consumption. Consumption (Fig. 3.1 A) generally decreased with latitude with significant differences in variation by population ( $F_{3,262} = 39.68$ ,  $P = <0.0001$ ) and temperature ( $F_{2,262} = 17.04$ ,  $P = <0.0001$ ; covariate: initial mass,  $F_{1,262} = 11.62$ ,  $P = 0.0008$ ) treatments with no significant interaction terms across the 4 populations reared on diets ACE. On average, populations from the central part of the collection gradient had highest overall consumption (Fig. 3.1 B).

Mass-specific consumption. Diet type did not alter significantly the total consumption. However, mass-specific consumption (Fig. 3.2) differed significantly for all three fixed treatment effects (population:  $F_{3,262} = 37.61$ ,  $P = <0.0001$ ; temperature:  $F_{2,262} = 17.15$ ,  $P = <0.0001$ ; diet:  $F_{2,262} = 3.04$ ,  $P = 0.0497$ ; covariate, initial mass:  $F_{1,262} = 112.28$ ,  $P = <0.0001$ ). Diet was only marginally significant across the 4 populations reared on diets A, C, and E. Population\*diet ( $F_{6,262} = 2.01$ ,  $P = 0.0648$ ) and temperature\*diet ( $F_{4,262} = 2.13$ ,  $P = 0.0778$ ) interactions were only marginally insignificant

### ***Consumption Rate***

Whole organism. Total consumption rate (Fig. 3.3) varied by population ( $F_{3,264} = 5.21$ ,  $P = 0.0016$ ) and temperature ( $F_{2,264} = 493.26$ ,  $P = <0.0001$ ) and also showed a population\*temperature ( $F_{6,264} = 2.96$ ,  $P = 0.0081$ ) interaction. Whole organism consumption rate increased as temperature increased for all populations. However, at low temperatures (25, 31°) all populations consumed food at approximately similar rates ( $\text{mg d}^{-1}$ ). There was a latitudinal trend with the highest consumption rates observed in individuals from populations collected at lower latitudes with decreasing consumption rates as latitude increased (Fig. 3.3).

Mass specific. Significant variation by population ( $F_{3,263} = 4.44$ ,  $P = 0.0046$ ) and temperature ( $F_{2,263} = 580.34$ ,  $P = <0.0001$ ) as well as a population\*temperature interaction effect ( $F_{6,263} = 2.11$ ,  $P = 0.0527$ ; covariate: initial mass  $F_{1,263} = 53.66$ ,  $P = <0.0001$ ) were detected for mass specific consumption rate. Individuals from northern populations consumed more diet per gram body mass than all other populations (Fig. 3.4).

### ***Diet preference***

Most populations along the latitudinal gradient selected balanced diets when given the opportunity to choose between two complementary but imbalanced diets (in terms of proportion protein: carbohydrate). However, there was a shift from more carbohydrate-biased ratios at lower latitudes to protein-biased ratios at higher latitude (Fig. 3.5). ND individuals selected protein-biased diets (25°:  $t = -4.21$ ,  $d.f. = 8$ ,  $P = 0.0029$ ; 31°:  $t = -2.97$ ,  $d.f. = 8$ ,  $P = 0.0179$ ; 37°:  $t = -3.35$ ,  $d.f. = 9$ ,  $P = 0.0085$ ) while TX populations selected carbohydrate-biased diets (25°:  $t = 1.67$ ,  $d.f. = 8$ ,  $P = 0.1341$ ; 31°:  $t = 2.99$ ,  $d.f. = 9$ ,  $P = 0.0151$ ; 37°:  $t = 1.05$ ,  $d.f. = 9$ ,  $P = 0.3192$ ) across all temperatures treatments. Individuals from centrally located populations selected balanced diets. There was a shift in preference as temperature increased for KS populations from

almost completely balanced to a preference for a more carbohydrate biased diet (25°:  $t = -0.41$ ,  $d.f. = 8$ ,  $P = 0.6915$ ; 31°:  $t = 1.86$ ,  $d.f. = 9$ ,  $P = 0.0953$ ; 37°:  $t = 2.37$ ,  $d.f. = 7$ ,  $P = 0.0498$ ).

### ***Post-Ingestive Mechanisms***

#### ***Approximate Digestibility (AD)***

Approximate digestibility (AD, also known as assimilation efficiency) is a measure of diet retention (Table 3.1). Overall, AD (Fig. 3.6) varied significantly by population ( $F_{3,262} = 4.55$ ,  $P = 0.0040$ ), temperature ( $F_{2,262} = 5.79$ ,  $P = 0.0013$ ) and diet ( $F_{2,262} = 43.91$ ,  $P = <0.0001$ ) with a significant population\*diet ( $F_{6,262} = 3.89$ ,  $P = 0.0010$ ; covariate: consumption,  $F_{1,262} = 914.21$ ,  $P = <0.0001$ ) interaction for populations reared on ACE diets. All populations tended to exhibit the greatest AD at the intermediate temperature treatment (31°C) for all diet types. Northern populations consistently achieved higher AD's at moderate temperatures while there was more variation at the lowest (25°C) and highest (37°C) temperature treatments.

#### ***Efficiency of Conversion of Ingestion (ECI)***

Efficiency of conversion of ingestion (ECI, also known as gross growth efficiency) is the measure of biomass gain given ingestion (Table 3.1). Overall, populations reared on diets A, C, and E exhibited population ( $F_{3,263} = 6.87$ ,  $P = 0.0002$ ), temperature ( $F_{2,263} = 20.05$ ,  $P = <0.0001$ ), diet ( $F_{2,263} = 4.20$ ,  $P = 0.0161$ ) and population\*temperature interaction effects ( $F_{6,263} = 2.16$ ,  $P = 0.0477$ ; covariate: consumption:  $F_{1,263} = 139.10$ ,  $P = <0.0001$ ) for ECI (Fig 3.7). When individuals were reared on either balanced or low protein diets, ECI increased with temperature. Populations from the extremes of the gradient (ND and TX) generally showed the highest ECIs, especially under the highest temperature treatment (37°).

### ***Efficiency of Conversion of Digestion (ECD)***

Efficiency of conversion of digestion (ECD, also known as net growth efficiency) is the measure of biomass gained given actual assimilation (Table 3.1). Overall, population ( $F_{3,263} = 6.73$ ,  $P = 0.0002$ ) and temperature ( $F_{2,263} = 18.63$ ,  $P = <0.0001$ ; covariate: assimilation:  $F_{1,263} = 118.91$ ,  $P = <0.0001$ ) effects are driving the responses for ECD (Fig. 3.8). There is no effect of diet or any interaction effects for the 4 populations reared on A, C, or E diets. Increased temperatures enhanced ECD for all populations. Texas grasshoppers demonstrated higher ECD than Northern populations even though Northern populations often exhibited highest AD.

### ***Nitrogen use efficiency (NUE)***

Nitrogen use efficiency (NUE) of converted N is measured as the amount of N biomass gained given the amount of N ingested (Table 3.1) and was greatest overall for grasshoppers reared on diets containing the lowest protein ratios (Fig. 3.9). There were significant population ( $F_{3,262} = 3.44$ ,  $P = 0.0175$ ), temperature ( $F_{2,262} = 8.19$ ,  $P = 0.0004$ ), and diet ( $F_{2,262} = 31.08$ ,  $P = <0.0001$ ) effects as well as population\*temperature ( $F_{6,262} = 2.36$ ,  $P = 0.0310$ ) and population\*diet ( $F_{6,262} = 4.20$ ,  $P = 0.0005$ ) interaction effects (covariate, mg N consumed:  $F_{1,262} = 113.04$ ,  $P = <0.0001$ ). Although overall population trends in NUE are variable across treatments, grasshoppers from populations at the extremes of the sample gradient tended to achieve highest NUE compared to populations at central locations.

## **Discussion**

Populations of broad ranging species should exhibit ecotypes enabling them to match performance to local environmental conditions that differ geographically because organisms adapt to the specific local environment (Endler 1977). Latitudinal gradients provide classic

opportunities to study such responses (Gaston et al. 2009, Chown and Gaston 2010), where clinal patterns become quite informative about the importance of critical environmental drivers to population performance. Latitudinal variation in temperature and growing season length provide a natural environmental gradient in which to study thermal adaptation(s) or physiological responses to temperature for organisms that can exist in a variety of thermal environments (Sibly and Atkinson 1994, Arnett and Gotelli 1999a, Blackburn et al. 1999, Addo-Bediako et al. 2000, Walters and Hassall 2006, Bidau and Marti 2008, Terblanche et al. 2009). I compared responses to temperature for feeding and digestion among populations from along a 2000 km latitudinal gradient because temperature is a major driver for most behavioral and physiological processes in insects, including those that dictate food acquisition (Harrison and Fewell 1995, Chapman 1998, Karasov and del Rio 2007, Angilletta 2009, Kingsolver 2009). I predicted that because energetic demands increase with temperature and increased metabolic rates, grasshoppers from warmer, lower latitudes should adjust consumption and assimilation capacities by shifting intake to foods that better meet their dietary/energetic demands by increasing intake targeting carbohydrate (energy) rich dietary ratios. However, all populations should not respond in the same fashion. Northern ecotypes experience cooler environments overall and are limited by growing season length, requiring them to process foods rapidly and efficiently. In this sense, I predicted a shift from C (energy)-limitation at the southern, high temperature end of the gradient to N (protein)-limitation in northern populations, reflected as shifts in preferred diet in choice tests to meet these expectations. Because a common-garden experimental design was used in this study, significant differences in population responses indicate local adaptation.

