

AN ABSTRACT OF THE THESIS OF

Arthur Lomis Pelegrin for the degree of Master of Science in Zoology presented on December 5, 2006.

Title: Phenotypic Evolution as a Response to Thermal Ecology in the Ferocious Waterbug *Abedus herberti* (Hemiptera: Belostomatidae)

Abstract approved:

---

David A. Lytle

Over 150 years ago, C. Bergmann observed a within-species pattern of increasing body size as elevation and latitude increased. Bergmann's data came from numerous endothermic taxa, but the pattern has since been documented among numerous poikilotherms and invertebrates. The ferocious waterbug *Abedus herberti* Hidalgo (Hemiptera: Belostomatidae) lives in isolated populations distributed across gradients of latitude and altitude, and as such is an excellent model organism to examine the application of Bergmann's Rule to a large aquatic insect.

This study documents the variance in adult length and width of fifteen disparate populations of *Abedus herberti* in southeastern Arizona, USA and northern Sonora, Mexico. It also examines the life-history traits of two cadres of populations: those living in mountain runoff creeks, the typical habitat for this species; and those living in spring-fed desert ponds and marshes, called ciénagas.

During 2004-2006 my colleagues and I collected and measured 611 individual adult *A. herberti* from fifteen populations in the Madrean Sky Islands. I

took live insects from six populations back to Oregon and raised their offspring in a common-garden experiment, to measure length, width, and time between molts. Temperature probes were placed in the pools from which these six populations were taken, to provide a record of annual thermal fluctuation.

I used these data to examine several hypotheses concerning body size and growth in insects:

Are there significant size differences between mountain-stream bugs and those living in ciénagas?

Do these insects obey Bergmann's Rule, *i.e.* does body size increase as local mean temperature decreases, as predicted by altitude and latitude?

Does a larger final body size correlate with a longer development time?

Are the size differences between bugs from different habitat types resulting from phenotypic plasticity or are the traits heritable?

How does Dyar's Rule apply to this organism?

Does body size provide any evidence of isolation or speciation within these populations?

I also used these data to create a rearing and care program for *Abedus herberti* that will serve future researchers working with this organism. This program, as well as an investigation of the latter two hypotheses, is relegated to the appendix of this work.

I found the mean sizes of individuals within *Abedus herberti* populations to be consistent with Bergmann's Rule. Adult length and width increased with the altitude of the population. Even omitting the ciénaga populations from the analysis produced significant results. As the populations were distributed over a minor gradient in latitude, I tested for significant correlation but found only a

weak relationship between latitude and length, and no significant relationship between altitude and width.

The thermal regimes between mountain and ciénaga habitats differed in the following ways: higher annual mean temperature and greater thermal stability in ciénagas, lower annual mean temperature and greater fluctuations in mountain streams. These differences may contribute to selective pressures that result in size disparities between conspecifics living in the two habitats.

The mean adult length and width varied significantly between mountain-type populations and ciénaga-type populations. These differences were perpetuated by offspring raised under identical conditions, suggesting the traits in question are inherited, rather than resulting from phenotypic plasticity.

Larger-sized nymphs took significantly longer to develop than smaller nymphs. Intermolt time between hatching and first molt, and between first and second molt, was longer for the mountain-type populations. Further intermolt times were not included in the analysis because high mortality interfered with design balance. This animal undergoes five nymph instars before attaining adulthood.

There was a 1.29x increase in length and 1.27x increase in width between molts ( $\pm 1.2\%$  depending on population), lower than the maximum allowed by Dyar's Rule, which posits a maximal 1.4x increase in size between instars.

I hypothesize that the differences in body size between populations living under different thermal regimes could be due to three main factors: 1. A physiological response to colder environments and selection for a particular

optimal body size for a given mean annual temperature. 2. Selection for smaller size and faster development in warmer environments to optimize the number of generations possible during the growth season, balanced by the stored-resource requirements of overwintering adults. 3. Selection for larger egg-size and first-instar nymph size in colder habitats to favor increased energy content of early offspring, as a hedge against fluctuating conditions; this is balanced in the warmer habitats by selection for small egg-size that allows females to fit more eggs onto the males' hemelytra, the surface area of which limits the fecundity of this species. The results of this study suggest all these factors may be in play to some degree, but the strongest evidence supports a physiological and life-history response in body size based strictly on local thermal regime.

© Copyright by Arthur Lomis Pelegrin  
December 5, 2006  
All Rights Reserved

Phenotypic Evolution as a Response to Thermal Ecology in the Ferocious Waterbug  
*Abedus herberti* (Hemiptera: Belostomatidae)

by  
Arthur Lomis Pelegrin

A THESIS  
submitted to  
Oregon State University

in partial fulfillment of  
the requirements for the  
degree of  
Master of Science

Presented December 5, 2006  
Commencement June 2007

Master of Science thesis of Arthur Lomis Pelegrin presented on December 5, 2006.

APPROVED:

---

Major Professor, representing Zoology

---

Chair of the Department of Zoology

---

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

---

Arthur Lomis Pelegrin, Author

## ACKNOWLEDGEMENTS

The lion's share of my gratitude goes to Dr David Lytle. He invited me to participate in his research after I took his Aquatic Entomology class and had signed on to be part of the annual Arizona Expedition. Dave took a chance on me, a student of the humanities with very little in the way of standard scholastic science background, and I am eternally grateful for the opportunity to prove myself. Under Dave's guidance I have grown as a scholar and a scientist. He has a keen eye for the interesting questions and the perspicacity to guide his students around corners and obstacles. His laissez faire policy toward lab hours and schedules has allowed me the flexibility to work at my own pace, often after midnight, and to explore the fundamentals of the Russian language as a side-project. Dave also provided multiple opportunities for adventure and exploration, in Arizona, Oregon and Alaska. Being savaged by mosquitoes while we climbed a glacier under the midnight sun is a treasured memory. In addition to his fine guidance and leadership, Dave is a friend and a supporter, who has shared laughs and wonder and history through my years at OSU.

My colleague and friend Michael T. Bogan has been a big brother to me in the best way throughout this project. His invaluable knowledge of the study system and the region has helped me in more ways than I can count. His company in the field, on long car trips and late nights at the lab has been my privilege.

Dr Debra Finn has assisted me tremendously in working with sequence data and analytical methods. My other labmates, Asako Yamamuro and Laura McMullen have patiently dealt with my constant questions about statistics and



methodology. Thanks are due to other graduate students and post-docs, including Ryan Kepler, Kimberly Tanner, Brian Knaus, Mario Ambrosino, Melissa Scherr and Joshua Ogawa, who all helped me get up to speed as a graduate student and shared their insights into the ways of the scholarly world. Jeremy Henderson helped feed my nymphs and Sabrina Helm taught me bug-handling basics. My deep gratitude also goes to my friend, former housemate and companion Marieke Steuben, for her constant support, her tolerance of my late hours, and her honest wonder and delight in the natural world.

Dr Andrew V.Z. Brower got me interested in systematics and has been an enormous help as a mentor. I owe him special thanks for encouraging me to apply for graduate school and for his many insights. Dr Brower has also been a tremendous help in the generation of trees and the use of phylogenetic software.

Dr Aaron Liston made me think about taxonomy and systematics in a new way in his Speciation class, and I am proud to have him on my committee. Dr Christopher Marshall has been a source of knowledge and energy since his arrival at OSU, and I am very pleased he could join this project on short notice. Dr Peter McEvoy taught me to think critically about scientific papers and to expand my perceptions of the insect's role in ecology. I welcome his advice and guidance.

Dr Jack Lattin has acted as my Hemipterist Guru, helping me tell an embolium from a connexivum and providing me with valuable connections around the world. Dr Norm Anderson's avuncular good cheer and encyclopedic knowledge of local fauna have brightened many of my Corvallis mornings. Dr Arthur Boucot has always treated me as a colleague and has proved an amazing

repository of palaeology and experience, and his dedication to natural history is exemplary. Dr Sujaya Rao has been a champion of creating venues to let me get my bugs and other amazing insects into the public eye.

Dr Dawn Wilson at American Museum of Natural History Southwest Research Station was a wonderful host for the three summers I was in Arizona. Dr George Mittendorf, Dr Lee Dyer, Drs Fred and Nancy Gelbach, and Dr Piotr Jablonksi contributed to an amazing atmosphere of shared knowledge and curiosity. Kelly Lasater was instrumental in creating and sustaining the feeling of shared adventure and exploration that permeated my time at SWRS.

Dr John Dunley at Washington State University Tree Fruit Research and Extension Center hired me as a field hand and insect photographer in 2001 and told me I'd miss my calling if I didn't become an entomologist. Through his support and encouragement I found my way to graduate school at OSU.

Dr Peter Wimberger, Dr Stuart Merrill, Dr Dennis Paulson, and Dr Alexa Tullis at University of Puget Sound welcomed a young Asian Studies undergraduate's curiosity about biology and always pointed me in the direction of more knowledge.

Finally, the deepest and warmest gratitude to my parents, Jon and Karl Pelegrin, for their faith in me over the years.

## CONTRIBUTION OF AUTHORS

Dr. David A. Lytle gave valuable advice in the design, implementation, and editing of this thesis, and provided financial support to Arthur L. Pelegrin in the form of research assistantships during Fall 2004, Winter and Summer of 2005, and Spring and Summer 2006. Dr. Lytle is the co-author of Chapters 2 and 3.

## TABLE OF CONTENTS

	<u>Page</u>
CHAPTER 1:	
Introduction.....	2
 CHAPTER 2: Phenotypic response to two distinct thermal ecologies in a giant waterbug .....	 7
Abstract.....	7
Introduction.....	8
Study system.....	9
Materials and Methods.....	13
Results.....	20
Discussion.....	30
Summary.....	38
 CHAPTER 3: Bergmann's Rule in allopatric populations of the giant waterbug <i>Abedus herberti</i> .....	 39
Abstract.....	39
Introduction.....	40
Materials and Methods.....	44
Results.....	46
Discussion.....	52
Summary.....	59
 CHAPTER 4: Rearing techniques for <i>Abedus herberti</i> .....	62
 CHAPTER 5: Summary and Conclusions.....	76
 Acknowledgements.....	80
 Bibliography.....	81

## TABLE OF CONTENTS (Continued)

	<u>Page</u>
APPENDICES.....	85
Appendix I: A case against speciation.....	86
Appendix II: Dyar's Rule and nymph morphometry.....	90

## LIST OF FIGURES

	<u>Page</u>
1.1. The Madrean Sky Islands are a series of isolated mountain ranges in between the Mogollon Rim and the Sierra Madre Occidental.....	2
2.1. Madrean Sky Islands and Study Population Localities.....	15
2.2. A diagram showing where length and width were measured on <i>Abedus herberti</i> adults and nymphs.....	16
2.3. Wild-caught adult <i>Abedus herberti</i> morphometry.....	21
2.4. Lab-bred <i>Abedus herberti</i> first-instar nymph morphometry.....	22
2.5. Lab-bred <i>Abedus herberti</i> second-instar nymph morphometry.....	23
2.6. Mean intermolt times of lab-bred <i>Abedus herberti</i> nymphs.....	24
2.7. A strict consensus tree.....	27
2.8. Comparison of thermal regimes in three mountain-type ( <i>M</i> ) and three ciénaga-type ( <i>C</i> ) environments.....	29
2.9. Temperature fluctuations during the same period of 2004 in an exemplary mountain stream (M3) and a ciénaga creek (C1).....	30
3.1. Map of collection localities in southern Arizona (USA) and northern Sonora (Mexico).....	45
3.2. The mean lengths and widths of 611 <i>Abedus herberti</i> individuals from fifteen populations in southeastern Arizona and northern Mexico.....	48
3.3. A linear regression of length as a function of elevation in 15 populations of <i>Abedus herberti</i> in the Madrean Sky Islands.....	48
3.4. A linear regression of width as a function of elevation of 15 populations of <i>Abedus herberti</i> in the Madrean Sky Islands.....	49
3.5. <i>A. herberti</i> body length positively correlated with pop. latitude.....	50
3.6. <i>A. herberti</i> width insignificantly correlating with pop. latitude .....	51

## LIST OF FIGURES (Continued)

	<u>Page</u>
4.1 Silver markings on <i>Abedus herberti</i> .....	62
4.2 <i>Abedus herberti</i> oviposition.....	65
4.3 Second-instar nymph and exuvia shortly after molting.....	68
4.4 Portion cups with bolts for nymph segregation.....	68
4.5 Porous tubs allow nymphs to share water reservoir.....	69
4.6 Darkened wingpads of fifth-instar indicate imminent molt.....	70

## LIST OF TABLES

	<u>Page</u>
2.1. Six populations of <i>Abedus herberti</i> , number of live adults captured, and average adult dimensions.....	14
2.2. Mortality in <i>Abedus herberti</i> nymphs descended from six native populations, reared at 20°C and 10°C in the laboratory.....	24
2.3. Sample size, haplotype richness, and numbers of haplotypes found in each population.....	26
3.1. Size and locality information for fifteen populations of <i>Abedus herberti</i> , collected 2004-2006.....	47
3.2a. ANOVA of length as a function of elevation .....	49
3.2b. ANOVA of width as a function of elevation .....	49
3.3. A summary of regression analyses performed on eleven non-ciénaga populations of <i>Abedus herberti</i> .....	51
3.4. <i>t</i> -test results comparing males and females on the parameters of length and width, per population.....	52



## LIST OF APPENDIX TABLES

	<u>Page</u>
A1.1. Results of 13 crosses of <i>Abedus herberti</i> from different populations.....	87
A2.1. Number of broods, length and width of each instar in millimeters, and number of nymphs surviving at each instar ( <i>n</i> ), grouped by population.....	89
A2.2. Dyar's Values for length and width of six populations of <i>Abedus</i> <i>herberti</i> raised in the laboratory.....	90

## Dedication

I dedicate this thesis to my parents, Jon Slaughter Pelegrin and Karl Arthur Pelegrin. They taught me how to view the world in fine detail, and instilled me with a sense of adventure and love of nature that helps me find my path every day. My mother showed me the secret worlds under stones and rotten logs, showed me how to cup an insect in my hand, and let me carry little jars of mosquito larvae and embalmed octopi to school. My father regaled me with tales of inner New Guinea, Greenland, the Sudan, Egypt, Kurdistan and Paraguay, and taught me how to make tools and tell stories. There is more than mere heritability at work here. I see the magic of the universe through my parents' eyes.

**Phenotypic Evolution as a Response to Thermal Ecology in the Ferocious  
Waterbug *Abedus herberti* (Hemiptera: Belostomatidae)**

**by  
Arthur Lomis Pelegrin**

## CHAPTER 1

### INTRODUCTION

The Madrean Sky Islands of southeastern Arizona and northern Sonora, Mexico are a series of abrupt mountain ranges that rise from expanses of flat, arid desert. Because they are largely unconnected to each other, they form insular habitats as determined by elevation. This is especially true in the case of aquatic habitats such as streams, which persist perennially in the Madrean Sky Islands between elevations of 1400 and 2200 meters. Except when flooded, these streams do not converge, so there is not a continuum of flow between river deltas and headwaters; rather, the streams are islands of water in a sea of desert.