### ***Diet Selection and Ingestion***

Grasshoppers are not indiscriminant feeders and readily exhibit diet selection with respect to host plant species (Bailey and Mukerji 1976, 1977, Boutton et al. 1978, Joern 1979, Chapman 1990, Raubenheimer and Simpson 2003, Barbehenn et al. 2004, Berner et al. 2005, Clissold 2007, Fielding and Defoliart 2008, Jonas and Joern 2008) or nutritional characteristics of available diets (Waldbauer and Friedman 1991, Behmer and Joern 1994, Bernays and Chapman 1994, Lee 2007, Miller et al. 2009, Franzke et al. 2010). Seasonal constraints on growth and/or developmental rate or variation in locally available food quality should result in adaptive shifts in protein:carbohydrate nutritional intake targets to meet dietary needs. In this study, experimentally determined intake targets for diet selection varied by geographic location (Fig 3.5). Individuals from ND always selected diet ratios that were protein-biased under all temperature treatments. Because season length in ND is short (Fig 1.1), targeting a protein-biased diet allows these individuals to meet protein requirements necessary for growth and development within the time allotted for development and successful reproduction. This also requires fewer feeding events than would be needed if individuals selected lower p:c intake ratios. Individuals from North and South Dakota are also generally smaller as adults (Fig 2.4), further facilitating the ability to reach adult maturity over a shorter growing season (Chapter 2). Developing rapidly may thereby prove advantageous for populations experiencing short growing seasons (Dingle 1986, Dingle et al. 1990, Fielding and Defoliart 2007, Whitman 2008) and allow escape from other mortality risks such as size-selective predation (Belovsky and Slade 1993, Oedekoven and Joern 1998, Fielding and Defoliart 2007).

Conversely, individuals from the southern end of the sample gradient (TX) selected carbohydrate-biased diets (Fig. 3.5). The southern climate has higher average temperatures and vastly longer growing seasons than the north. Higher daily temperatures coupled to larger body

size in southern populations leads to increased energetic demands. It is unlikely that populations in TX experience thermal limitations to time available for foraging on a daily basis, but the potential to experience energetic limitations and restricted foraging because of generally high temperatures and resulting increased metabolic rate could be great (Harrison and Fewell 1995). In all temperature treatments used here, grasshoppers from the southern-most location in TX selected an intake target that was carbohydrate-biased when given the choice of imbalanced, complementary diets (Fig 3.5). Grasshoppers from a geographically central population in KS selected nearly equivalent p:c diets at low temperatures, but then shifted to a preference for carbohydrate-biased diet as temperatures increased, suggestive of energy limitation. Although, it is difficult to pinpoint a geographically definitive transition zone of preference from protein-biased to carbohydrate-biased diet along the gradient, the shift toward more carbohydrate rich preference with increasing temperature in KS populations suggests that central populations along this gradient might be transitional. In a previous study, *M. femurrubrum* individuals from western Nebraska preferred a slightly protein biased diet (p:c, 1.7:1) at ~30°C (Behmer and Joern 2008). While those grasshoppers from a cooler site in western NE exhibited a protein-biased choice selection that was more pronounced than any preference results for populations along my gradient, the results were consistently protein-biased north of KS.

Body size can dictate the quantity and perhaps quality of resources needed for energetic and maintenance costs (Fielding and Defoliart 2007, Boswell et al. 2008, Ho et al. 2010). For example, a general latitudinal trend existed where consumption decreased with increasing latitude (Fig. 3.1), larger individuals ate more overall. However, overall mass-specific consumption differed among populations but did not follow the same latitudinal trends as body size. Populations along this gradient had similar mass-specific metabolic rates (Chapter 2). But,



even though these populations have similar metabolic demands on a per gram basis, nutritional demands vary latitudinally. When populations are restricted to diets that offer p:c ratios that are imbalanced, but not in the direction that matches preference, a mass-specific compensatory feeding response can be observed among populations across different diet treatments (Fig. 3.2). For example, when examining mass-specific consumption for individuals restricted to carbohydrate-biased (p14:c28) diets, an increasing latitudinal trend can be observed across all increasing temperature treatments where TX populations consume less diet (per gram body mass) than ND populations. As diet treatments become more protein-biased (p28:c14), the clinal pattern transitions to an decreasing latitudinal trend where individuals from TX are now consuming more per gram than ND populations are. These populations are employing a compensatory feeding to adjust diet intake to meet preferred targeted diet ratios. This shifting compensatory feeding response is also consistent with the shift in diet ratio preference that resulted in choice feeding experiments along the gradient.

Although, body size followed the inverse Bergmann's Rule with decreasing body size as latitude increased (see chapter 2), gross differences in mass-specific consumption rate are reduced across lower latitude populations along the gradient, but high latitude populations consumed at significantly greater rates across temperatures (Fig 3.4).

### ***Internal compensation for unbalanced diets***

A consumer may cope with low quality food through compensation by adjusting post-ingestive processing at one or more levels in digestion and assimilation. Yang and Joern (1994a) demonstrated that individuals of the grasshopper *Melanoplus differentialis* that fed on poor quality (low nitrogen, high carbohydrate) diet developed a larger gut and exhibited increased gut passage rate of digesta when compared to individuals fed higher quality diet. The modification

of a larger gut allows greater quantities of food to reside in the gut for longer periods while nutrients are extracted, while faster gut passage rate allows individuals to replace partially digested food with decreased nutrient concentrations with new food of high levels. Such mechanisms are beneficial in environments with poor quality forage.

Overall approximate diet digestibility was highest for low protein and balanced diets (Slansky and Feeny 1977, Waldbauer and Friedman 1991, Fielding and Defoliart 2008) with AD peaking at 31°C for most populations (Fig. 3.6). Miller and colleagues (2009) found similar results for peak nutritional efficiencies in locusts at moderate (32 °C) temperature compared to feeding done at lower (26 °C) and higher (38 °C) temperatures. They hypothesized that while locusts preferred body temperatures around 38 °C, it may be metabolically more costly to operate in ambient temperatures close to the preferred body temperature because heat production during active metabolism could push individuals past operational body temperatures, thus reducing overall nutritional efficiencies. There were no strong latitudinal trends for AD in my study, but there was a slight trend for increasing AD with latitude in some treatments, and populations at the extremes of the gradient (ND and TX) both demonstrated the highest AD in other treatments. However, the trend for increasing AD with latitude for the high protein diet might also suggest that these northern populations were perhaps more efficient at processing higher quality food than were southern populations. Gross growth efficiency (ECI) generally increased with temperature for low and balanced protein diets but varied by population and diet without regard to any distinct latitudinal trends. In many cases, populations from the extremes of the gradient, like in AD, exhibited the greatest gross growth efficiencies although decreasing and increasing trends were seen under different treatment combinations. While certain populations might be better able to process food into grasshopper biomass, the direction of this trend is difficult to

interpret. A similar trend for increasing net growth efficiency (ECD) with increasing temperature was also present with a significant population\*temperature interaction. ECD of populations from the extremes of the gradient increased the most dramatically at highest temperature treatment, regardless of diet type. These populations also developed most rapidly and may be more sensitive to temperature effects but perhaps for different reasons. This suggests that northern populations typically experience fewer days with very high temperatures and thus respond quickly when conditions (or temperatures) are optimal. Populations in the south likely experience high temperature conditions regularly for the majority of their lifetime, leading to standard increases in metabolic state for all temperatures.

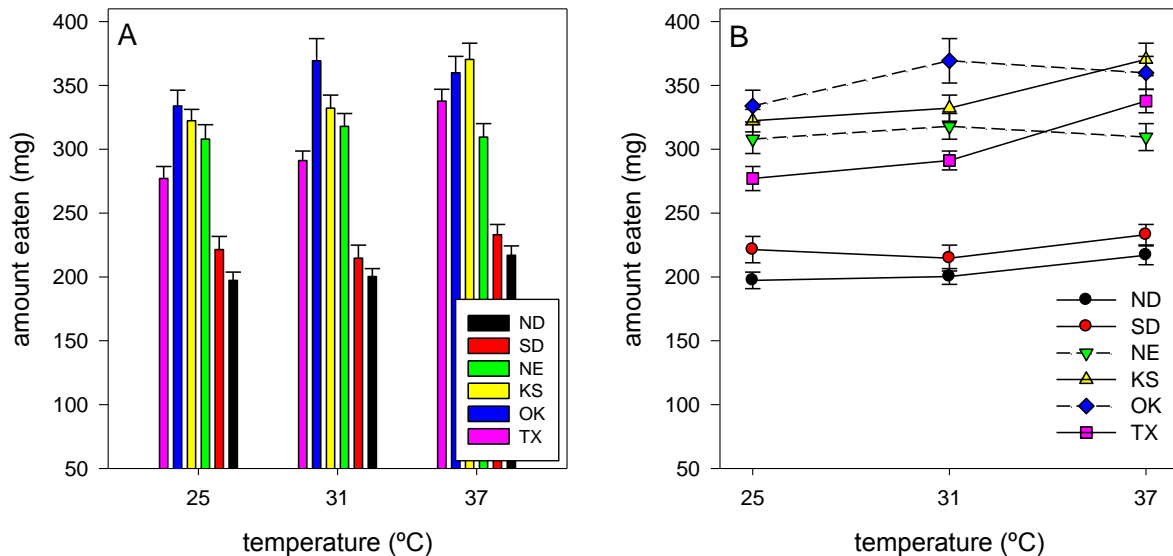
## Conclusions

Grasshoppers employ multiple behavioral and post-ingestive mechanisms to compensate for environmental heterogeneity for food quality and temperature. *Melanoplus femurrubrum* populations demonstrated a latitudinal shift in diet preference from carbohydrate-biased diets in low latitudes to protein-biased diets in northern latitudes. Grasshoppers adjusted consumption and consumption rates in response to temperature induced energetic demands by increasing consumption and also used compensatory feeding adjustments when restricted to unbalanced diets that did not match their selected diet preference. Nutritional efficiencies were variable across latitude but AD and NUE were optimal for low protein high carbohydrate diet ratios. Efficiency was also highest for moderate temperature treatments for AD and sometimes for NUE but ECI and ECD increased with temperature. Overall, the ability to compensate for unbalanced diets during digestion and using compensatory and selective feeding allows populations to tolerate a variety of environments and likely contributes to the broad distribution of *M. femurrubrum*.

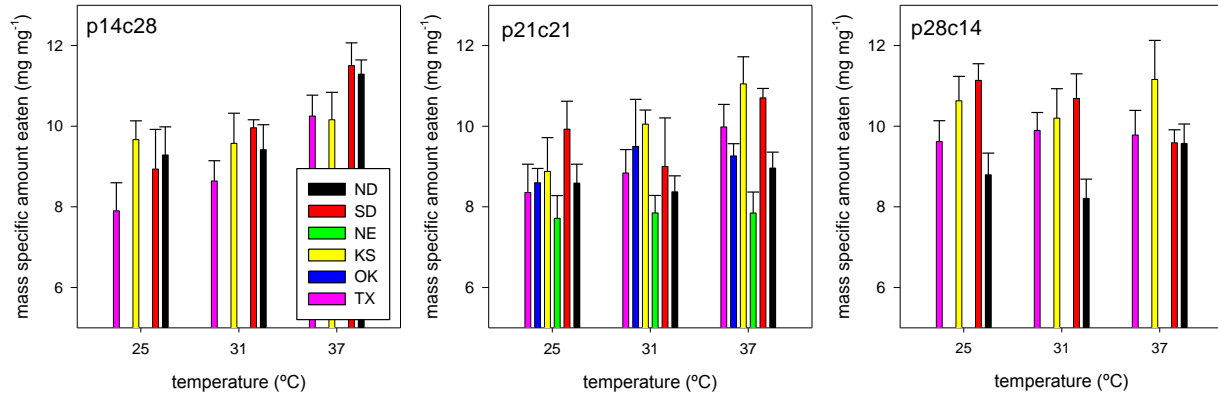
## Acknowledgements

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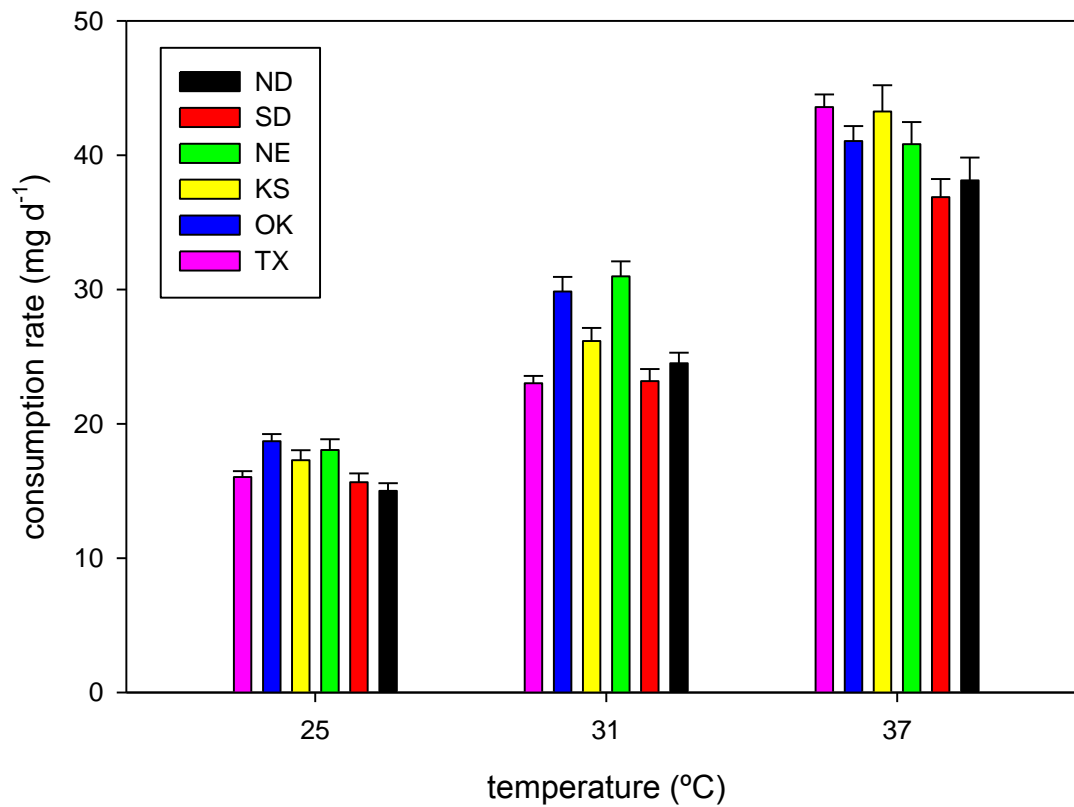
## Figures and Tables



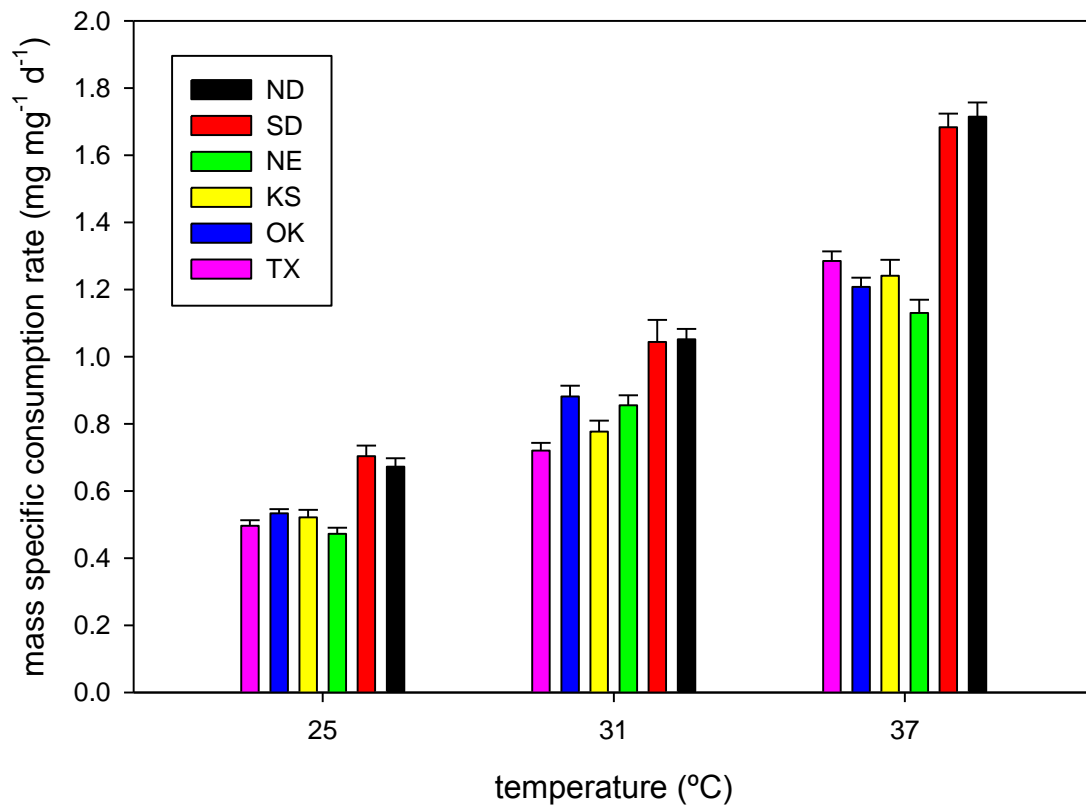
**Figure 3.1** Total consumption (mg) over the duration of the Vth juvenile instar for individuals from laboratory feeding trials. All diet treatments were pooled by population. Bars (A) represent population means ( $\pm$  SE) arranged in increasing latitude (L to R; TX in pink, OK in blue, KS in yellow, NE in green, SD in red, ND in black) by temperature treatment. Reaction norms are presented with symbol and color combinations corresponding to population means ( $\pm$  SE).