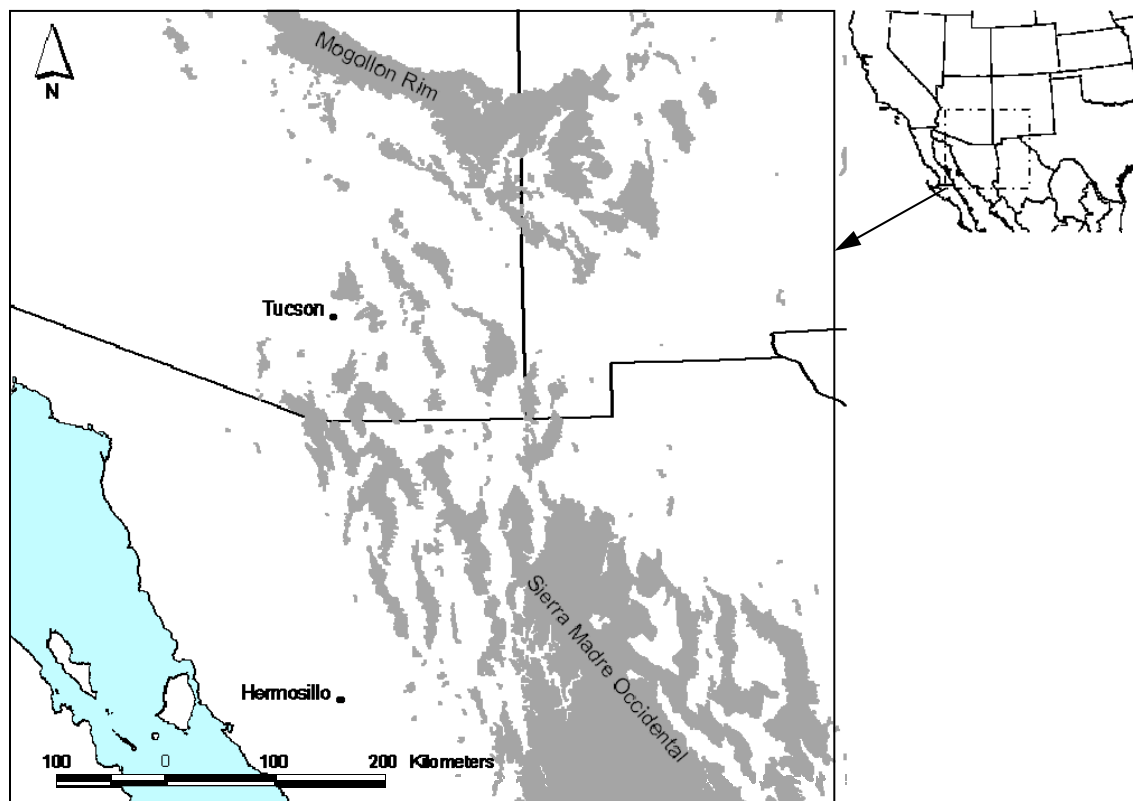


Figure 1.1: The Madrean Sky Islands are a series of isolated mountain ranges in between the Mogollon Rim and the Sierra Madre Occidental.

Aquatic animals living in these streams are effectively stranded, forming isolated populations that are subjected to highly-localized conditions of temperature regime and flow. If these animals have no way to disperse over miles of dry desert, then they are relegated to their natal waterway. For invertebrates that lay dozens or hundreds of eggs per clutch, intraspecific competition for limited resources is typically high, resulting in strong selective pressure favoring individuals who are more suited to the particular conditions of their stream.

Living in streams and pools from southern Utah to northern Mexico is the ferocious waterbug, *Abedus herberti* Hidalgo (Hemiptera: Belostomatidae). This is a large predatory insect with an appetite for any animal it can overpower. Though it has wings, it is flightless, which seriously impairs its ability to disperse (Menke, 1961; Lytle, 1999). It breathes air through retractable straps on the tip of its abdomen and is thus freed from dependency on dissolved oxygen. It can tolerate a wide range of thermal conditions and has few natural enemies.

Members of our laboratory on field expeditions to discover populations of *Abedus herberti* began to encounter individuals in some populations that appeared small in size. Several other *Abedus* species are known from the area, but breathing-strap chaetotaxy and male genital morphology indicated that these diminutive bugs were small individuals of *A. herberti*. Populations of small bugs were rarely encountered; they appeared to coincide with low-elevation, spring-fed marshes and creeks known locally as *ciénagas*. Preliminary data from previously-deployed temperature probes suggested the *ciénaga* habitats were warmer and more thermally stable than the creeks at higher elevation in the mountains.

Temperature has repeatedly been cited as a major factor in the evolution and ecology of insects (Hynes, 1970; Mitchell, 1974; Markarian, 1979; Ward and Stanford, 1982) and poikilotherms in general (Ray, 1960; Atkinson, 1994). The general trend, first noted by C. Bergmann in 1847, is that populations within a species tend to comprise larger individuals as altitude and latitude increased. Bergmann's Rule originally applied to mammals; however, the pattern has been observed in a diversity of taxa (Atkinson, 1994).

In addition to temperature, other ecological factors could also be affecting body size in *A. herberti*. The seasonal shift of community composition in the mountain streams as flow abated and aquatic habitats shrank to predator-dominated, oxygen-poor pools (Bogan and Lytle, in press) and the longer winter at higher altitudes both might exert selective pressures on the body size of these insects.

The first step in any study investigating phenotypic variation and selection is to determine if the variation witnessed in natural populations is due to heritable differences or habitat-dictated phenotypic plasticity. To assess this, I employed a common-garden experiment using offspring of wild-caught individuals from ciénagas and mountain streams to illustrate some aspects of the nature of the differences in size between the two types. Specifically, I sought to answer whether the size disparity was a heritable character or the result of individual response to environmental cues (phenotypic plasticity) and whether the offspring of larger-sized bugs would develop more slowly.

I further hypothesized that the size disparity in *Abedus herberti* populations in southern Arizona and northern Mexico would follow Bergmann's Rule, independent of special ecological considerations like the difference between mountain and ciénaga habitat types. To test this, I measured the length and width of 611 individuals from fifteen populations distributed across a gradient of elevation, and to a lesser degree, a gradient of latitude. I employed knowledge about the habits and ecology of *A. herberti* to explore possibilities of why Bergmann's Rule would serve this animal in an adaptive capacity.

Chapter Two handles the ecotypic differences between mountain and ciénaga populations, examining adult body length and width among three of each type, and comparing that to the body dimensions of nymphs raised in a common-garden experiment. The intermolt times of the two types of nymphs are compared to see if there is a difference in the developmental rate between ecotypes. Chapter 2 also examines mitochondrial DNA to establish relationships between mountain and ciénaga populations, so as to assess whether the body-size similarities are due to convergence or to shared ancestry.

Chapter Three examines the length and width of 611 adult *Abedus herberti* from fifteen disjunct populations, distributed across an elevational gradient of 929-1900 meters and a latitudinal gradient of approximately 2.7°. This was undertaken to see whether *Abedus herberti* obeys Bergmann's Rule with respect to altitude and/or latitude.

Chapter Four describes in detail the rearing techniques I developed for maintaining a culture of *Abedus herberti* in the laboratory. Future researchers may use this as a reference to sidestep many potential catastrophes.

The differences in body size between mountain and ciénaga bugs were found to be significant. In the common-garden experiment, the significance of these differences persisted in nymphs raised under identical conditions. This suggests the size differences are inherited, rather than a phenotypically plastic response to environmental cues. The ciénaga bugs developed significantly more quickly than the mountain-type bugs. Mitochondrial DNA analysis did not provide evidence for common ancestry among ciénaga bugs or among mountain bugs with respect to the character of body size. Body size was found to correlate significantly with elevation on metrics of both length and width. Body size was found to correlate significantly, albeit weakly, with latitude on the metric of length, but insignificantly on the metric of width. These results support the general predictions of Bergmann's Rule and suggest that these populations of insects are evolving in response to their thermal environments by increasing or decreasing their mean body size. Further research to pinpoint the mechanism for this response might include the formulation of a degree-day model for the different ecotypes and an extensive survey of *Abedus herberti* body size throughout its range.



## CHAPTER 2

Phenotypic response to two distinct thermal ecologies in a giant waterbug

### ABSTRACT

This study documents the magnitude of variation in size and in life-history traits between populations of the ferocious waterbug *Abedus herberti* living in two distinct regimes of temperature and flow: mountain-runoff stream and ciénaga outflows. Mountain populations are characterized by larger body size and slower development. The thermal regime of mountain populations fluctuates throughout the year more so than that of ciénaga populations, and the mean annual temperature is lower. Common-garden experiments showed that body size is not a phenotypically plastic trait, in that the broods of mountain and ciénaga bugs raised under identical conditions consistently maintain significantly different body sizes. Furthermore, comparison of intermolt time between all individuals shows mountain populations take significantly longer to molt than ciénaga populations. Comparative examination of mitochondrial DNA show that the ciénaga populations do not form a monophyletic group, so the differences in body size are likely due to convergence rather than shared inheritance. AMOVA results indicate that genetic variation does not account for the variation in body size, suggesting that the body size and life history traits are shared by the ciénaga populations via a similar evolutionary response to similar conditions. These results support the hypothesis that *Abedus herberti* populations have repeatedly

and independently evolved body sizes suited to local environmental conditions, possibly due to selection for optimal thermal physiology (smaller body size favored at higher mean temperatures) or size at maturity (ability to reproduce year-round favors earlier maturity at a smaller size).

## INTRODUCTION

While collecting individuals of the ferocious waterbug *Abedus herberti* from disparate populations in the Madrean Sky Islands over several years, members of our research team began to note that individuals some populations appeared to vary in size from the typical norm for this species. This appeared to correlate with a specific, uncommon habitat-type: the outflows of thermally-stable springs called ciénagas. Comparative measurement of length and width of hundreds of individuals confirmed the initial observation: populations inhabiting ciénagas are significantly smaller than their counterparts living in thermally-variable runoff streams.

The morphological adaptation of these populations is either an evolutionary response to environmental conditions that has become ensconced in the genes themselves, or it is a result of phenotypic plasticity on the part of individuals reared under conditions that evoke a developmental response. I designed a common-garden rearing experiment to examine these possibilities: under identical conditions, the morphometric differences between populations should be diminished if their response is due to phenotypic plasticity. Alternately,

if the size variation has a strong heritable component (genetic or maternal) the significance of the variation will persist. Molecular analysis will support multiple autapomorphic origins of small body size, i.e. repeated convergence on similar size-characters in similar environments, rather than populations of like body size being related through common descent.

### STUDY SYSTEM

The present-day fragmented habitat of *Abedus herberti* Hidalgo in southeastern Arizona consists of isolated streams and pools in the Madrean Sky Islands, a series of northwest-southeast trending tilted fault-block mountain ranges separated by expanses of arid desert. The streams themselves run through rocky oak and pine forests and are not part of a perennial river system and dry up before they reach the desert, except under flood conditions (Lytle & Bogan, in press). The aquatic habitats are insular, being unconnected to any greater waterway for most of the year.

Although current populations of *Abedus herberti* are generally isolated, they were likely connected as recently as the late Pleistocene. Some of the basins between Sky Island ranges contain ephemeral lakes during the wet season and playas during the dry. Considerable evidence suggests these basins used to be vast pluvial lakes during the Pleistocene. Palynology (Martin, 1963), radiocarbon stratigraphy (Long, 1966), marl analysis (Woosley and Waters, 1990), and archaeology (*ibid.*) all support the presence of perennial lentic habitats during the

Wisconsin Age (35-10 kya). While *A. herberti* is not usually a lake-dweller, the quondam lakes are evidence of a much wetter climate in the region. Ancient arroyos located in contemporary arid desert (Waters and Hayne, 2001) indicate a much greater degree of connectedness between waterways. *Abedus herberti* or its ancestor existing during this era probably formed large, panmictic populations with greatly-diminished barriers to gene flow compared to present-day. By 8 kya, however, the lakes had begun to dry up as the climate began its shift to current conditions. Perennial bodies of water became ephemeral, and aquatic organisms followed the retreating waterways into the hills where at least some water persisted throughout the year.

To date, approximately 40 currently disjunct populations have been identified (Lytle, unpublished data). At least two of these populations, Ash Canyon in the Galiuros Mountains and French Joe Canyon in the Whetstones, have likely gone extinct during the last five years, as their habitat dried up during droughts (Lytle, unpublished data). Although these animals are able to walk on land and breathe air, they are not well-suited to terrestrial migration. Successful dispersal by walking from one reach to another across miles of arid terrain, undefended against predators and dehydration, is unlikely. However, they are able to traverse overland short distances during rainstorms, when their flash-flood avoidance response has been triggered (Lytle, 1999) and conditions are moist. While the dried reaches may again become habitable in wetter years, no satisfactory mechanism of recolonization has been posited. It is conceivable that sustained diluvial events could cause widely separated drainages to unite,

allowing animals like *A. herberti* to disperse, exchange genes and establish in new streams. However, mitochondrial analysis is consistent with post-Pleistocene isolation (Lytle et al, in review); migration and miscegenation appear to be rare.

Nymphs undergo five immature instars before attaining adulthood. There was a 1.29x increase in length and 1.27x increase in width between molts ( $\pm 1.2\%$  depending on population, Appendix 3).

Menke (1960) splits *Abedus herberti* into two subspecies, *A. herberti herberti* and *A. herberti utahensis*, on the basis of size and breathing strap morphology. The latter is confined to the Colorado River drainage of southern Utah and northern Arizona, and comprises the shortest populations in the length distribution. *A. herberti herberti* ranges from 29mm to 39mm between population. Unless otherwise stated, references to *Abedus herberti* refer only to *Abedus herberti herberti sensu* Menke, 1960.

These insects are typically large ( $\bar{X} = 35\text{-}39\text{mm}$  adult body length) but several populations of small ( $\bar{X} < 32\text{mm}$ ) bugs have been found. The small bugs tend to be found in thermally-stable spring-fed streams called ciénagas, whereas the more typical large bugs are found in mountain runoff streams. The bugs belong to the same biological species and are capable of interbreeding (Appendix 1). The difference in body size between the large (mountain-type, hereinafter *M*-type) and small (ciénaga-type, hereinafter *C*-type) bugs probably derives from one of two conditions: first, selection acts differently on body size between the different ecological regimes, resulting in genetic fixation of size-related traits, or second, individual bugs respond to their particular environments during the course

of development, via phenotypic plasticity. If the first condition is true, and there is a heritable component to body size, two scenarios could explain the geographic distribution of this trait: first, repeated, isolated optimization of body size to local environment; or second, ancestral selection with subsequent dispersal or vicariance to their current locations.

In addition to examining the heritability of body size, this study also seeks to examine the possible mechanisms by which temperature might directly and indirectly affect body size. Even if body size is found to be a heritable trait, there are a number of ways that the local environmental conditions could exert selective pressure so as to favor a different average body size. One possibility is that mean temperature, as predicted by altitude, drives selection for a particular optimal body size via strictly physiological considerations (Thermal response hypothesis, Chapter 3). A second hypothesis is that local thermal ecology causes selection for an earlier age of maturity in *ciénaga* regimes because the period of the year favorable to growth and reproduction is much longer than that of mountain environments, thereby providing the possibility of multiple generations and hence greater fitness (Quick Ripening hypothesis). A third possibility is that adult size is the result of ancillary selection (*sensu* Smith, 1997) and that direct selection is acting upon the size of the eggs or the first-instar nymphs by favoring a higher energy-content egg and first-instar in colder environments, and a smaller egg-size in warmer environments to accommodate the constraint of limited male back space (Ancillary Selection hypothesis). The fourth hypothesis is a corollary to the third, such that selection favors larger nymphs in the mountain habitats because of

the intense competition between conspecific nymphs as well as other predators that occurs during the low flow phase of the river before the summer monsoons arrive (Tough Nymph hypothesis).

This study approaches these possibilities by testing the hypothesis that body size in *Abedus herberti* is heritable, and not strictly a result of phenotypic plasticity, and that intermolt time decreases with adult body size. A common-garden rearing design will show that body size differences between populations are conserved, even when individuals are raised from eggs under identical thermal regimes. This study also examines the mitochondrial heritage of the populations in question, to rule out the possibility of shared inheritance as an agent for similarity of body size among populations. This would further bolster the hypothesis that the body size and life-history differences between mountain bugs and ciénaga bugs have a heritable component. Aspects of the ecology, habits and environment of *Abedus herberti* are taken into consideration to assay which of the evolutionary scenarios (Thermal Response, Quick Ripening, Ancillary Selection and Tough Nymph) is best supported.

## MATERIALS and METHODS

Six wild populations of *Abedus herberti* were selected, three *C*-type and three *M*-type, from southeastern Arizona (Figure 2.1). *C*-type populations were chosen on the basis of availability: at the outset of the study, only these three populations had been definitively identified as *C*-type. Three *M*-type populations

were chosen to balance the three *C*-type populations. When possible, *C*-type populations were paired with nearby *M*-type populations, for purposes of comparing putative genetic likeness in morphologically-different, geographically-proximal populations. Adult lengths and widths were extensively surveyed (Chapter 3). Twenty-five live adult individuals were taken from each location, except C2 (Leslie Canyon NWR) because only ten adults and nine fifth-instar bugs could be found after an exhaustive search of the habitat; C3 (French Joe Canyon) similarly yielded only 21 adult individuals. Bugs were transported in coolers with a layer of damp leaves at the bottom.

The fifth-instar *C*2 bugs were reared to adults in Arizona in plastic tubs on diets of tadpoles, crickets, mealworms and aquatic Hemiptera. All adult bugs were transported to Oregon in sealed plastic food containers lined with layers of damp paper towel.

Table 2.1: Six populations of *Abedus herberti*, number of live adults captured, and average adult dimensions.

Population	Type/Code	Adult <i>n</i>	Average length (cm)	Length s.e.	Average width (cm)	Width s.e.
Gardner	M1	25	3.504	0.012	1.911	0.009
Rucker	M2	25	3.619	0.014	1.99	0.010
East Turkey	M3	25	3.5	0.010	1.924	0.007
Ciénaga Cr	C1	25	2.96	0.021	1.593	0.014
Leslie	C2	19	3.274	0.034	1.77	0.020
French Joe	C3	21	3.243	0.026	1.709	0.019



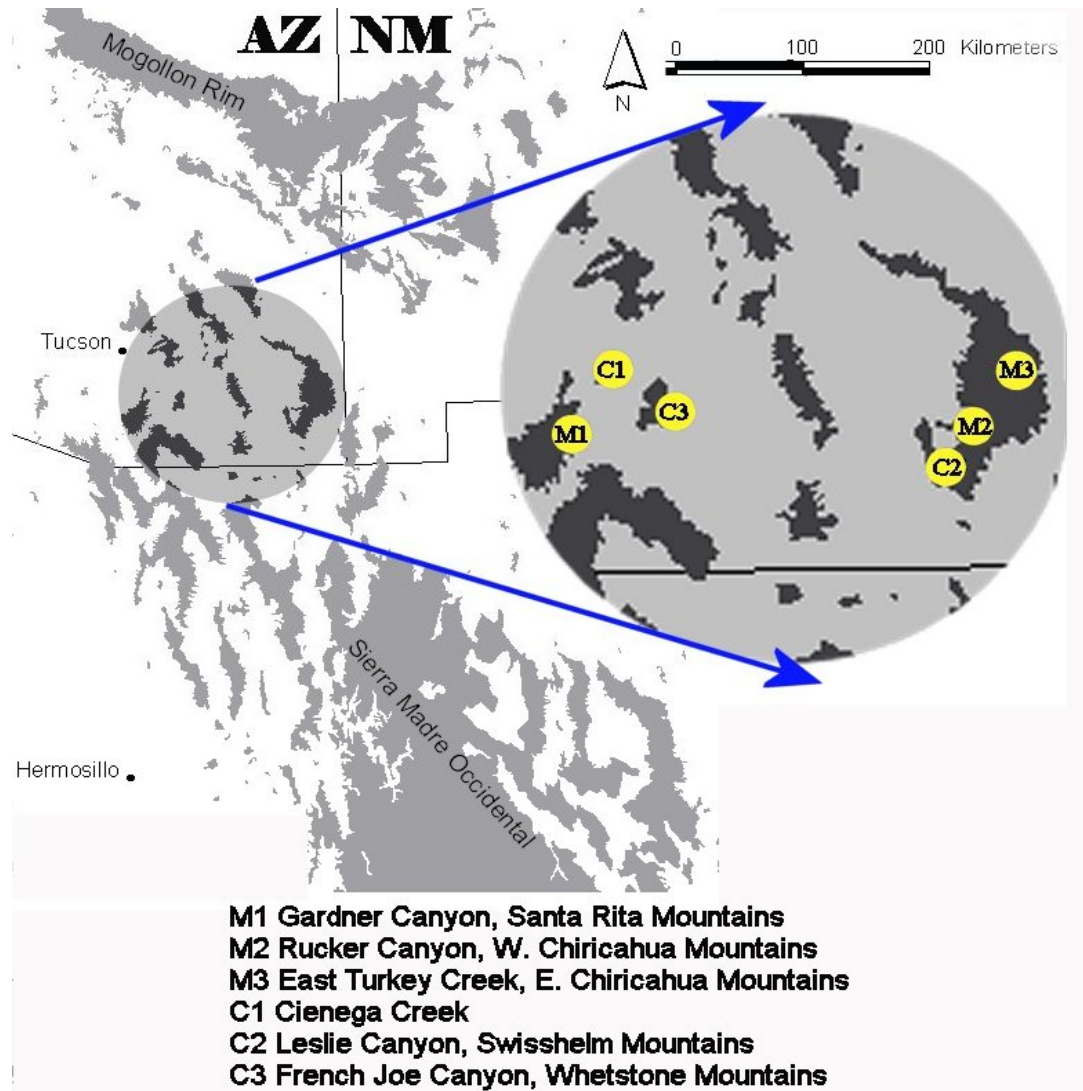


Figure 2.1: Madrean Sky Islands and Study Population Localities

Chapter 4 of this work describes in detail maintenance, husbandry and rearing of these animals. The bugs were placed in white plastic tubs (36cm x 29cm x 18cm) containing two red bricks and water up to the level of the brick. This simulated their native rocky substrate while also preventing the bugs from wedging themselves under anything, a problem when trying to ensure all individuals had eaten.

Bugs were marked with nontoxic silver Sharpie® on their hemelytra. Each bug received a distinct caste-mark to indicate population, a unique number, and a gender symbol. Length and width were recorded for each individual. Following the technique of Keffer and Macpherson (1988), length was measured on bugs at rest, from the tip of the tylus to the posterior edge of the connexivum, exclusive of air-straps (Fig. 2.2). Of 100 re-measured individuals, 40 had a maximum error of 1mm, yielding a mean error rate of  $\pm 0.4$ mm.

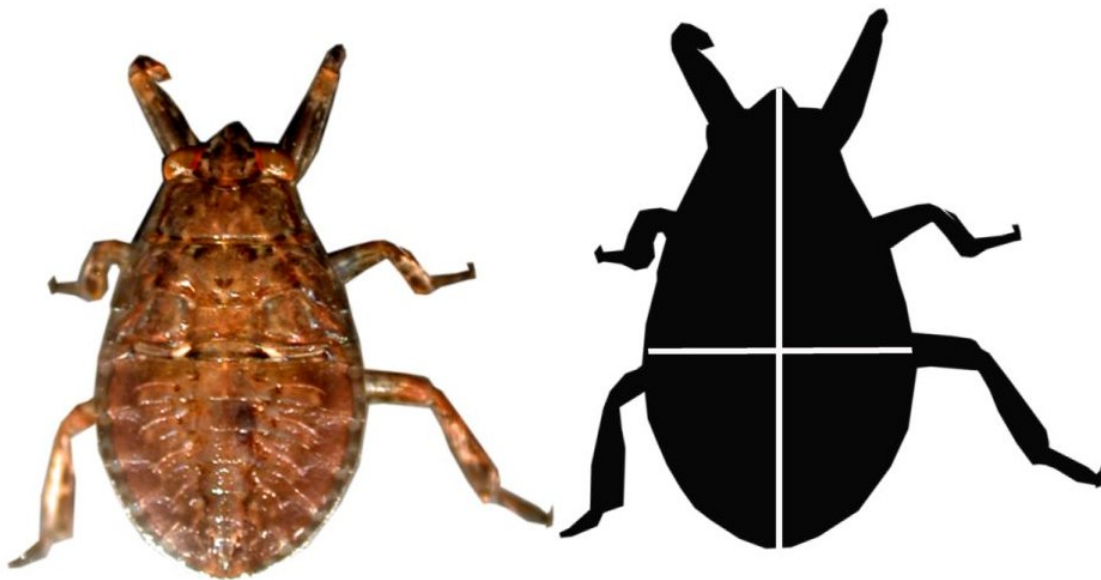


Figure 2.2: A diagram showing where length and width were measured on *Abedus herberti* adults and nymphs. Left: a fourth-instar nymph.

All adults were kept at 20°C on a 14/10 light/dark regime, to mimic summertime in Arizona. Bugs were fed twice a week on crickets and mealworms raised on Total® cereal to ensure nutritional repletion. Water was changed once a week.

### *Mating*

Males and females were kept in separate tubs at first, then after three weeks in their new environment, were combined per population in single tubs and allowed to mate *ad libitum* until there were two broods per tub.

Brooding males were isolated in clear plastic obstetric tubs (8cm tall, 13cm basal diameter, 1 liter) with an apple-sized river rock in the middle. This allowed the males to rest with the eggs partially submerged.

The eggs hatched 24-28 days after deposition. Twenty-four nymphs per brood were randomly selected after the entire brood had hatched, and sequestered in 4-ounce (118mL) portion cups. Emergent vegetation was standardized by placing an upside-down steel hex bolt (3cm long, .9cm wide, 7 threads/cm) in the center of the cup. The threads provided grasping-substrate for the nymphs and also served as an anchor for molting. Each nymph was assigned a patronymic number and an individual letter, written on its cup, and its length and width were measured to the nearest 0.1mm through an ocular micrometer affixed to a dissecting microscope. Error was  $\pm 0.1$ mm. Each brood-set of 24 nymphs was randomly divided into two groups of twelve, and then each group was randomly assigned to a “warm” (20°C) room or “cool” (10°C) room. These thermal regimes were chosen to reflect typical summertime ciénaga temperatures and late spring mountain temperatures. The lighting remained identical in both rooms.

There were two broods per population used in this analysis. Some of the populations produced three broods during the reproductive window; in such cases,

one egg-bearing male was randomly excluded from the study and had his eggs stripped off his back.

First-instar nymphs were fed one *Drosophila melanogaster* per day. Later instar nymphs received young crickets, young mealworms, and carpenter ants, in the amount of one prey-item per day. Bugs were checked daily in late afternoon to tally molts and deaths. When a bug molted, the date was recorded and the nymph given time to darken before being removed from the water and measured.

After reaching the fourth instar, nymphs were transferred to 16-ounce (473 mL) round clear plastic tubs with 5mm holes drilled into the base. These were weighted with a small rock, which also served as substrate for the nymph, and placed in a larger tub filled with water to just below the level of the rock. In this way several nymphs could share the same reservoir of water.

Teneral imago bugs were kept sequestered from other adults for at least three weeks, because individuals who were integrated too quickly were eaten.

### *Molecular techniques*

Nine to 31 individuals from each population were stored in 95% ethanol for transport to the lab. Juveniles were taken from populations with few adults, and samples were augmented with specimens from a tissue library, wherein a single hind leg of previously collected individuals was preserved in 100% ethanol in a -20°C freezer. Live individuals from C2 and C3 that died in captivity were similarly preserved and sequenced. A single hind leg was removed from each incoming specimen and ca. 25 mg of coxal muscle tissue was used for DNA

extraction using a Qiagen DNEasy kit. I PCR-amplified a mitochondrial product that included slightly  $>1/3$  of the COI gene at the 3' end, the entire tRNA-leucine, and most of the COII gene using primers C1-J-2441 ("Dick") and C2-N-3661 ("Barbara") listed in Simon *et al.* (1994). PCR reactions included 36.4  $\mu$ l ddH<sub>2</sub>O, 6  $\mu$ l 10X buffer, 9.6  $\mu$ l 25 mM MgCl<sub>2</sub>, 1.2  $\mu$ l 10 mM dNTPs, 1.2  $\mu$ l each primer (10  $\mu$ M), 0.4  $\mu$ l *Taq* polymerase, and 4  $\mu$ l template DNA at 1/10 dilution. These were subjected to the following program: 95°C for 3 min, 50°C for 1 min, 72°C for 1.5 min, 30 cycles of {95°C for 1 min, 50°C for 1 min, 72°C for 1.5 min}, 72°C for 4 min, final hold at 4°C. Products were purified using the UltraClean PCR Clean-up Kit (Mo Bio Laboratories) and sequenced on an ABI Prism 3730 DNA analyzer using PCR primers. Consistently, I was able to obtain reliable single-strand sequences extending to 700-750 base pairs using PCR primers such that pairwise alignment of complementary strands was reliable using BioEdit 7.0.5.3 (Hall, 1999). After removing questionable sequence at the extreme 5' and 3' ends, I retained a 990-bp interior fragment for molecular analyses.