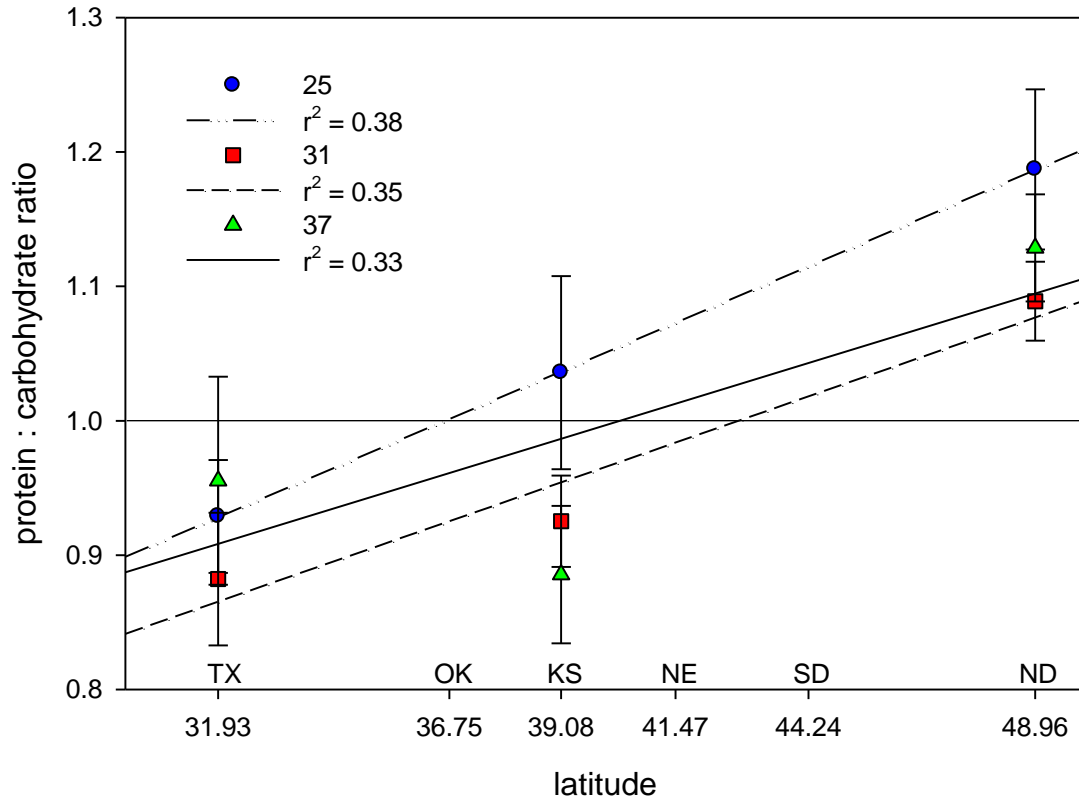
**Figure 3.2 Mass-specific consumption ( $\text{mg mg}^{-1}$ ) over the duration of the Vth juvenile instar for individuals from laboratory feeding trials. Bars represent population means ( $\pm$  SE) arranged in increasing latitude (L to R; TX in pink, OK in blue, KS in yellow, NE in green, SD in red, ND in black) by temperature treatment. Plots are separated by diet treatment (L to R; p14:c28, p21:c21; p28:c14).**



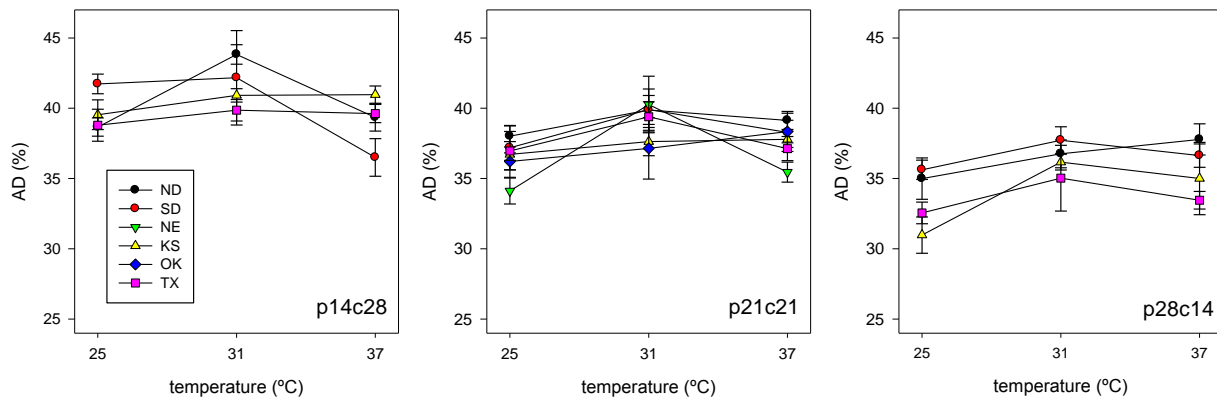
**Figure 3.3** Whole organism consumption rate (mg d<sup>-1</sup>) for individuals from laboratory feeding trials. Bars represent population means ( $\pm$  SE) arranged in increasing latitude (L to R; TX in pink, OK in blue, KS in yellow, NE in green, SD in red, ND in black) by temperature treatment. Treatments have been pooled by diet treatment.



**Figure 3.4** Mass-specific consumption rate ( $\text{mg mg}^{-1} \text{d}^{-1}$ ) for individuals from laboratory feeding trials. Bars represent population means ( $\pm$  SE) arranged in increasing latitude (L to R; TX in pink, OK in blue, KS in yellow, NE in green, SD in red, ND in black) by temperature treatment. Treatments have been pooled by diet treatment.



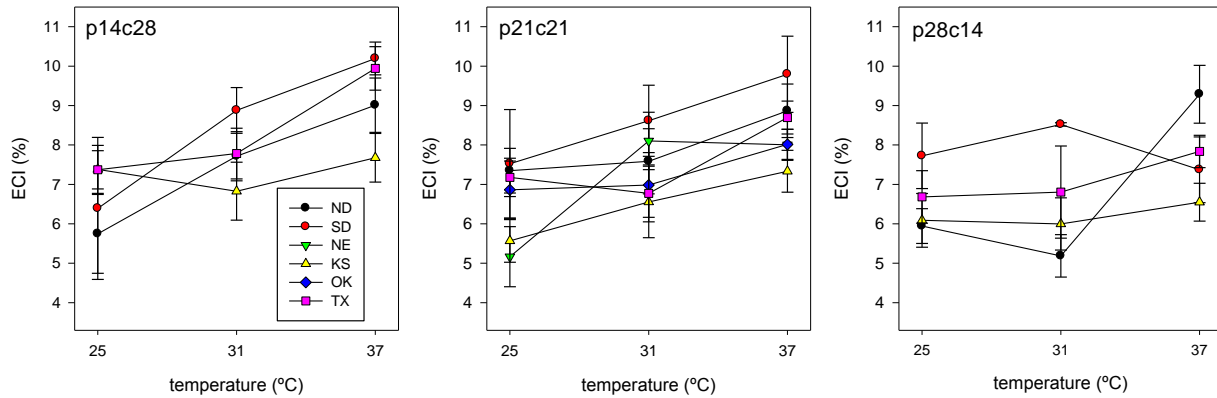
**Figure 3.5** Diet preference (p:c ratio (mg N: mg C consumed); mean  $\pm$  SE) for individuals from laboratory choice feeding trials. Populations are plotted by temperature treatment (25°C - blue circle; 31°C – red square; 37°C – green triangle) along latitude.



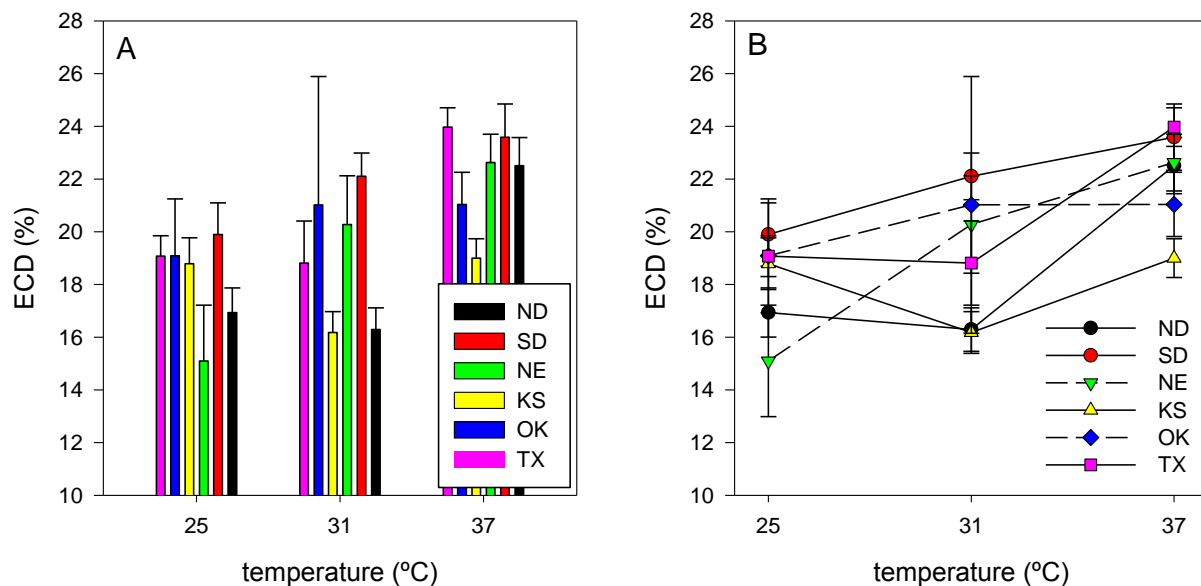
**Figure 3.6** Approximate digestibility (AD as a percentage) for individuals from laboratory feeding trials. Reaction norms are presented with symbol and color combinations



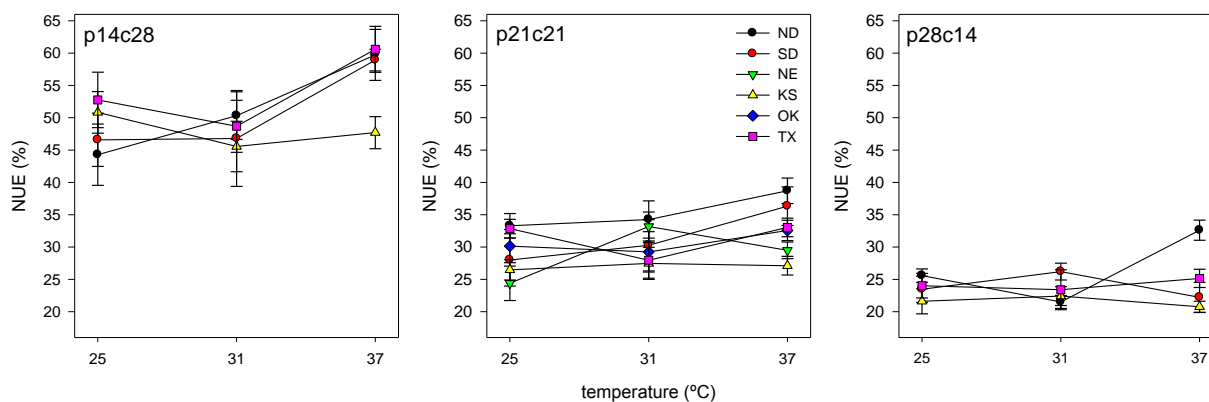
corresponding to population means  $\pm$  SE (symbols: ND – black circle; SD – red circle; NE green inverted triangle; KS – yellow triangle; OK – blue diamond; TX – pink square). Plots are separated by diet treatment (L to R; p14:c28, p21:c21; p28:c14).