The phylogenetic tree was generated using a strict consensus of 126 equally-parsimonious trees in PAUP (length = 267 steps, CI excluding uninformative characters = 0.6697, RI = 0.8065, based on a heuristic search of 1000 random addition replications and equal weights; Swofford, 1993). The subspecies *Abedus herberti utahensis* and the electric light bug *Lethocerus medius* were used as outgroups. Kishino-Hasegawa comparisons were performed in PAUP with alpha set to 0.05.

### *Temperature*

Thermal variation in native habitats was recorded by placing a Hobo Probe® data logger into a deep pool in each reach. Temperature was logged hourly for at least six months. In some cases, due to relentless probe theft, flash flooding, and drying down, data for the particular reaches in the study was unavailable. When possible, data from nearby reaches at the same elevation in the same drainages were used as proxies.

### *Statistical Analysis*

Analysis of variance (ANOVA) was used to assay difference between ecotypes. ANOVA was performed using S-Plus 7.0. Populations were separated into cadres of *M*-type and *C*-type for purposes of comparison. Analysis of molecular variance (AMOVA, Weir, 1996) was used to test whether size variation could be explained by genetic variation.

## RESULTS

### *Morphometry and Life History*

Wild-caught *M*-type bugs differed significantly from *C*-type bugs in both length and width (ANOVA:  $F = 433.176$ ,  $df = 262$ ,  $p < 0.001$  for length and  $p <$

0.001 for width, Fig. 2.3). Variance within populations was inconsequential (s.e < 0.04 in all cases).

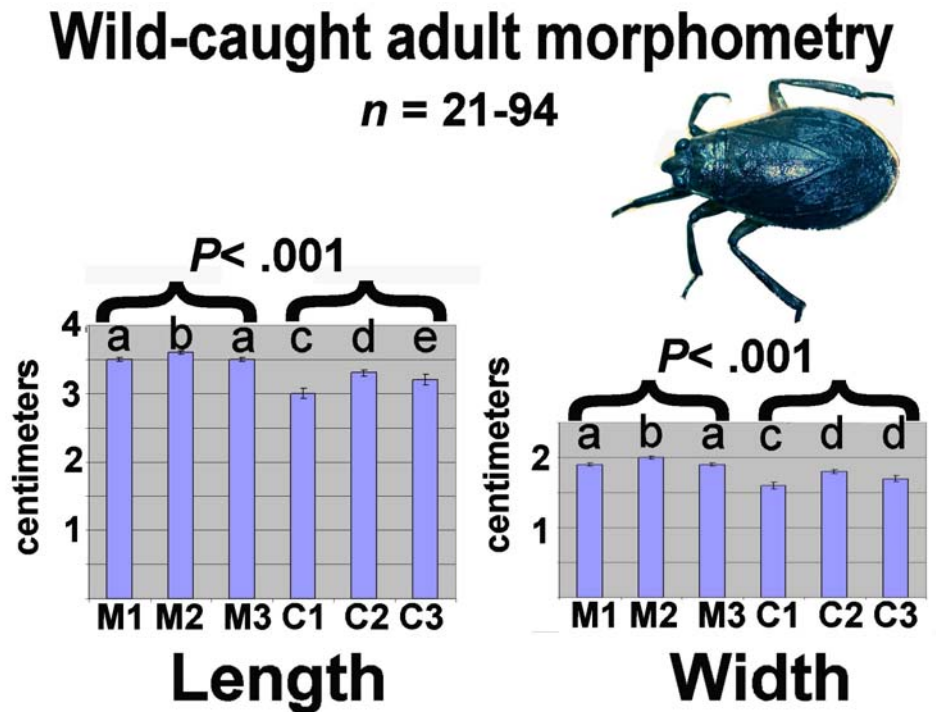


Figure 2.3: Wild-caught adult *Abedus herberti* morphometry. Mean lengths and widths are shown for three mountain stream populations (M) and three ciénaga populations (C). Brackets indicate significant differences between groups M and C. Mountain populations were significantly longer and wider than ciénaga populations. Lower-case letters indicate significant within-group differences.

Most of the bugs in the 10°C room (93%) did not molt a single time, and of those that did ( $n=13$ ), none of them molted a second time. Of thirteen molting nymphs, seven were from M1, five from M2, and one from C1. All second-instar measurements fell within the same range of variance as those of second-instars in the warm treatment. Average time to first molt was 47 days at 10°C. Nine other nymphs attempted the molting process, as evidenced by the assumption of a molting-pose (Smith, 1975) and the dorsally-split ecdysial suture, but these

individuals died before completing the molt. All bugs in the cool room continued to eat daily.

First-instar nymphs in both temperature regimes and second-instar nymphs in the 20°C regime perpetuated parental variance in both length and width (ANOVA: First-instar length  $F = 392.73$ ,  $df = 280$ ,  $P < 0.001$ ; First-instar width  $F = 233.96$ ,  $df = 280$ ,  $P < 0.001$ ; Second-instar length  $F = 153.90$ ,  $df = 141$ ,  $P < 0.001$ ; Second-instar width  $F = 149.37$ ,  $df = 141$ ,  $P < 0.001$ ; Figs. 2.4 and 2.5). There was a 1.29x increase in length and 1.27x increase in width between molts ( $\pm 1.2\%$  depending on population).

## Lab-bred first-instar nymph morphometry

$n = 48$

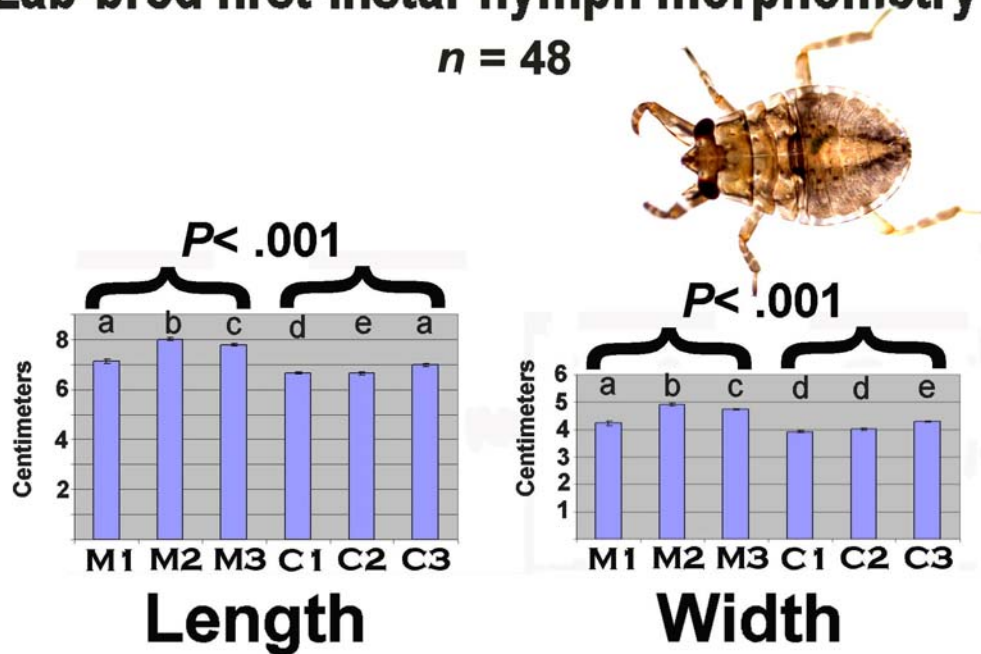


Figure 2.4: Lab-bred *Abedus herberti* first-instar nymph morphometry, measured before nymphs were sorted into temperature treatments. Mean lengths and widths are shown for captive descendants of three mountain stream populations (M) and three ciénaga populations (C). Brackets indicate significant differences between groups M and C. Nymphs of mountain ancestry were significantly longer and wider than nymphs of ciénaga ancestry. Lower-case letters indicate significant within-group differences.



## Lab-raised second-instar nymph morphometry

$n = 17-25$

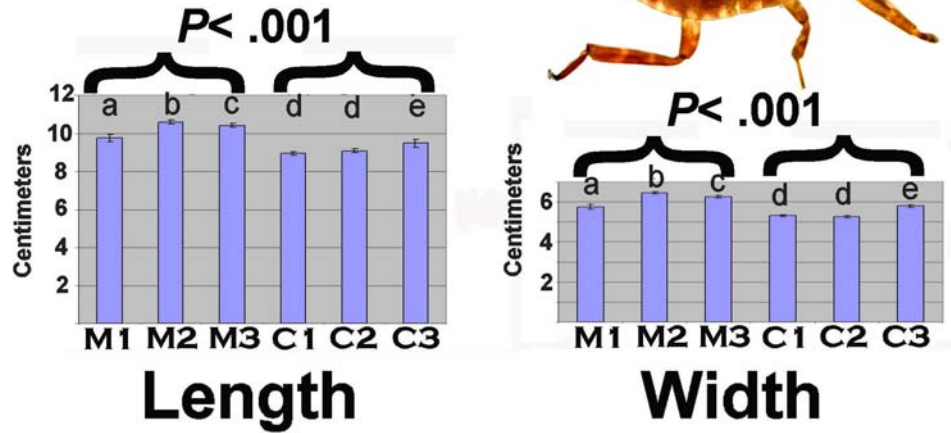


Figure 2.5: Lab-bred *Abedus herberti* second-instar nymph morphometry from the 20°C treatment. Mean lengths and widths are shown for captive descendants of three mountain stream populations (M) and three ciénaga populations (C). Brackets indicate significant differences between groups *M* and *C*. The mountain nymphs are significantly longer and wider than the ciénaga nymphs. Lower-case letters indicate significant within-group differences.

At 20°, nymphs molted regularly and without apparent difficulty. Age at first molt varied significantly between the two ecotypes (ANOVA:  $F = 15.23$ ,  $df = 130$ ,  $P = < 0.001$ , Fig. 2.6) as did the interval between first molt and second molt (ANOVA:  $F = 4.81$ ,  $df = 53$ ,  $P = 0.0326$ , figure 2.6). High mortality in the third instar interfered with experimental design balance, so third-instars and above were excluded from the analyses (Table 2.2).

Table 2.2: Survival in *Abedus herberti* nymphs descended from six native populations, reared at 20°C and 10°C in the laboratory. There were two broods per population, 24 randomly-selected nymphs per brood.

Population	<i>n</i> 1 <sup>st</sup> instars		<i>n</i> 2 <sup>nd</sup> instars		<i>n</i> 3 <sup>rd</sup> instars		<i>n</i> 4 <sup>th</sup> instars	
	20°C	10°C	20°C	10°C	20°C	10°C	20°C	10°C
M1	24	24	15	7	11	0	8	0
M2	24	24	20	5	9	0	5	0
M3	24	24	20	0	6	0	0	0
C1	24	24	21	1	8	0	3	0
C2	24	24	21	0	11	0	2	0
C3	24	24	17	0	10	0	3	0

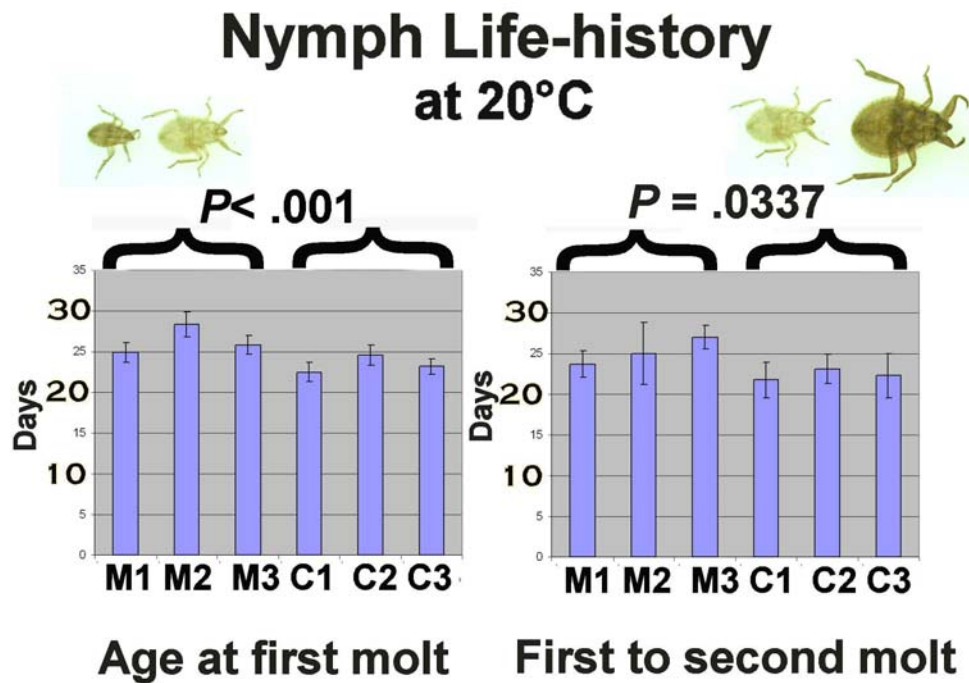


Figure 2.6: Mean intermolt times of lab-bred *Abedus herberti* nymphs raised at 20°C, shown for captive descendants of three mountain stream populations (M) and three ciénaga populations (C). **Left:** Age at first molt. **Right:** Interval between first and second molt. Brackets indicate significant differences between groups *M* and *C*. In both cases, the ciénaga nymphs molted significantly sooner than the mountain nymphs.

### *Molecular analysis*

The number of individuals typed from each population ranged from 9 to 31, and there were two to six haplotypes found per population (Table 2.3). There were 23 distinct haplotypes. A replete haplotype network that includes several more populations can be found in Lytle, Finn and Bogan (in review). Most of the haplotypes were private, or unique to a single population. This yielded 126 equally-parsimonious trees of length = 267 steps, CI excluding uninformative characters = 0.6697, RI = 0.8065, based on 1000 random addition replications, equal weights, rooted with the subspecies *Abedus herberti utahensis* (Menke, 1960) and the electric light bug *Lethocerus medius*. A strict consensus of these trees is presented in Fig. 2.7. None of the haplotypes grouped together by population or by habitat type, supporting the hypothesis that body size is a convergent trait rather than a character shared by common descent.

To further bolster this hypothesis, I performed a monophyly constraint test to see whether the length of an artificially-constrained tree (with respect to groups *M* and *C*) would be longer than the unconstrained tree. With group *C* constrained as a monophyletic group, the resulting tree was a consensus of 43 trees of 281 steps, CI = 0.822, RI = 0.731. I performed a Kishino-Hasegawa test to compare the trees and found the original tree to be significantly shorter than the *C*-constrained tree ( $t = 3.7661$ ,  $P < 0.001$ ). With group *M* constrained as monophyletic, the resulting tree was a consensus of 41 trees of 283 steps, CI = 0.8163, RI = 0.7204. A Kishino-Hasegawa test comparing this to the original tree

supported the hypothesis that the artificially-constrained tree was significantly longer ( $t = 3.7963$ ,  $P = 0.0002$ ).

Table 2.3: Sample size ( $N$ ), haplotype richness (% of total haplotypes present), and numbers of haplotypes found in each population. \* indicates private haplotype.

Population	M1	M2	M3	C1	C2	C3
$N$ :	25	20	20	19	14	15
Richness	.32	.27	.18	.18	.09	.18
Haplotype						
1	5					3
2				5		6
3		2				
4	1			4		4
5*	5					
6*	5					
7*						2
8*			7			
9*			10			
10*	3					
11*			3			
12*					13	
13*					1	
14*	5					
15*		13				
16*		2				
17*		1				
18*		1				
19*				4		
20*				6		
21*	1					
22*		1				

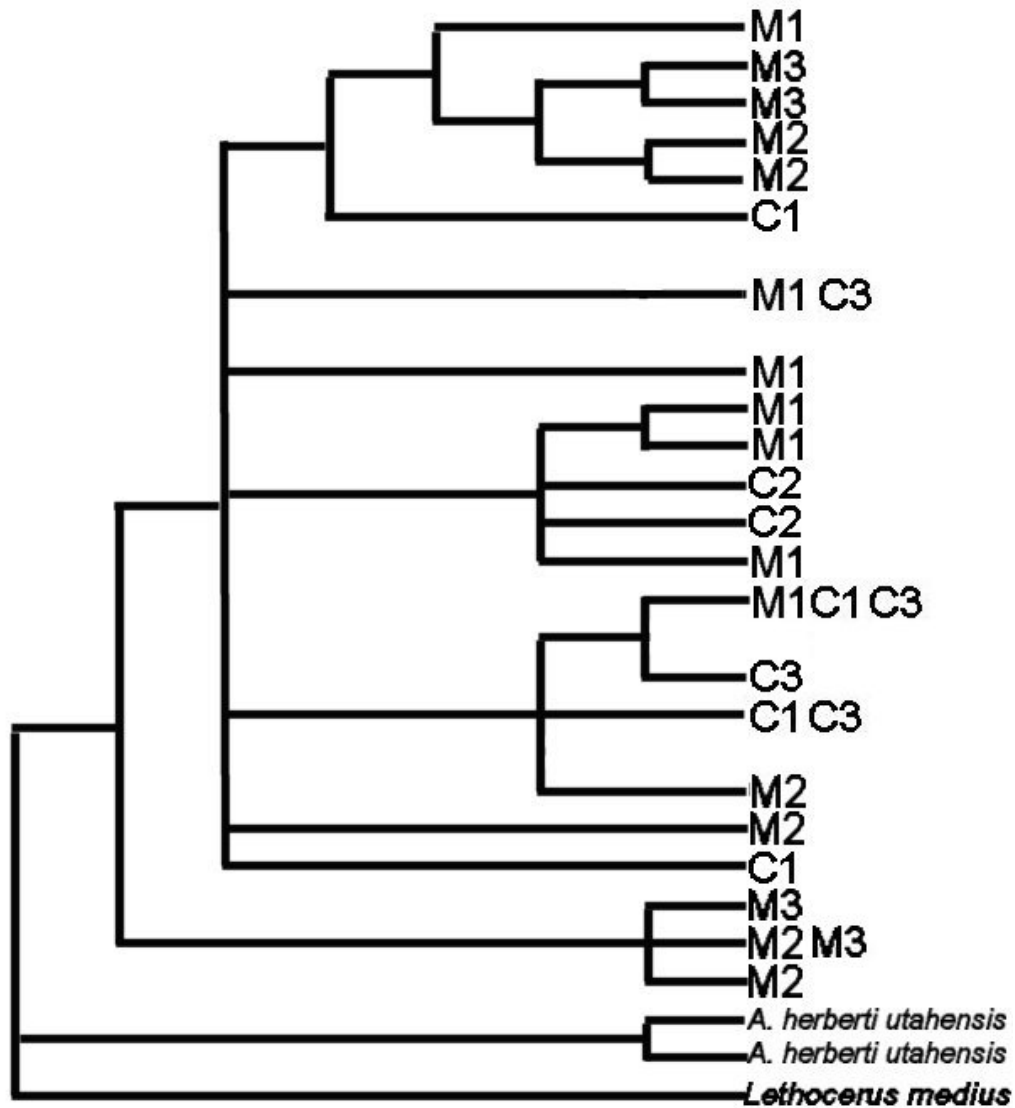


Figure 2.7: A strict consensus tree (126 equally-parsimonious trees of length = 267 steps, CI excluding uninformative characters = 0.6697, RI = 0.8065, based on 1000 random addition replications, equal weights, rooted with *A. herberti utahensis* and *Lethocerus medius*) *M*-type and *C*-type show arbitrary distributions relative to one another, indicating no common ancestry among types.

AMOVA results comparing groups *M* and *C*, to examine whether phenotypic variation can be explained by genetic variation, yielded an insignificant *p*-value of 0.196, indicating that phenotypic variation cannot be explained by genetic variation.

### *Temperature*

The data logger at *M2* washed away in a flash flood, so *M2* must be excluded from analyses. At the time of writing, a new data logger has been deployed and this final component of the analysis will eventually be in hand. For the moment *M2* shall remain a gap in the thermal data. However, its geography and local ecology are consistent with other *M*-type reaches in the region.

Mean annual temperature of the five remaining reaches showed a general trend of *C*-type habitat as warmer than *M*-type; the standard deviation of *C*-type and *M*-type varied substantially (Fig. 2.8). A comparison of *M*-type and *C*-type temperature fluctuation over a seasonal shift showed the greater extremes reached in *M*-type habitats, although the general trend appeared the same (Fig. 2.9).

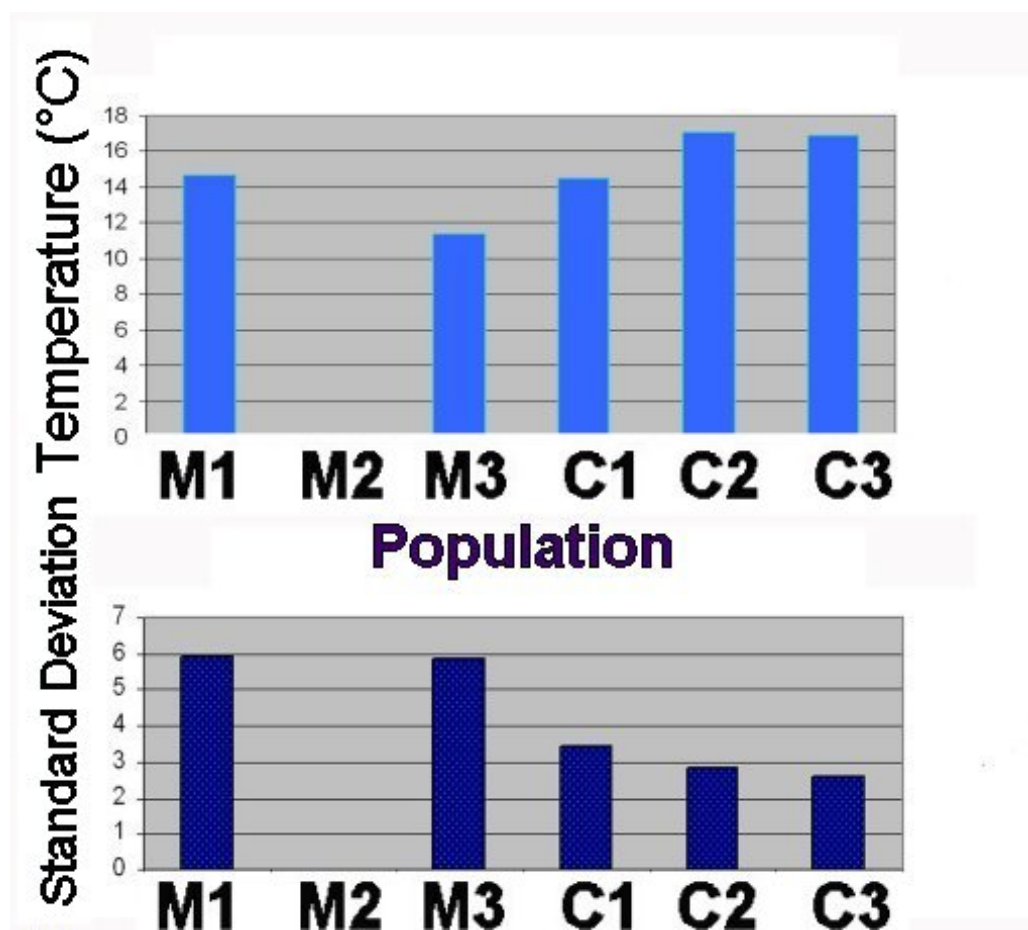


Figure 2.8: Comparison of thermal regimes in three mountain-type (*M*) and three *ciénaga*-type (*C*) environments. Top: Mean annual temperature. Bottom: Standard deviation of temperature.

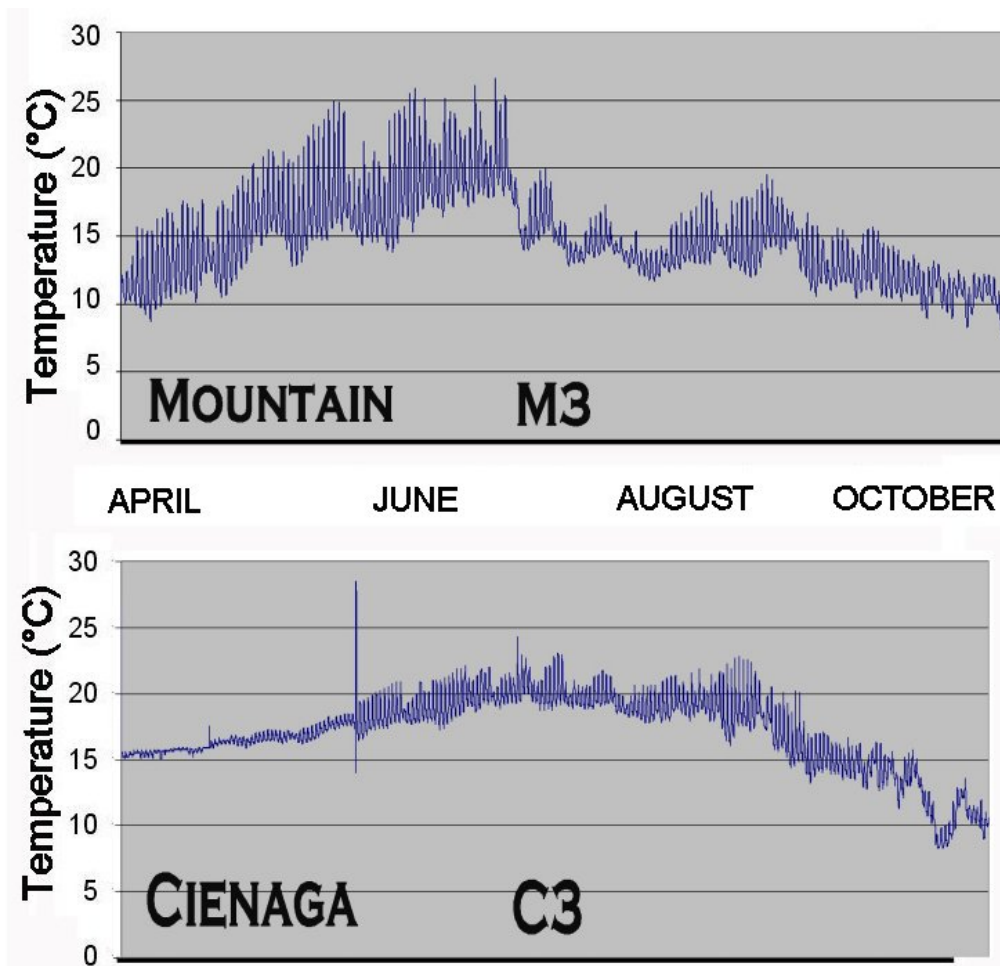


Figure 2.9: Temperature fluctuations during the same period of 2004 in an exemplary mountain stream (M3) and a ciénaga creek (C1). While the general seasonal trend appears similar, the extremes are more pronounced in the mountain environment.

## DISCUSSION

### *Morphometry and Life History*

The extremely-restricted development of bugs kept at 10°C suggests that there is a temperature threshold for these insects to molt and grow, and that threshold is somewhere around 10°C. Partridge et al (1994) successfully cultivated multiple generations of *Drosophila* at 16.5°C, and Davidson (1944) reared them as low as 14.95°C. Bayoh and Lindsay (2003) reported developmental abatement of the culicid *Anopheles gambiae* at 18°C. Purcell and



Welter (1990) found the lower threshold for the mirid *Calocoris norvegicus* to be 6.39°C. There is evidently wide variation among insect taxa, and thus far there is a paucity of degree-day models for belostomatids. However, since *some* bugs molted in the 10°C chamber, the threshold is very likely just below 10°.

Wild-type body size variance was indistinguishable from that of lab-raised bugs. This suggests the primary source of variation between populations can be accounted for by genetically or maternally heritable factors, rather than phenotypic plasticity. Subsequent generations of lab-raised individuals continue to maintain the size disparity of the parental stocks (Figs. 2.4 and 2.5). The implication is that *M*- and *C*-type bugs have been selected for different optima.

Intermolt time is not known for wild *A. herberti*. The pattern observed in the laboratory was consistent with the morphometric patterns of both wild and lab-raised bugs: that is, larger bugs have significantly longer development times. A large individual needs more time to attain sexual maturity than a small individual. If field development times mirror those measured in the laboratory, *M*-type bugs are constrained to fewer generations per breeding season. This is compounded by the annual temperature variation in the different thermal environments. Figure 2.9 shows the mountain temperature steadily declining as a function of time, whereas the ciénaga temperature, though also decreasing as winter approaches, is less variable. In mountain streams during the winter, most nymph instars are present but no encumbered males are seen whereas in ciénagas, egg-bearing males occur even in winter, demonstrating that reproduction can occur there throughout the year (Lytle, pers. observation). Individuals living in

warmer environments can apparently more easily accrue the requisite number of degree-days for development.

### *Thermal Response hypothesis*

Independent of *M* or *C* habitat type, *Abedus herberti* populations distributed across a gradient of elevation show a morphological response, apparently converging on an optimal size for a given elevation and presumably an associated thermal regime (Chapter 3). The persistent pattern of significant difference between M-type and C-type offspring raised under identical conditions suggests the final body size of these insects is a product of generations of selection under a particular regime, rather than a response to external cues triggered at the individual level. This experimental design did not address selection because among other things, it lacked a competitive component. Each brood of *Abedus herberti* comprises 50-140 eggs (Pelegrin, unpublished data) and like any creature with a geometric rate of increase in a closed system, competition must necessarily be a major ingredient of the selective regime.