**Figure 3.7** Efficiency of conversion of ingestion (ECI as a percentage) for individuals from laboratory feeding trials. Reaction norms are presented with symbol and color combinations corresponding to population means  $\pm$  SE (symbols: ND – black circle; SD – red circle; NE green inverted triangle; KS – yellow triangle; OK – blue diamond; TX – pink square). Plots are separated by diet treatment (L to R; p14:c28, p21:c21; p28:c14).



**Figure 3.8** Efficiency of conversion of digestion (ECD as a percentage) for individuals from laboratory feeding trials. All diet treatments were pooled by population. Bars (A) represent population means ( $\pm$  SE) arranged in increasing latitude (L to R; TX in pink, OK in blue, KS in yellow, NE in green, SD in red, ND in black) by temperature treatment. Reaction norms are presented with symbol and color combinations corresponding to population means  $\pm$  1SE (symbols: ND – black circle; SD – red circle; NE green inverted triangle; KS – yellow triangle; OK – blue diamond; TX – pink square).



**Figure 3.9** Nitrogen use efficiency (NUE as a percentage) for individuals from laboratory feeding trials. Reaction norms are presented with symbol and color combinations corresponding to population means  $\pm$  SE (symbols: ND – black circle; SD – red circle; NE

green inverted triangle; KS – yellow triangle; OK – blue diamond; TX – pink square).  
 Plots are separated by diet treatment (L to R; p14:c28, p21:c21; p28:c14).

**Table 3.1 Post-ingestive efficiency indices calculations (from Slansky and Feeny 1977) used to estimate efficiency of grasshoppers from feeding trials. Feeding efficiencies are listed followed by abbreviations used in the text and calculations for each term.**

Feeding efficiency	Abbrev.	calculation
Approximate Digestibility	AD	$\frac{(\text{food ingested (in dry mg)} - \text{feces (in dry mg)})}{(\text{food ingested (in dry mg)})} \times 100$
Efficiency of Conversion of Digestion	ECD	$\frac{(\text{biomass gained (in dry mg)})}{(\text{food ingested (in dry mg)} - \text{feces (in dry mg)})} \times 100$
Efficiency of Conversion of Ingestion	ECI	$\frac{(\text{biomass gained (in dry mg)})}{(\text{food ingested (in dry mg)})} \times 100$
Nitrogen Utilization Efficiency	NUE	$\frac{(\text{biomass N gained (in dry mg)})}{(\text{N ingested (in mg)})} \times 100$

## Chapter 4 - Conclusions

Temperature and food quantity and quality are two of the primary ecological axes that have predictable and often directional influences on insect herbivore performance and life histories. However, studies investigating insect responses are often limited to single population manipulations, and comparisons across broad geographic ranges are generally lacking. In my studies, I compared grasshopper responses to temperature and food quality by six populations of a generalist feeding grasshopper, *Melanoplus femurrubrum* using a common garden experimental approach to evaluate clinal variation in size, color, development, growth, performance and efficiency.

Chapter 2 explored body size and grasshopper performance in response to temperature and diet along a latitudinal gradient. In the field, I measured internal body temperature, pronotum coloration and body size in adult grasshoppers sampled from each of the six field sites along a broad latitudinal gradient spanning 6 states and 18° of latitude. I found a body size cline that followed the Converse Bergmann's Rule (decrease in body size as latitude increases), which is common in many insect species. All populations achieved similar body temperatures given their ambient conditions but darker body coloration was observed in northern versus southern populations suggesting possible adaptive coloration. I also used F1 offspring from those same six populations in artificial diet feeding trials conducted in temperature controlled chambers. Overall body size in grasshoppers from feeding trials followed the Converse Bergmann's Rule, but there was a plateau in body size. North and South Dakota grasshoppers increased in size as latitude decreased, but NE, KS, OK, and TX grasshoppers all achieved similar sizes. By eliminating the environmental component experienced in the field by using the common garden

design, this plateau suggests that environmental contributions to grasshopper body size are important along this gradient. The extent to which temperature or food quality influences this trait is difficult to determine, however, because temperature and diet were not significant effects in body size for my feeding trials.

Grasshopper development rate was shortest for Northern populations. These populations with the shortest development time were also smallest in body size. Seasonal environmental constraints likely influence these traits simultaneously. Growing seasons vary latitudinally with shorter seasons in the north versus the south. Because grasshoppers are limited to one growing season for reproduction, individuals must reach sexual maturity faster in environments with limited season lengths, showing a trade-off in body size. Northern populations also exhibit greater RGRs than southern populations emphasizing the obvious benefit for faster maturity, although these grasshoppers did not show trade-offs for mass-specific metabolic rates. However, Northern populations were able to optimize growth by selecting protein-biased diets to fuel rapid development.

Chapter 3 considered the role of feeding and post-ingestive mechanisms associated with whole-animal digestion used to compensate for environmental heterogeneity. I found overall consumption was body-size dependent. Diet quality did not influence total consumption overall, but, diet did significantly alter mass-specific consumption. Grasshoppers made adjustments in the amount of diet consumed when restricted to a diet that was imbalanced in a way that did not match selection preferences. A latitudinal shift in preference from carbohydrate-biased diet selection at low latitudes to protein-biased diet selection at higher latitude was observed in my study. These preference shifts may result from environmental constraints on development; rapid development is possible with higher protein ingestion and warmer environments increase

metabolic rates which require higher energy input. Grasshopper populations from TX increased mass-specific consumption of high protein diets across all temperature treatments while ND populations increased mass-specific consumption of high carbohydrate diets when restricted to these diets as a means to compensate for dietary needs by increasing feeding on the resource available, even though it was of an insufficient preferred ratio.

Lastly, I examined post-ingestive nutritional use efficiencies for all grasshoppers from the feeding trials. I hypothesized that grasshoppers from northern (cooler) environments would demonstrate the highest processing efficiencies as an adaptation to seasonal constraints. My results on this point were variable. Temperature increased efficiency for ECI and ECD and sometimes for NUE, but AD was optimal at moderate temperatures (31°C). Diet quality influenced AD, NUE and ECI, with highest efficiency on low protein (p14:c28) and decreased as protein content increase. In many cases, ND and TX populations achieved similar, and often greatest, efficiencies. This is not what I would have expected given these populations exhibit significantly different body sizes and diet preferences, but perhaps these types of “variation” act as a buffer to population survival when environmental changes can be drastic, frequent, and highly unpredictable.

Overall, my research aimed to tease out the mechanisms that grasshoppers use to adapt to variable environments across a broad geographic range. I found body size to follow the Converse Bergmann’s Rule, which is consistent with other insect body size patterns, but found a plateau in size suggesting ecotypic variation among populations. This body size pattern may result from season length and temperature constraints in combination with plant quality variation that exists along latitudinal gradients. Grasshopper populations have adapted strategies to cope with geographic constraints by adjusting development times and growth rates and may ultimately

rely on selective feeding to optimize performance. I found processing efficiency responses to be highly variable, but highest for populations at the extremes of my gradient

## References

- Abrams, P. A. and O. J. Schmitz. 1999. The effect of risk of mortality on the foraging behaviour of animals faced with time and digestive capacity constraints. *Evolutionary Ecology Research* **1**:285-301.
- Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**:739-745.
- Alho, J. S., G. Herczeg, F. Soderman, A. Laurila, K. I. Jonsson, and J. Merila. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *Bmc Evolutionary Biology* **10**.
- Angilletta, M. J., M. W. Sears, and T. D. Steury. 2003. Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life history puzzle. *Integrative and Comparative Biology* **43**:923-923.
- Angilletta, M. J., T. D. Steury, and M. W. Sears. 2004. Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integrative and Comparative Biology* **44**:498-509.
- Angilletta, M. J. J. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, New York.
- Arnett, A. E. and N. J. Gotelli. 1999a. Bergmann's rule in the ant lion *Myrmeleon immaculatus* DeGeer (Neuroptera : Myrmeleontidae): geographic variation in body size and heterozygosity. *Journal of Biogeography* **26**:275-283.
- Arnett, A. E. and N. J. Gotelli. 1999b. Geographic variation in life-history traits of the ant lion, *Myrmeleon immaculatus*: Evolutionary implications of Bergmann's rule. *Evolution* **53**:1180-1188.
- Arnett, A. E. and N. J. Gotelli. 2003. Bergmann's rule in larval ant lions: testing the starvation resistance hypothesis. *Ecological Entomology* **28**:645-650.
- Atkinson, D. and R. M. Sibly. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology & Evolution* **12**:235-239.