The tantalizing question, addressed by a multitude of authors (Discussion, Chapter 3) is what drives selection for larger size at lower temperatures. The pattern is well-established, though it falls short of being a biological law (See Atkinson, 1994 for review). The reports of Partridge et al. (1994) and Alpatov (1930) indicate an increase in cell-size, but not in cell number, of *Drosophila melanogaster* cultivated for years under a colder regime than the genetically-identical control line. If *A. herberti* is responding on the cellular level to its

thermal environments, such a response may constitute an adaptation that is exapted into service for other purposes.

In the case of *Abedus herberti* *M* in particular, a larger body is a hedge against a perennially hazardous environment. In addition to the trials of a long winter, the mountain runoff stream habitat is inconstant in the summertime; flow can abate to almost nothing, stranding aquatic organisms in small isolated reaches. Under common hot summer conditions, these small pools can cook down or even dry up, forcing the residents to seek moisture elsewhere. Desiccating conditions have been shown to favor larger body size in cricket frogs, ants, grasshoppers, and fruit flies (Atkinson, *ibid*) and a dry stream in Arizona summertime certainly qualifies as a desiccating condition. Larger bugs might be able to disperse longer distances. The *ciénagas*, which are perennial, do not impose such migratory necessities upon their inhabitants. In this case the *M*-type environment is not only evoking larger body size through thermal regime, but also favoring larger individuals in other environmental struggles.

#### *Quick Ripening Hypothesis*

The attainment of earlier sexual maturity, even at the cost of advantages conferred by larger size, leads to a shorter generation time and a net of more offspring. Nymphs which hatch earlier will form a greater fraction of the population in subsequent generations, thereby increasing their fitness (Lewontin, 1965). The *C*-type populations are able to develop significantly faster under identical conditions; under typical yearly cycles in nature they are likely to attain

a minimum degree-day threshold faster than their *M*-type conspecifics and thereby increase their development rate still further. In addition, the quicker the nymph can molt, the sooner it has size advantage over other nymphs competing for the same resources. A second-instar is at much less risk of injury when attacking first-instars than it is when engaging in combat with individuals its own size. If this is in effect, it is likely balanced by a minimal effective size threshold, smaller than which a nymph can no longer successfully compete.

*Abedus herberti* living in *M*-type environments have a smaller window of viable development time as determined by seasonal temperatures. Additionally, the off-season adults must persist for greater periods of the year that are unfavorable for growth or reproduction. There may be a trade-off between a maximally-optimal development time and a minimum size requirement for overwintering, as predicted by length of the cold season. Nymphs left “stranded” at the onset of the cold season, i.e. unable to develop to adulthood due to degree-day constraints, may be forced to allocate energy to somatic growth instead of reproductive tissue, favoring larger bodies as repositories of greater stores of energy. By contrast, conditions in *C*-type habitats are more stable and moderate, without the freezing or near-freezing periods of winter or the hot anoxic zero-flow pools of summer, both of which would favor larger individuals.

#### *Ancillary Selection Hypothesis and Tough Nymph Hypothesis*

In a variety of organisms, larger eggs produce larger offspring (Berven, 1981). This study did not undertake the measurement of egg-size in *M*-type as

compared to *C*-type bugs; however the mothers, fathers, and offspring were all significantly smaller in the *C*-type populations, so it is likely the eggs were similarly minute. The benefit of producing smaller eggs is that clutch size is increased, as the surface area of the male's hemelytra is the factor that limits the number of offspring per brood (Smith, 1979; Kraus, 1985). More eggs confer numerical advantage, but again there is likely a balancing factor involving the minimal size required for *Abedus herberti* to effectively fill its niche.

Selection in colder regimes for larger early-instar nymphs, as predicted by larger eggs, would give these insects a greater hedge against fluctuating conditions at the beginning of the growth season. However, it is not merely lower temperatures that present additional challenges to *A. herberti* inhabiting mountain streams. After runoff flow ceases, the pools inhabited by *M*-type bugs grow hot and oxygen-poor. The community shifts from a diverse mix of collector-gatherers, collector filterers, scrapers, shredders and predators, to a fiercely competitive pool dominated by air-breathing predators (Bogan, 2005; Bogan and Lytle, in press). *Abedus herberti* must compete with other powerful bugs and beetles, as well as conspecifics. Cannibalism is not uncommon for this animal (Smith, 1974). Their voracious appetite for nymphs of their own species (Velasco and Millan, 1998) and the necessity to eat or be eaten by other predators would favor the physical advantage conferred by larger body size.

These hypotheses are not mutually exclusive, so attempts to tease one apart from the others in terms of its likelihood is akin to trying to remove the most important ingredient from a baked cake. Bergmann's Rule and the Thermal

Response Hypothesis have numerous corroborations elsewhere in the animal kingdom, suggesting that body size as inversely proportional to temperature regime is a physiological, cellular response independent of niche or habits of the animal in question. If *Abedus herberti* is responding on an organismal, cellular level to selective pressure in its thermal environment by altering its body size, the “new” body sizes may confer competitive advantage in a number of arenas unrelated to a strictly thermal response. The faster development time of the C-type nymphs lends support to the Quick Ripening Hypothesis; under identical conditions, in the absence of other selective pressures, the C-type bugs would numerically outstrip the M-types and thereby increase their relative fitness. The Ancillary Selection Hypothesis and Tough Nymph Hypothesis do not draw much support from the evidence gathered in this experiment, save for the higher molting rate of M-type bugs in the 10°C treatment. To test the Tough Nymph Hypothesis more conclusively, nymphs from both M- and C-type ancestry could be subjected to artificial rigor treatments, wherein they were variously deprived of food, exposed to predators in their same size class (corixids or notonectid nymphs, often found in pools with *Abedus*), and kept at a temperature slightly above their developmental threshold. This would yield some metric of performance that could be assayed between mountain and ciénaga populations.

*Molecular analysis*

While the tree in Figure 6 may be riddled with polytomies and shows very little resolution as to patterns of descent, it serves to show that small body size is autapomorphic, arising multiple times among several lineages. The tree clearly shows the *C*-type populations falling out on numerous disparate branches, providing no evidence for common ancestry of the character. Monophyly constraint tests for both groups *M* and *C* yielded significantly longer trees when constrained than when unconstrained, supporting the polyphyletic nature of these groups.

The number of private haplotypes in Table 2.3 supports the hypothesis of severely restricted gene flow among populations. Some of the shared haplotypes are curiously distributed, such as those found in *M1* and *C3*. These locations are separated by formidable stretches of mountain and desert. A more thorough treatment of patterns of descent among these populations and several others is given in Finn and Lytle (in preparation.)

The AMOVA reiterates the claim supported by the phylogenetic tree, that the morphological variation cannot be attributed to genetic variation. The *p*-value of 0.196 fails to disprove the null hypothesis that genetic patterns corroborate phenotypic traits.

## SUMMARY

Body size in *Abedus herberti* is linked to the specific thermal ecology of a given population. Whether this aspect of the ecology is determined by altitude (this work, Chapter 3) or ecotype, the correlation of smaller size in warmer environment is significant. Selection has acted on these animals to winnow out a particular optimal size for a particular thermal regime. These optimal sizes are not determined by phenotypic plasticity; they are likely heritable via genetic or maternal effects, indicative of an evolutionary plasticity on the population level.

Selection has also adjusted the life-histories of these animals, such that smaller individuals can reach adulthood more quickly and propagate faster, though this may be a serendipitous by-product of smaller body size. For animals living in more thermally-stable, predictable environments, faster development can yield more offspring and potentially more generations throughout the year.

The instance of a series of isolated populations replicated among two separate habitat types provides a ripe system for examination of the evolution of finely-tuned responses to particular environments. Further study is needed to determine more subtle properties of these insects, such as a degree-day model and the precise mechanism for a shift in body size related to local temperature.



## CHAPTER 3

Bergmann's Rule in allopatric populations of the giant waterbug *Abedus herberti*

### ABSTRACT

Geographical variation in body size across gradients of elevation and latitude were surveyed for fifteen discrete populations of the ferocious waterbug *Abedus herberti* Hidalgo (Hemiptera: Belostomatidae) collected from the Madrean Sky Islands of southeastern Arizona and northern Sonora, Mexico, across an elevation gradient from 929 to 1900 meters, and a modest latitudinal gradient of approximately 2.7°. A total of 611 individual adults were collected in the wild and measured for length and width. The purpose was to see whether this organism obeys Bergmann's Rule, and if so, whether a particular explanatory mechanistic hypothesis of the rule is supported by aspects of the insect's ecology and life history.

Length and width were significantly different among populations, and were found to correlate positively with elevation. Length also correlated positively with latitude, but width did not. Variation within populations was low. The mean size of females was significantly greater than males in four populations. These results support the general predictions of Bergmann's Rule and indicate that this species manifests a morphological response to thermal environment as predicted by altitude and latitude.

## INTRODUCTION

The various factors influencing temperature of aquatic habitats, such as elevation, latitude, local flow conditions and seasonality, have affected the evolution and ecology of insects living in these habitats. In particular, thermal ecology is well-established as a determinant of body size for many animals (Imai, 1937; Ray, 1960; Ward and Stanford, 1982; Atkinson, 1994). Bergmann's rule, as stated in Mayr (1963), says that within species, larger individuals are generally found at higher elevations and latitudes. Populations distributed across gradients of altitude and latitude, and by association, temperature, commonly exhibit an increase in mean body dimensions associated with greater distance from the equator and higher elevation (Berven, 1982; Pettus & Angleton, 1967; Arnett & Gotelli, 1999; Cushman et al, 1993).

Bergmann's rule originally applied to endotherms, in which larger body size is thermodynamically advantageous in colder environments. The link between body size and heat conservation in ectotherms is unclear, as their body temperatures fluctuate in lockstep with environmental temperature (von Bertalanffy, 1960.) Many hypotheses have been proposed to explain Bergmann's rule as it applies to ectotherms, but none is firmly conclusive. Proposals include resistance to starvation (Kondoh, 1977; Kaplan, 1980), voltinism (Bradford & Roff, 1995), and responses to temperature (Masaki, 1967; Atkinson, 1994; Markarian, 1980; Partridge et al, 1994). Von Bertalanffy (1960) also suggests that growth is constrained at higher temperatures because the rate of anabolism is unable to keep pace with that of catabolism.

*Abedus herberti* is a flightless aquatic bug (Hemiptera: Belostomatidae) in a geographical region dominated by desert and arid mountains. It lives in streams and pools in the Madrean Sky Islands of southeastern Arizona and northern Mexico. Isolated populations are confined to waterways distributed across a gradient of elevation and latitude. Unlike most aquatic insects, it is flightless throughout its life cycle (Lytle 1999). It is able to make short walking forays out of the water into riparian and lacustrine areas, but it generally perishes of desiccation if away from its native element for more than 12 hours (personal observation of escaped laboratory specimens, 2004-2006). Because dispersal between habitable environments is extremely difficult for an animal that must stay at least partially submerged in water, gene flow between populations is limited (Lytle et al., in review). As such, separate populations are likely to occupy unique evolutionary trajectories, influenced by highly-localized conditions of altitude, flow regime, and precipitation (*ibid.*).

To date, approximately 40 disjunct populations have been identified (Lytle, unpublished data). The insects are typically large ( $\bar{X} = 35\text{-}39\text{mm}$  adult body length) but several populations of small ( $\bar{X} < 32\text{mm}$ ) bugs have been found. The small bugs tend to be found in outflows of ciénagas (spring-fed desert marshes or ponds) whereas the more typical large bugs are found in mountain runoff streams. Ciénaga habitats tend to be more thermally stable throughout the year, whereas runoff streams fluctuate between seasonal extremes. A more thorough treatment of specific differences between mountain and ciénaga

ecotypes is given in Chapter 2. The populations belong to the same biological species and are capable of interbreeding (Appendix 1).

The distribution of a species across a gradient, in this case elevation, affords the opportunity to observe serial replications of different natural “treatments.” The area encompassed by the study is small (approximately 35000 km<sup>2</sup>) relative to the known distribution of *A. herberti* (approximately 220000 km<sup>2</sup>, Menke, 1960) and there is not a great diversity of habitat types that might confound the findings related to altitude. Elevation, and by association local mean temperature, has been shown to correlate with adult body size and developmental speed in populations of *A. herberti* (Chapter 2.)

This study examines the allometric variability of a large predatory aquatic insect across an elevation gradient in the Madrean Sky Islands. This gradient makes this an ideal system in which to examine the application of Bergmann’s rule. This study surveys the length and width of individuals in fifteen discrete populations of *Abedus herberti* to see if the variance can be explained by differences in altitude and latitude.

The evolutionary significance of the differences in body size may be approached through various hypotheses that are not necessarily mutually exclusive. One possibility is that mean temperature, as predicted by altitude, drives selection for a particular optimal body size via strictly physiological considerations (Thermal Response hypothesis), such as relatively higher rates of development versus growth at higher temperatures, leading to a smaller adult body size (Vannote and Sweeney, 1980). A second hypothesis is that local

thermal ecology causes selection for an earlier age of maturity in warmer regimes because the period of the year favoring growth and reproduction is lengthened, thereby providing the possibility of multiple generations and hence greater fitness (Quick Ripening hypothesis). A third possibility is that adult size is the result of ancillary selection (*sensu* Smith, 1997) and that direct selection is acting upon the size of the eggs or the first-instar nymphs in the following ways: larger eggs contain nymphs with greater energy content which will fare better at colder temperatures, and in warmer regimes, selection will favor smaller eggs because more of them can fit onto a male's back in a single brood (Ancillary Selection hypothesis). This study combines measurement of adult body length and width with observed patterns of behavior and ecology of *A. herberti* to assay the potential accuracy of these three alternative hypotheses. If the Thermal Response hypothesis is true, this means altitude or latitude should be a good predictor of adult size of these insects and marks this species as a follower of Bergmann's rule. The Quick Ripening hypothesis suggests that size-at-maturity is the character under selection, conferring an increasing reproductive benefit as size and development time diminish. In this case, one would expect to see populations living in warmer habitats reproducing and developing over a greater fraction of the yearly cycle than those living in cooler habitats with shorter growing seasons. The Ancillary Selection hypothesis predicts that the observable differences in adult size are mere artifacts of selection acting upon an earlier stage of the animal. Under ancillary selection, one would see larger eggs and nymphs at higher altitudes, but if this were the only selective regime in action, one might expect to

see more variability in adult body size because the primary selective pressure would have occurred already at earlier stages and later-stage instars and adults would not necessarily have an optimal size as determined by thermal regime.

These hypotheses are not mutually exclusive and may be acting in tandem. Other explanations may exist, such as those related to thermal stability and flow regime, but these are not related specifically to mean temperature and are treated extensively in Chapter 2. Among-population body-size patterns related to latitude and altitude and within-population sex differences are examined in this study.

## MATERIALS AND METHODS

*Abedus herberti* individuals were collected from fifteen sites in the Madrean Sky Islands (Figure 3.1) over three years. Sites were chosen to represent a large range of elevations. Five to 57 bugs were collected per site.

Following the technique of Keffer and McPherson (1988) length was measured from the tip of the tylus to the distal edge of the connexivum, not including air straps. Width was measured across the widest part of the hemelytra, at the border between the second and third abdominal segments. For 100 re-measured individuals, 40 had a maximum error of 1mm, yielding a mean error rate of  $\pm 4$ mm. Bugs were categorized into three gender classes: males, females, and egg-laden males. Each insect was assigned a unique number which was painted onto the hemelytra using a silver Sharpie<sup>®</sup>.

Populations spanned a wide range of elevation, from 929 meters to 1900 meters. Sampling reaches were less than 100m long in all cases. Elevation was

taken by GPS at the midpoint of the sampling reach. One stream (East Turkey Creek, Chiricahua Mountains) contained two sampling reaches at different elevations; bugs from these two sites were treated as separate populations for sampling purposes. However, a two-sample pooled-variance t-test of length ( $t = -0.0901$ ,  $df = 112$ ,  $P = 0.9284$ ) and width ( $t = 1.1724$ ,  $df = 112$ ,  $P = 0.2435$ ) showed that no significant difference existed between the means of the two populations. Additionally, there is no physical barrier to gene flow or any point between the two reaches that recognizably divides one from the other during seasonal flow. Therefore, these two were combined into a single population for purposes of the analysis, and an average of their elevations was used.

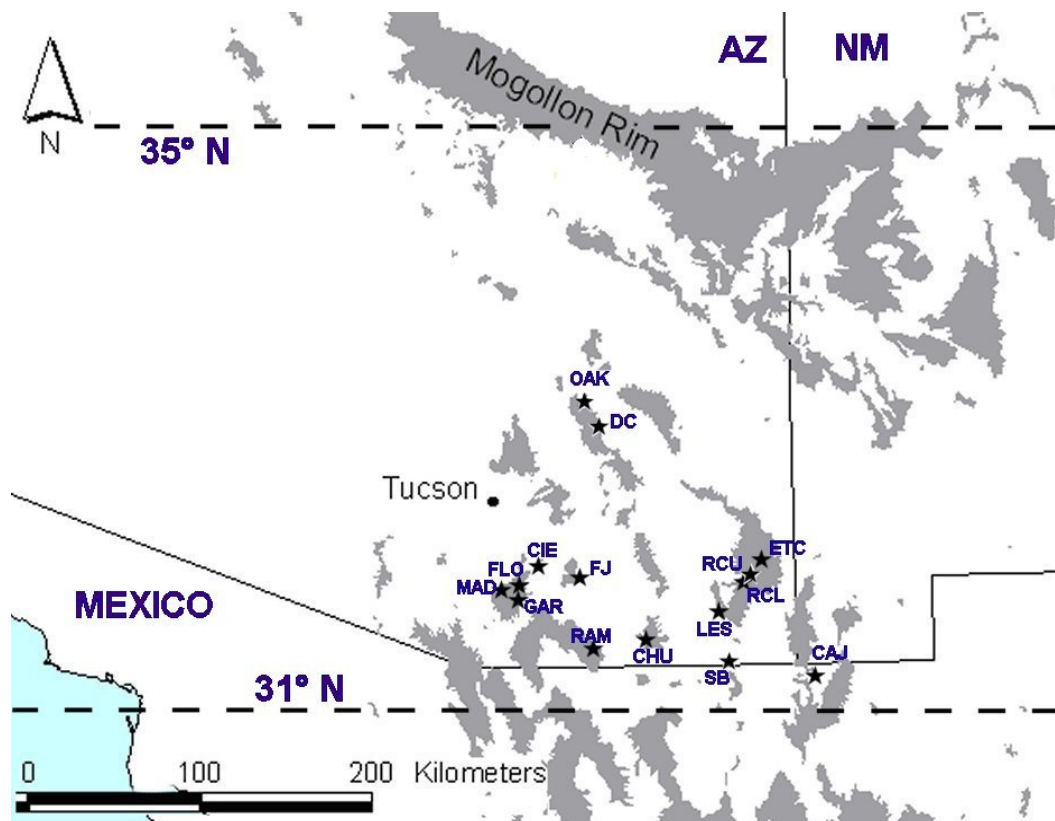


Figure 3.1: Map of collection localities in southern Arizona (USA) and northern Sonora (Mexico). The gray areas represent mountain ranges. See Table 3.1 for codes.

Another stream (Rucker Canyon, Chiricahua Mountains) contained two sampling reaches separated by ~5 kilometers of discontinuously flowing creekbed, an artificial dam, and 170 meters of elevation. A two-sample pooled-variance  $t$ -test of length ( $t = -3.3873$ ,  $df = 88$ ,  $P = 0.0011$ ) and width ( $t = -3.7838$ ,  $df = 88$ ,  $P = 0.0003$ ) showed significant difference in body size between the two reaches of Rucker Creek, so they were counted as two separate populations.

Statistical analyses were performed using S+. They included two-sample pooled-variance  $t$ -tests, linear regressions, and ANOVA. Alpha was set at 0.05, and all tests were two-tailed.

## RESULTS

### *Collection*

At least 21 individuals were collected from most sites (Table 3.1). Two sites yielded ten or fewer individuals; while these sites are discussed here for comparative purposes, they were excluded from some statistical analyses due to small sample size.

### *Population Morphometry*

Mean length and width among populations varied by up to 23% and 26%, respectively (Table 3.1). Figure 2 shows a comparison from least-to-greatest body size of all populations. Variation within populations was small (s.e. <.06 in all cases.)



Both indices of body size correlated positively with increasing elevation, as shown by a linear regression analysis (Figs. 3.3 and 3.4.) The individuals captured at Deer Creek buck the trend, as they live at the lowest elevation but do not have the smallest body size. However, the four populations whose mean size is smaller than those at Deer Creek are all living in ciénaga outflows, which may affect body size to a greater degree than altitude (Chapter 2)

Table 3.1: Size and locality information for fifteen populations of *Abedus herberti*, collected 2004-2006.

Population	Code	Mountain Range	Elevation (m)	Latitude (°N)	Length (cm)	Width (cm)	<i>n</i>
Cajon Bonito	CAJ	San Luis	1280	31.2780	3.215 s.e.= 0.017	1.746 s.e.= 0.011	41
Chulo Canyon	CHU	Mule Mts	1674	31.4854	3.439 s.e.= 0.015	1.867 s.e.= 0.010	48
La Ciénaga	CIE	N/A (Desert spring)	1400	31.7867	2.96 s.e.= 0.022	1.593 s.e.= 0.014	30
Deer Creek	DC	Galiuros	929	32.9121	3.41 s.e.= 0.038	1.87 s.e.= 0.021	10
E. Turkey Creek	ETC	Chiricahuas	1887	31.9174	3.5 s.e.= 0.014	1.924 s.e.= 0.010	114
Florida Canyon	FLO	Santa Ritas	1463	31.7508	3.574 s.e.= 0.018	1.922 s.e.= 0.011	50
French Joe Canyon	FJ	Whetstones	1700	31.8176	3.243 s.e.= 0.026	1.709 s.e.= 0.019	21
Gardner Canyon	GAR	Santa Ritas	1700	31.7022	3.504 s.e.= 0.012	1.911 s.e.= 0.009	46
Leslie Canyon	LES	Swisshelm	1410	31.5891	3.274 s.e.= 0.034	1.77 s.e.= 0.020	23
Madera Canyon	MAD	Santa Ritas	1504	31.7241	3.422 s.e.= 0.017	1.878 s.e.= 0.013	41
Oak Creek	OAK	Galiuros	1434	32.7159	3.475 s.e.= 0.015	1.877 s.e.= 0.011	56
Ramsey Canyon	RAM	Huachucas	1800	31.4339	3.592 s.e.= 0.016	2.019 s.e.= 0.014	36
Upper Rucker	RCU	Chiricahuas	1730	31.7567	3.543 s.e.= 0.015	1.923 s.e.= 0.015	40
Lower Rucker	RCL	Chiricahuas	1900	31.7644	3.619 s.e.= 0.017	1.99 s.e.= 0.011	50
San Bernardino	SB	Rio Batepito	1138	31.4229	2.92 s.e.= 0.058	1.58 s.e.= 0.037	5

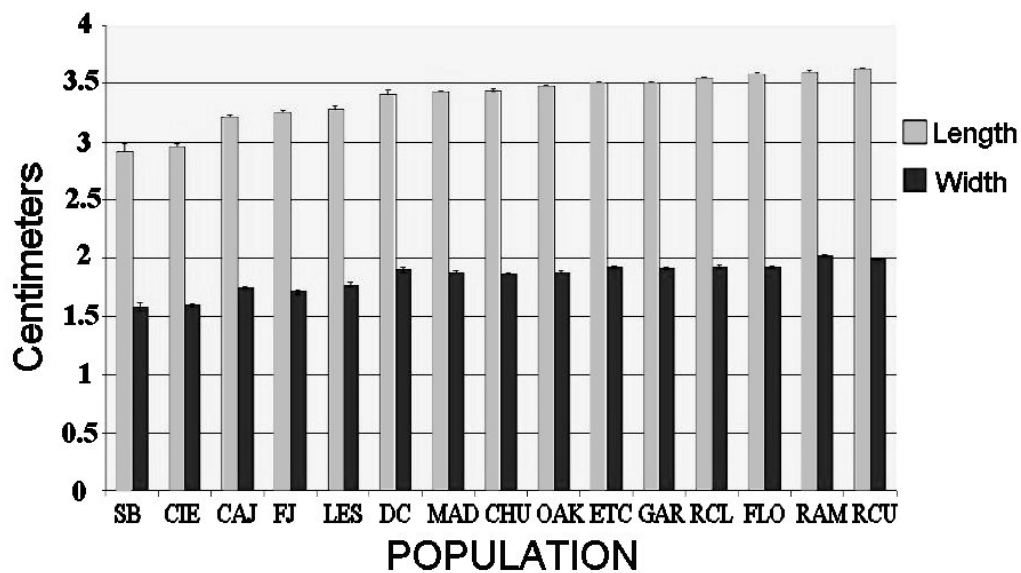


Figure 3.2: The mean lengths and widths of 611 *Abedus herberti* individuals from 15 populations in southeastern Arizona and northern Mexico.

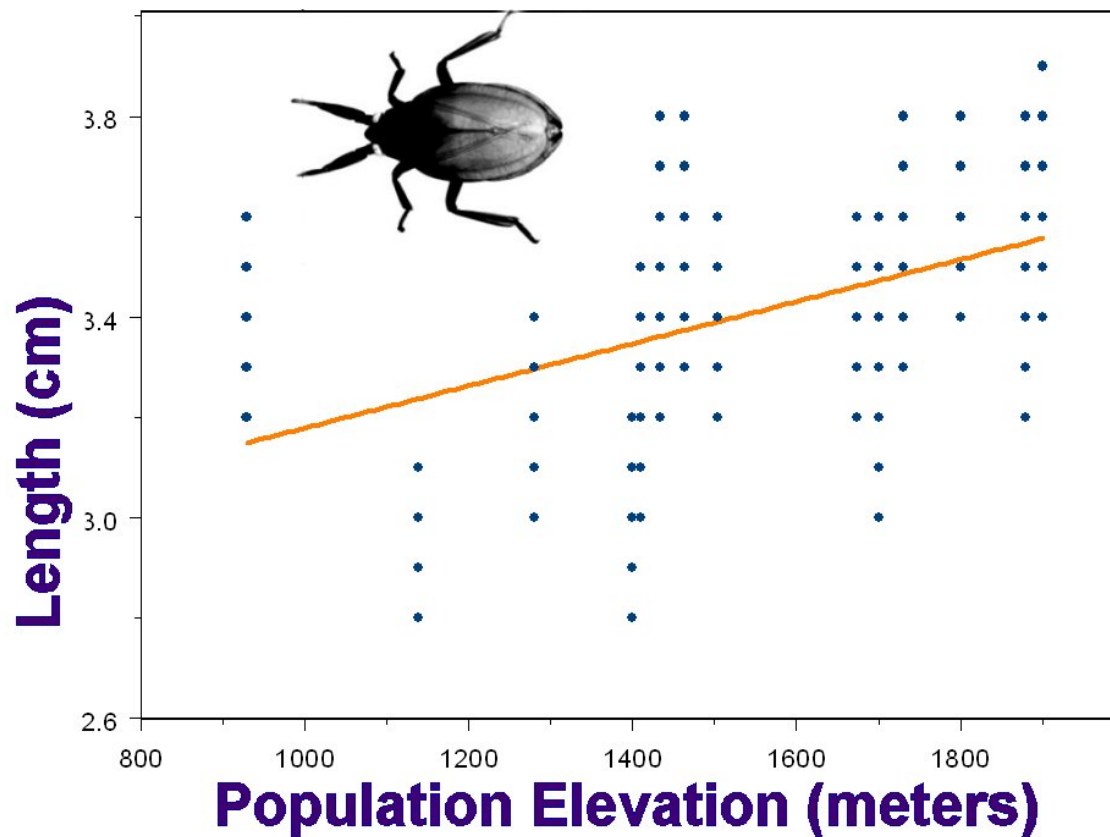


Figure 3.3: A linear regression of length as a function of elevation in 15 populations of *Abedus herberti* in the Madrean Sky Islands. Each dot may represent overlapping values from several individuals. Multiple  $R^2 = 0.2306$ .  $df = 609$ ,  $P < 0.001$