- Ayres, M. P. and J. M. Scriber. 1994. Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera, Papilionidae). *Ecological Monographs* **64**:465-482.
- Bailey, C. G. and M. K. Mukerji. 1976. Feeding-habits and food preference of *Melanoplus bivittatus* and *Melanoplus femurrubrum* (Orthoptera Acrididae). *Canadian Entomologist* **108**:1207-1212.
- Bailey, C. G. and M. K. Mukerji. 1977. Energy dynamics of *Melanoplus bivittatus* and *Melanoplus femurrubrum* (Orthoptera Acrididae) in a grassland ecosystem. *Canadian Entomologist* **109**:605-614.
- Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, J. Farrar, J. E. G. Good, R. Harrington, S. Hartley, T. H. Jones, R. L. Lindroth, M. C. Press, I. Symrnioudis, A. D. Watt, and J. B. Whittaker. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**:1-16.
- Barbehenn, R. V., D. N. Karowe, and Z. Chen. 2004. Performance of a generalist grasshopper on a C-3 and a C-4 grass: compensation for the effects of elevated CO<sub>2</sub> on plant nutritional quality. *Oecologia* **140**:96-103.
- Barimo, J. F. and D. R. Young. 2002. Grasshopper (Orthoptera : Acrididae)-plant-environmental interactions in relation to zonation on an Atlantic coast barrier island. *Environmental Entomology* **31**:1158-1167.
- Bear, A., A. Simons, E. Westerman, and A. Monteiro. 2010. The Genetic, Morphological, and Physiological Characterization of a Dark Larval Cuticle Mutation in the Butterfly, *Bicyclus anynana*. *Plos One* **5**.
- Beckerman, A. P. 2002. The distribution of *Melanoplus femurrubrum*: fear and freezing in Connecticut. *Oikos* **99**:131-140.
- Beckerman, A. P., M. Uriarte, and O. J. Schmitz. 1997. Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America* **94**:10735-10738.
- Behmer, S. T. 2009. Insect Herbivore Nutrient Regulation. *Annual Review of Entomology* **54**:165-187.
- Behmer, S. T., E. Cox, D. Raubenheimer, and S. J. Simpson. 2003. Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Animal Behaviour* **66**:665-675.

- Behmer, S. T. and A. Joern. 1993. Diet choice by a grass-feeding grasshopper based on the need for a limiting nutrient. *Functional Ecology* **7**:522-527.
- Behmer, S. T. and A. Joern. 1994. The influence of proline on diet selection - sex-specific feeding preferences by the grasshopper *Ageneotettix deorum* and *Phoetaliotes nebrascensis* (Orthoptera, Acrididae). *Oecologia* **98**:76-82.
- Behmer, S. T. and A. Joern. 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proceedings of the National Academy of Sciences of the United States of America* **105**:1977-1982.
- Behmer, S. T., D. Raubenheimer, and S. J. Simpson. 2001. Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Animal Behaviour* **61**:995-1005.
- Behmer, S. T., S. J. Simpson, and D. Raubenheimer. 2002. Herbivore foraging in chemically heterogeneous environments: Nutrients and secondary metabolites. *Ecology* **83**:2489-2501.
- Belovsky, G. E. and J. B. Slade. 1993. The role of vertebrate and invertebrate predators in a grasshopper community. *Oikos* **68**:193-201.
- Bernays, E. A. and R. F. Chapman. 1994. *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, Inc., New York.
- Berner, D., W. U. Blackenhorn, and C. Körner. 2005. Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos* **111**:525-533.
- Bidau, C. J. and D. A. Marti. 2007a. 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.
- Bidau, C. J. and D. A. Marti. 2007b. *Dichroplus vittatus* (Orthoptera : Acrididae) follows the converse to Bergmann's rule although male morphological variability increases with latitude. *Bulletin of Entomological Research* **97**:69-79.
- Bidau, C. J. and D. A. Marti. 2008. Geographic and climatic factors related to a body-size cline in *Dichroplus pratensis* Bruner, 1900 (Acrididae, Melanoplinae). *Journal of Orthoptera Research* **17**:149-156.

- Blackburn, T. M., K. J. Gaston, and N. Loder. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions*:165-174.
- Blanckenhorn, W. U. and M. Demont. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integrative and Comparative Biology* **44**:413-424.
- Boggs, C. L. 2009. Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* **23**:27-37.
- Boswell, A. W., T. Provin, and S. T. Behmer. 2008. The relationship between body mass and elemental composition in nymphs of the grasshopper *Schistocerca americana*. *Journal of Orthoptera Research* **17**:307-313.
- Boutton, T. W., G. N. Cameron, and B. N. Smith. 1978. Insect herbivory on C3 and C4 grasses. *Oecologia* **36**:21-32.
- 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. 2010. Density-Dependent Effects of an Early Season Insect Herbivore on a Later Developing Insect Herbivore. *Environmental Entomology* **39**:346-350.
- Brooks, J. L. and S. I. Dodson. 1965. Predation body size and composition of plankton. *Science* **150**:28-&.
- Brose, U. 2010. Body-mass constraints on foraging behaviour determine population and food-web dynamics. *Functional Ecology* **24**:28-34.
- Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. *Ecology* **85**:1771-1789.
- Brust, M. L., W. W. Hoback, and R. J. Wright. 2009. Degree-day requirements for eight economically important grasshoppers (Orthoptera: Acrididae) in Nebraska using field data. *Environmental Entomology* **38**:1521-1526.
- Capinera, J. L. and D. R. Horton. 1989. Geographic-variation in effects of weather on grasshopper infestation. *Environmental Entomology* **18**:8-14.
- Capinera, J. L., R. D. Scott, and T. J. Walker. 2004. *Field Guide to Grasshoppers, Katydid, and Crickets of the United States*. Comstock Publishing Associates, Ithaca and London.

- Castaneda, L. E., M. A. Lardies, and F. Bozinovic. 2005. Interpopulational variation in recovery time from chill coma along a geographic gradient: A study in the common woodlouse, *Porcellio laevis*. *Journal of Insect Physiology* **51**:1346-1351.
- Cease, A. J., S. Hao, L. Kang, J. J. Elser, and J. F. Harrison. 2010. Are color or high rearing density related to migratory polyphenism in the band-winged grasshopper, *Oedaleus asiaticus*? *Journal of Insect Physiology*.
- Chambers, P. G., S. J. Simpson, and D. Raubenheimer. 1995. Behavioural mechanisms of nutrient balancing in *Locusta migratoria* nymphs. *Animal Behaviour* **50**:1513-1523.
- Chapman, R. F. 1990. Food selection. Pages 39-72 in R. F. Chapman and A. Joern, editors. *Biology of Grasshoppers*. John Wiley & Sons, New York.
- Chapman, R. F. 1998. *The Insects: Structure and Function*. Cambridge University Press, Cambridge, United Kingdom.
- Chapman, R. F. and A. Joern. 1990. *Biology of grasshoppers*. John Wiley & Sons, New York.
- Chown, S. L. and K. J. Gaston. 2010. Body size variation in insects: a macroecological perspective. *Biological Reviews* **85**:139-169.
- Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. R. B. Lighton, and T. M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Functional Ecology* **21**:282-290.
- Chown, S. L. and S. W. Nicolson. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, New York.
- Clarke, A. 2003. Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology & Evolution* **18**:573-581.
- Clarke, A. 2006. Temperature and the metabolic theory of ecology. *Functional Ecology* **20**:405-412.
- Clissold, F. J. 2007. The biomechanics of chewing and plant fracture: Mechanisms and implications. *Advances in Insect Physiology: Insect Mechanics and Control* **34**:317-372.
- Clissold, F. J., G. D. Sanson, J. Read, and S. J. Simpson. 2009. Gross vs. net income: How plant toughness affects performance of an insect herbivore. *Ecology* **90**:3393-3405.
- Clusella Trullas, S., J. H. van Wyk, and J. R. Spotila. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology* **32**:235-245.

- Colombo, P. C. 1997. Exophenotypic effects of chromosomal change: the case of *Leptysmia argentina* (Orthoptera). *Heredity* **79**:631-637.
- Conover, D. O. and T. M. C. Present. 1990. Countergradient variation in growth-rate - compensation for length of the growing-season among Atlantic Silversides from different latitudes. *Oecologia* **83**:316-324.
- Cotter, S. C., S. J. Simpson, D. Raubenheimer, and K. Wilson. 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. *Functional Ecology* **25**:186-198.
- Crawford, K. M., J. M. Land, and J. A. Rudgers. 2010. Fungal endophytes of native grasses decrease insect herbivore preference and performance. *Oecologia* **164**:431-444.
- De Block, M., S. Slos, F. Johansson, and R. Stoks. 2008. Integrating life history and physiology to understand latitudinal size variation in a damselfly. *Ecography* **31**:115-123.
- del Rio, C. M. 2008. Metabolic theory or metabolic models? *Trends in Ecology & Evolution* **23**:256-260.
- Dingle, H. 1986. The evolution of insect life cycle syndromes. Pages 187-203 in F. Taylor and R. Karban, editors. *The evolution of insect life cycles*. Springer-Verlag, New York.
- Dingle, H. and T. A. Mousseau. 1994. Geographic-variation in embryonic-development time and stage of diapause in grasshopper. *Oecologia* **97**:179-185.
- Dingle, H., T. A. Mousseau, and S. M. Scott. 1990. Altitudinal variation in life-cycle syndromes of California populations of the grasshopper, *Melanoplus sanguinipes* (F). *Oecologia* **84**:199-206.
- Dixon, A. F. G., A. Honek, P. Keil, M. A. A. Kotela, A. L. Sizling, and V. Jarosik. 2009. Relationship between the minimum and maximum temperature thresholds for development in insects. *Functional Ecology* **23**:257-264.
- Elser, J. J., W. F. Fagan, A. J. Kerkhoff, N. G. Swenson, and B. J. Enquist. 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist* **186**:593-608.
- Elser, J. J., R. W. Sterner, E. Gorokhova, W. F. Fagan, T. A. Markow, J. B. Cotner, J. F. Harrison, S. E. Hobbie, G. M. Odell, and L. J. Weider. 2000. Biological stoichiometry from genes to ecosystems. *Ecology Letters* **3**:540-550.

- Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton.
- Fielding, D. J. 2004. Developmental time of *Melanoplus sanguinipes* (Orthoptera : Acrididae) at high latitudes. *Environmental Entomology* **33**:1513-1522.
- Fielding, D. J. and L. S. Defoliart. 2005. Density and temperature-dependent melanization of fifth-instar *Melanoplus sanguinipes*: interpopulation comparisons. Pages 107-113, *Journal of Orthoptera Research*.
- Fielding, D. J. and L. S. Defoliart. 2007. Growth, development, and nutritional physiology of grasshoppers from subarctic and temperate regions. *Physiological and Biochemical Zoology* **80**:607-618.
- Fielding, D. J. and L. S. Defoliart. 2008. Discriminating tastes: self-selection of macronutrients in two populations of grasshoppers. *Physiological Entomology* **33**:264-273.
- Fisher, J. R. 1994. Temperature effect on postdiapause development and survival of embryos of 3 species of *Melanoplus* (Orthoptera, Acrididae). *Annals of the Entomological Society of America* **87**:604-608.
- Fleming, P. A. and P. W. Bateman. 2007. Just drop it and run: the effect of limb autotomy on running distance and locomotion energetics of field crickets (*Gryllus bimaculatus*). *Journal of Experimental Biology* **210**:1446-1454.
- Forsman, A. 1999. Variation in thermal sensitivity of performance among colour morphs of a pygmy grasshopper. *Journal of Evolutionary Biology* **12**:869-878.
- Franzke, A., S. B. Unsicker, J. Specht, G. Kohler, and W. W. Weisser. 2010. Being a generalist herbivore in a diverse world: how do diets from different grasslands influence food plant selection and fitness of the grasshopper *Chorthippus parallelus*? *Ecological Entomology* **35**:126-138.
- Frentiu, F. D. and S. F. Chenoweth. 2010. Clines in cuticular hydrocarbons in two *Drosophila* species with independent population histories. *Evolution* **64**:1784-1794.
- Gaston, K. J., S. L. Chown, P. Calosi, J. Bernardo, D. T. Bilton, A. Clarke, S. Clusella-Trullas, C. K. Ghalambor, M. Konarzewski, L. S. Peck, W. P. Porter, H. O. Poertner, E. L. Rezende, P. M. Schulte, J. I. Spicer, J. H. Stillman, J. S. Terblanche, and M. van Kleunen. 2009. Macrophysiology: A Conceptual Reunification. *American Naturalist* **174**:595-612.

- Gates, D. M. 1980. *Biophysical Ecology*. Springer-Verlag, New York.
- Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. *Science* **293**:2248-2251.
- Goranson, C. E., C. K. Ho, and S. C. Pennings. 2004. Environmental gradients and herbivore feeding preferences in coastal salt marshes. *Oecologia* **140**:591-600.
- Gouveia, S. M., S. J. Simpson, D. Raubenheimer, and F. P. Zanotto. 2000. Patterns of respiration in *Locusta migratoria* nymphs when feeding. *Physiological Entomology* **25**:88-93.
- Grant, A., M. Hassall, and S. J. Willott. 1993. An alternative theory of grasshopper life-cycles. *Oikos* **66**:263-268.
- Gunn, D. L. 1942. Body temperature in poikilothermal animals. *Biological Reviews* **17**:293-314.
- Hahn, D. A. 2005. Larval nutrition affects lipid storage and growth, but not protein or carbohydrate storage in newly eclosed adults of the grasshopper *Schistocerca americana*. *Journal of Insect Physiology* **51**:1210-1219.
- Hao, S. G. and L. Kang. 2004. Effects of temperature on the post-diapause embryonic development and the hatching time in three grasshopper species (Orth., Acrididae). *Journal of Applied Entomology* **128**:95-101.
- Harrison, J. F. and J. H. Fewell. 1995. Thermal effects on feeding-behavior and net energy-intake in a grasshopper experiencing large diurnal fluctuations in body-temperature. *Physiological Zoology* **68**:453-473.
- Ho, C. K., S. C. Pennings, and T. H. Carefoot. 2010. Is diet quality an overlooked mechanism for Bergmann's rule? *American Naturalist* **175**:269-276.
- Horton, D. R. and R. A. Redak. 1993. Further comments on analysis of covariance in insect dietary studies. *Entomologia Experimentalis Et Applicata* **69**:263-275.
- Hui, D. F. and R. B. Jackson. 2007. Uncertainty in allometric exponent estimation: A case study in scaling metabolic rate with body mass. *Journal of Theoretical Biology* **249**:168-177.
- Huizenga, K. M., M. D. Shaidle, J. S. Brinton, L. A. Gore, M. A. Ebo, A. J. Solliday, P. J. Buguey, D. W. Whitman, and S. A. Juliano. 2008. Geographic differences in the body sizes of adult *Romalea* microptera. *Journal of Orthoptera Research* **17**:135-139.
- Irlich, U. M., J. S. Terblanche, T. M. Blackburn, and S. L. Chown. 2009. Insect Rate-Temperature Relationships: Environmental Variation and the Metabolic Theory of Ecology. *American Naturalist* **174**:819-835.

- Joern, A. 1979. Feeding patterns in grasshoppers (Orthoptera, Acrididae) - factory influencing diet specialization. *Oecologia* **38**:325-347.
- Joern, A. 1981. Importance of behavior and coloration in the control of body-temperature by *Brachystola magna* Girard (Orthoptera, Acrididae). *Acrida* **10**:117-130.
- Joern, A. and S. T. Behmer. 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 S. T. Behmer. 1998. Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. *Ecological Entomology* **23**:174-184.
- Jonas, J. L. and A. Joern. 2008. Host-plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera : Acrididae). *Ecological Entomology* **33**:546-554.
- Karasov, W. H. and C. M. del Rio. 2007. *Physiological ecology: how animals process energy, nutrients, and toxins*. Princeton University Press, Princeton.
- Kemp, W. P. 1986. Thermoregulation in 3 rangeland species. *Canadian Entomologist* **118**:335-343.
- Kemp, W. P. and J. A. Onsager. 1986. Rangeland grasshoppers (Orthoptera, Acridade) - modeling phenology of natural-populations of 6 species. *Environmental Entomology* **15**:924-930.
- Kingsolver, J. G. 2009. The Well-Tempered Biologist. *American Naturalist* **174**:755-768.
- Kingsolver, J. G. and R. B. Huey. 2008. Size, temperature, and fitness: three rules. *Evolutionary Ecology Research* **10**:251-268.
- Kingsolver, J. G. and R. B. Srygley. 2000. Experimental analyses of body size, flight and survival in pierid butterflies. *Evolutionary Ecology Research* **2**:593-612.
- Kivelä, S. M., P. Välimäki, D. Carrasco, M. I. Mäenpää, and J. Oksanen. 2011. Latitudinal insect body size clines revisited: a critical evaluation of the saw-tooth model. *Journal of Animal Ecology* **80**:1184-1195.
- Lactin, D. J. and D. L. Johnson. 1995. Temperature-dependent feeding rates of *Melanoplus sanguinipes* nymphs (Orthoptera: Acrididae) in laboratory trials. *Physiological and Chemical Ecology* **24**:1291-12696.



- Lactin, D. J. and D. L. Johnson. 1997. Response of body temperature to solar radiation in restrained nymphal migratory grasshoppers (Orthoptera: Acrididae): influences of orientation and body size. *Physiological Entomology* **22**:131-139.
- Lee, K. P. 2007. The interactive effects of protein quality and macronutrient imbalance on nutrient balancing in an insect herbivore. *Journal of Experimental Biology* **210**:3236-3244.
- Lighton, J. R. B. 2008. *Measuring metabolic rates : a manual for scientists*. Oxford University Press, New York.
- Logan, J. D., A. Joern, and W. Wolesensky. 2002. Location, time, and temperature dependence of digestion in simple animal tracts. *Journal of Theoretical Biology* **216**:5-18.
- Logan, J. D., A. Joern, and W. Wolesensky. 2003. Chemical reactor models of optimal digestion efficiency with constant foraging costs. *Ecological Modelling* **168**:25-38.
- Logan, J. D., A. Joern, and W. Wolesensky. 2004. Control of CNP homeostasis in herbivore consumers through differential assimilation. *Bulletin of Mathematical Biology* **66**:707-725.
- Logan, J. D., W. Wolesensky, and A. Joern. 2007. Insect development under predation risk, variable temperature, and variable food quality. *Mathematical Biosciences and Engineering* **4**:47-65.
- Masaki, S. 1967. Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution* **21**:725-741.
- Mattson, W. J. 1980. Herbivory in relation to plant nitrogen-content. *Annual Review of Ecology and Systematics* **11**:119-161.
- Melvin, R. G., W. A. Van Voorhies, and J. W. O. Ballard. 2007. Working harder to stay alive: Metabolic rate increases with age in *Drosophila simulans* but does not correlate with life span. *Journal of Insect Physiology* **53**:1300-1306.
- Miller, G. A., F. J. Clissold, D. Mayntz, and S. J. Simpson. 2009. Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. *Proceedings of the Royal Society B-Biological Sciences* **276**:3581-3589.
- Mitra, A. and K. J. Flynn. 2007. Importance of interactions between food quality, quantity, and gut transit time on consumer feeding, growth, and trophic dynamics. *American Naturalist* **169**:632-646.

- Moe, S. J., R. S. Stelzer, M. R. Forman, W. S. Harpole, T. Daufresne, and T. Yoshida. 2005. Recent advances in ecological stoichiometry: Insights for population and community ecology. *Oikos* **109**:29-39.
- Monaco, C. J., K. B. Brokordt, and C. F. Gaymer. 2010. Latitudinal thermal gradient effect on the cost of living of the intertidal porcelain crab *Petrolisthes granulosus*. *Aquatic Biology* **9**:23-33.
- Mousseau, T. A. 1997. Ectotherms follow the converse to Bergmann's Rule. *Evolution* **51**:630-632.
- Nespolo, R. F., D. A. Roff, and D. J. Fairbairn. 2008. Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). *Functional Ecology* **22**:624-631.
- O'Neill, K. M. and M. G. Rolston. 2007. Short-term dynamics of behavioral thermoregulation by adults of the grasshopper *Melanoplus sanguinipes*. *Journal of Insect Science* **7**.
- Oedekoven, M. A. and A. Joern. 1998. Stage-based mortality of grassland grasshoppers (Acrididae) from wandering spider (Lycosidae) predation. *Acta Oecologica-International Journal of Ecology* **19**:507-515.
- Partridge, L. and J. A. Coyne. 1997. Bergmann's rule in ectotherms: Is it adaptive? *Evolution* **51**:632-635.
- Pruess, K. P. 1983. Day-degree methods for pest management. *Environmental Entomology* **12**:613-619.
- Ragland, G. J. and J. G. Kingsolver. 2008. Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life-history timing in *Wyeomyia smithii*. *Evolution : International Journal of Organic Evolution* **62**:1345-1357.
- Raubenheimer, D. and S. J. Simpson. 1992. Analysis of covariance - an alternative to nutritional indices. *Entomologia Experimentalis Et Applicata* **62**:221-231.
- Raubenheimer, D. and S. J. Simpson. 1993. The geometry of compensatory feeding in the locust. *Animal Behaviour* **45**:953-964.
- Raubenheimer, D. and S. J. Simpson. 1994. The analysis of nutrient budgets. *Functional Ecology* **8**:783-791.
- Raubenheimer, D. and S. J. Simpson. 1996. Meeting nutrient requirements: The roles of power and efficiency. *Entomologia Experimentalis Et Applicata* **80**:65-68.

- Raubenheimer, D. and S. J. Simpson. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology* **206**:1669-1681.
- Raubenheimer, D. and S. J. Simpson. 2004. Organismal stoichiometry: Quantifying non-independence among food components. *Ecology* **85**:1203-1216.
- Raubenheimer, D., S. J. Simpson, and D. Mayntz. 2009. Nutrition, ecology and nutritional ecology: toward an integrated framework. *Functional Ecology* **23**:4-16.
- Reich, P. B. and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* **101**:11001-11006.
- Roff, D. 1980. Optimizing development time in a seasonal environment - the ups and downs of clinal variation. *Oecologia* **45**:202-208.
- Salgado, C. S. and S. C. Pennings. 2005. Latitudinal variation in palatability of salt-marsh plants: Are differences constitutive? *Ecology* **86**:1571-1579.
- Schmidt, D. J. and J. C. Reese. 1986. Sources of error in nutritional index studies of insects on artificial diets. *Journal of Insect Physiology* **32**:193-198.
- Schmitz, O. J., A. P. Beckerman, and K. M. Obrien. 1997. Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. *Ecology* **78**:1388-1399.
- Scriber, J. M. 2002. Latitudinal and local geographic mosaics in host plant preferences as shaped by thermal units and voltinism in *Papilio* spp. (Lepidoptera). *European Journal of Entomology* **99**:225-239.
- Scriber, J. M. and F. Slansky. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology* **26**:183-211.
- Sears, M. W. and M. J. Angilletta. 2004. Body size clines in *Sceloporus* lizards: Proximate mechanisms and demographic constraints. *Integrative and Comparative Biology* **44**:433-442.
- Sibly, R. M. and D. Atkinson. 1994. How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* **8**:486-493.
- Simpson, S. J. and D. Raubenheimer. 1993. A multilevel analysis of feeding-behavior - the geometry of nutritional decisions. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **342**:381-402.

- Simpson, S. J. and D. Raubenheimer. 2000. The hungry locust. Pages 1-44 *Advances in the Study of Behavior*, Vol. 29.
- Simpson, S. J. and D. Raubenheimer. 2001. The geometric analysis of nutrient-allelochemical interactions: A case study using locusts. *Ecology* **82**:422-439.
- Simpson, S. J., D. Raubenheimer, S. T. Behmer, A. Whitworth, and G. A. Wright. 2002. A comparison of nutritional regulation in solitary- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. *Journal of Experimental Biology* **205**:121-129.
- Slansky, F. and P. Feeny. 1977. Stabilization rate of nitrogen accumulation by larvae of cabbage butterfly on wild and cultivated foods. *Ecological Monographs* **47**:209-228.
- Sterner, R. W. and J. J. Elser. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton.
- Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. *Seasonal adaptations of insects*. Oxford University Press, New York.
- Taylor, F. and R. Karban, editors. 1986. *The Evolution of insect life cycles*. Springer-Verlag, New York.
- Terblanche, J. S. and S. L. Chown. 2010. Effects of flow rate and temperature on cyclic gas exchange in tsetse flies (Diptera, Glossinidae). *Journal of Insect Physiology* **56**:513-521.
- Terblanche, J. S., S. Clusella-Trullas, J. A. Deere, B. J. Van Vuuren, and S. L. Chown. 2009. Directional Evolution of the Slope of the Metabolic Rate-Temperature Relationship Is Correlated with Climate. *Physiological and Biochemical Zoology* **82**:495-503.
- Terblanche, J. S., C. Janion, and S. L. Chown. 2007. Variation in scorpion metabolic rate and rate-temperature relationships: implications for the fundamental equation of the metabolic theory of ecology. *Journal of Evolutionary Biology* **20**:1602-1612.
- Thompson, D. B. 1992. Consumption rates and the evolution of diet-induced plasticity in the head morphology of *Melanoplus femurrubrum* (Orthoptera: Acrididae). *Oecologia* **89**:204-213.
- Thompson, D. B. 1999. Genotype-environment interaction and the ontogeny of diet-induced phenotypic plasticity in size and shape of *Melanoplus femurrubrum* (Orthoptera : Acrididae). *Journal of Evolutionary Biology* **12**:38-48.
- Thompson, J. N. 2005. *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Chicago.

- Thompson, S. N. and R. A. Redak. 2008. Parasitism of an insect *Manduca sexta* L. alters feeding behaviour and nutrient utilization to influence developmental success of a parasitoid. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **178**:515-527.
- vanVoorhies, W. A. 1996. Bergmann size clines: A simple explanation for their occurrence in ectotherms. *Evolution* **50**:1259-1264.
- Vickery, V. R. and D. K. McE. Kevan. 1986. The grasshoppers, crickets, and related insects of Canada and adjacent regions: Ulonata, Dermaptera, Cheleutoptera, Notoptera, Dictuoptera, Grylloptera, and Orthoptera. Biosystematics Research Institute, Ottawa.
- Virtanen, T. and S. Neuvonen. 1999. Performance of moth larvae on birch in relation to altitude, climate, host quality and parasitoids. *Oecologia* **120**:92-101.
- Waldbauer, G. P. 1968. The consumption and utilization of food by insects. *Recent Advances in Insect Physiology* **5**:229-288.
- Waldbauer, G. P. and S. Friedman. 1991. Self-selection of optimal diets by insects. *Annual Review of Entomology* **36**:43-63.
- Walters, R. J. and M. Hassall. 2006. The temperature-size rule in ectotherms: May a general explanation exist after all? *American Naturalist* **167**:510-523.
- Whitman, D. W. 2008. The significance of body size in the Orthoptera: a review. *Journal of Orthoptera Research* **17**:117-134.
- Wigglesworth, V. B. 1957. The physiology of the insect cuticle. *Annual Review of Entomology* **2**:37-54.
- Woods, H. A. and J. G. Kingsolver. 1999. Feeding rate and the structure of protein digestion and absorption in lepidopteran midguts. *Archives of Insect Biochemistry and Physiology* **42**:74-87.
- Yang, Y. and A. Joern. 1994a. Gut size changes in relation to variable food quality and body-size in grasshoppers. *Functional Ecology* **8**:36-45.
- Yang, Y. L. and A. Joern. 1994b. Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. *Physiological Entomology* **19**:75-82.
- Yang, Y. L. and A. Joern. 1994c. Influence of diet quality, developmental stage, and temperature on food residence time in the grasshopper *Melanoplus differentialis*. *Physiological Zoology* **67**:598-616.

- Zanotto, F. P., S. M. Gouveia, S. J. Simpson, D. Raubenheimer, and P. C. Calder. 1997. Nutritional homeostasis in locusts: Is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *Journal of Experimental Biology* **200**:2437-2448.
- Zanotto, F. P., D. Raubenheimer, and S. J. Simpson. 1994. Selective egestion of lysine by locusts fed nutritionally unbalanced foods. *Journal of Insect Physiology* **40**:259-265.
- Zanotto, F. P., D. Raubenheimer, and S. J. Simpson. 1996. Haemolymph amino acid and sugar levels in locusts fed nutritionally unbalanced diets. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **166**:223-229.
- Zanotto, F. P., S. J. Simpson, and D. Raubenheimer. 1993. The regulation of growth by locusts through postingestive compensation for variation in the levels of dietary-protein and carbohydrate. *Physiological Entomology* **18**:425-434.