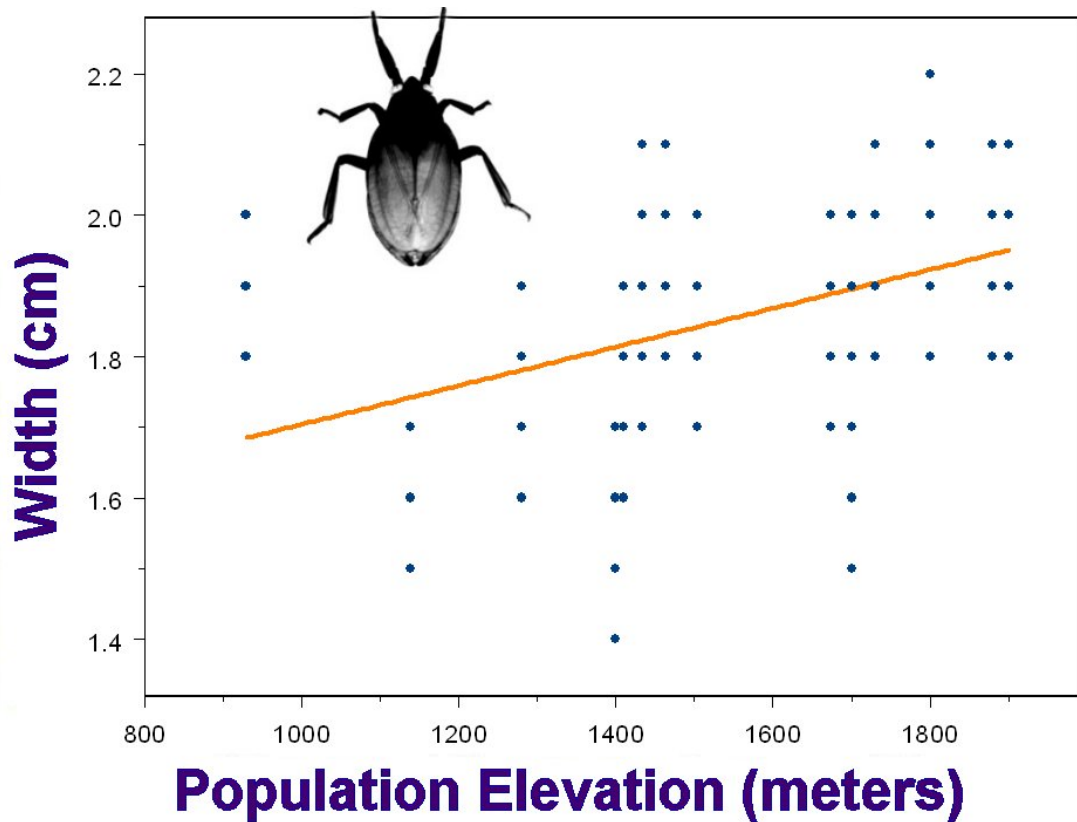


Figure 3.4: A linear regression of width as a function of elevation of 15 populations of *Abedus herberti* in the Madrean Sky Islands. Each dot may represent overlapping values from several individuals. Multiple  $R^2 = 0.2391$ ,  $df = 609$ ,  $P < 0.001$

Analysis of variance (ANOVA) comparing differences between the morphometric parameters of insects collected at different altitudes showed both length and width were significantly different across elevations (Table 3.2a, 3.2b). Deer Creek and San Bernardino bugs were excluded from this ANOVA because their small sample size compromised the balance of the design.

Table 3.2a: ANOVA of length as a function of elevation.

	Df	Sum of Sq	Mean Sq	F value	Pr (F)
<b>Elevation</b>	<b>1</b>	<b>5.22993</b>	<b>5.22993</b>	<b>187.4002</b>	<b>&lt;0.001</b>
<b>Residuals</b>	<b>594</b>	<b>16.57723</b>	<b>0.027908</b>		

Table 3.2b: ANOVA of width as a function of elevation.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
<b>Elevation</b>	<b>1</b>	<b>2.381477</b>	<b>2.381477</b>	<b>209.5033</b>	<b>&lt;0.001</b>
<b>Residuals</b>	<b>594</b>	<b>6.752148</b>	<b>0.011367</b>		

### Latitude

Length increased significantly with latitude (Figure 3.5.) Width did not correlate significantly with changes in latitude, by a very slim margin (Figure 3.6.)

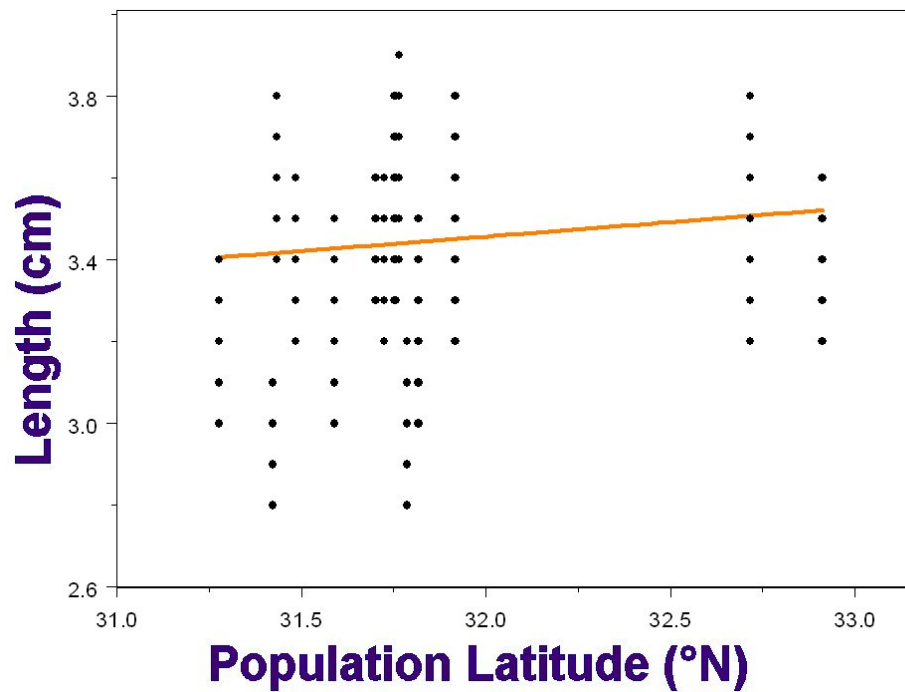


Figure 3.5: *Abedus herberti* body length is positively correlated with population latitude.  $R^2 = 0.01788$ ,  $df = 609$ ,  $P < 0.001$

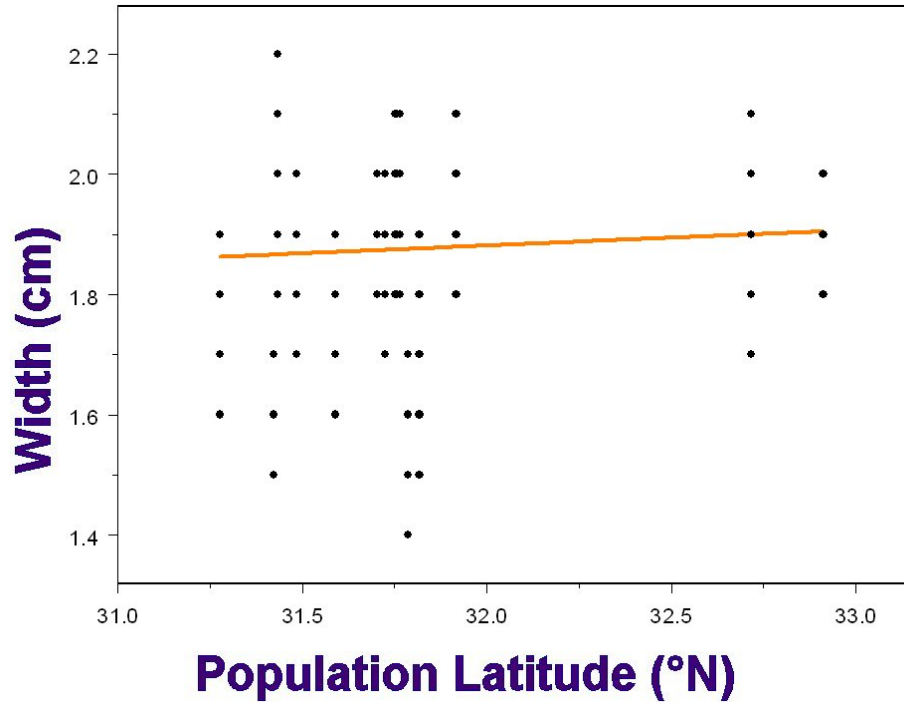


Figure 3.6: *Abedus herberti* width insignificantly correlating with population latitude.  $R^2 = 0.00593$ ,  $df = 609$ ,  $P = 0.057$

### *Ecotype Differences*

The differing thermal regimes of ciénaga environments and mountain runoff stream environments may have an effect on adult body size of these insects (Chapter 2). Spring-fed, thermally stable ciénagas comprise a fundamentally different thermal ecology, regardless of their elevation. To address this potentially confounding variable, I removed the ciénaga populations (FJ, CIE, SB, LES) from the data set and re-ran the regressions (Table 3.3). In each case, the significance of the all-inclusive analysis is preserved.

Table 3.3: A summary of regression analyses performed on eleven non-ciénaga populations of *Abedus herberti*. The significance of the earlier, all-inclusive analyses is preserved in all cases.

Regression	$R^2$	$df$	$P$
Length vs Elevation	0.1754	530	<0.001
Width vs Elevation	0.1998	530	<0.001
Length vs Latitude	0.01293	530	0.0086
Width vs Latitude	0.001176	530	0.4299

### *Sexual Differences*

Of 611 bugs measured, 209 were male and 402 were female. *t*-tests showed females were significantly longer than males in three populations, and significantly wider in two populations (Table 3.4). San Bernardino and Deer Creek bugs were excluded from the table for having too few representatives to make meaningful statistical comparisons.

Table 3.4: *t*-test results comparing males and females on the parameters of length and width, per population. Significant comparisons are shown in bold print

Population	Males vs. Females									
	Length					Width				
	t	df	P	$\bar{X}$ ♀ cm	$\bar{X}$ ♂ cm	t	df	P	$\bar{X}$ ♀ cm	$\bar{X}$ ♂ cm
CAJ	1.1531	39	0.2559	3.24	3.20	0.7574	39	0.4534	1.76	1.74
CHU	0.5962	46	0.554	3.45	3.43	1.0978	46	0.278	1.88	1.85
CIE	0.4284	28	0.6716	2.97	2.94	0.2886	28	0.775	1.60	1.59
ETC	2.6618	112	<b>0.0089</b>	<b>3.52</b>	<b>3.46</b>	1.5559	112	0.1226	1.94	1.90
FLO	3.9127	48	<b>0.0003</b>	<b>3.62</b>	<b>3.50</b>	4.0033	48	<b>0.0002</b>	<b>1.95</b>	<b>1.87</b>
FJ	1.3572	19	0.1906	3.29	3.22	0.616	19	0.5452	1.73	1.70
GAR	1.9167	44	0.0618	3.52	3.47	0.1578	44	0.8753	1.91	1.91
LES	0.425	21	0.6752	3.29	3.26	0.1118	21	0.912	1.77	1.77
MAD	2.4504	39	<b>0.0189</b>	<b>3.45</b>	<b>3.38</b>	1.6835	39	0.1003	1.90	1.85
OAK	1.4453	54	0.1542	3.49	3.44	2.8639	54	<b>0.0059</b>	<b>1.90</b>	<b>1.83</b>
RAM	0.6823	34	0.4997	3.60	3.58	-0.1888	34	0.8514	2.02	2.01
RCU	-0.1411	48	0.8884	3.62	3.63	0.1933	48	0.8475	1.99	1.98
RCL	0.4792	38	0.6345	3.55	3.53	0.886	38	0.3812	1.93	1.91

## DISCUSSION

### *Population morphometry: Altitude*

Size variation was conservative within each population, suggesting that each population of insects possessed a characteristic body size. The significant

positive correlation between body size and altitude supports the hypothesis that temperature, as predicted by altitude, may be involved with selection for larger body size.

#### *Thermal Response hypothesis*

These results provide another example of Bergmann's Rule applying to an ectothermic species. Ray (1960) found Bergmann's rule to apply to 27 of 36 poikilothermic species. Atkinson (1994) found the rule to hold true for 91 of 109 ectotherms, and suggested that those which did not might be reassessed more vigorously. Temperature is a necessary component of the rule, but there is a variety of ecological factors that may affect the selective pressure on populations in different geographical zones. Competition, rainfall, and food supply may be inconstant from one elevation or latitude to the next.

The response to temperature in *A. herberti* may be on the cellular level rather than the organismal level. Partridge et al. (1994) demonstrated that replicate lines of *Drosophila melanogaster*, reared in the laboratory under constant thermal regimes of 16.5°C and 25°C, differed significantly in size after five years, with the cooler regime favoring larger-bodied flies. Alpatov (1930) reports an increase in the cell size of *Drosophila* reared for several generations at low temperatures. The overall body size increase in *A. herberti* may be due to a per cell increase in volume and surface area.

The physical properties of water may be a factor in the size evolution of *A. herberti*. Water viscosity is lower at warmer temperatures, and thus buoyancy is

reduced. Floating aquatic organisms may offset the effects of diminished buoyancy by reducing body size (Atkinson 1994). However, *A. herberti* is large enough that the relatively small alteration of viscosity within its habitable temperature range probably does not drive selection.

A variety of selective pressures, in addition to temperature, may constrain body size in some *Abedus herberti* populations. Blanckenhorn (2000) posits a number of costs to larger size, including the viability cost of longer development due to predation, parasitism, or starvation; the viability cost of reduced agility or increased visibility; time and energy cost of supporting a larger body; viability cost of heat stress; and the costs to mating when reproducing later at a larger size. The heat stress (and conversely the cold stress) as related to body size must necessarily be minimal for a small poikilotherm like *Abedus herberti*, but why the other costs would apply unequally to populations at different elevations is unclear. Blanckenhorn (*ibid.*) also laments the paucity of evidence for selection and other processes that would limit body size in natural populations.

On the other hand, larger size also confers benefits. Larger size generally means greater fitness (Zeh and Zeh, 1988) and higher fecundity. Cooler water has higher viscosity, so aquatic organisms may become larger in cooler regimes to overcome the additional resistance (Atkinson, 1994). Seasonal shifts to lower daily mean temperature occur sooner at higher elevations, so organisms dwelling at higher altitudes must endure longer hibernation intervals. For such animals, larger size confers greater resistance to starvation by way of increased nutrient-storage volume.



### *Quick Ripening Hypothesis*

David and Cagy (1982) suggest that body size might decrease with latitude because tropical habitats are more likely to support populations at carrying capacity, increasing intraspecific competition. The attainment of earlier sexual maturity leads to a shorter generation time and a net of more offspring. Nymphs which hatch earlier will form a greater fraction of the population, thereby increasing their fitness (Lewontin, 1965).

*Abedus herberti* living at higher altitudes have a smaller window of viable development time as determined by seasonal temperatures. While such a regime might be expected to favor a faster development, the off-season adults must persist for greater periods of the year that are unfavorable for reproduction. There may be a trade-off between a maximally-optimal development time and a minimum size requirement for overwintering. Smith (1974) captured *A. herberti* from multiple central Arizona populations, cultivated them in the laboratory at 18°C, and raised them from egg to adult in 71-106 days. Preliminary evidence suggests development time in these animals is linked to a degree-day schedule, and summertime conditions in Arizona are typically warmer than Smith's laboratory (Chapter 2) so the expectation of two generations per season is not unrealistic, even for higher-altitude populations. For a large predator like *A. herberti*, the continued availability of food is as important as the accrual of degree days; at lower elevations, both requirements are met for a longer period. This increased margin may favor attainment of maturity at an earlier age and hence a

smaller size, by conferring greater fitness via more generations per season.

Additionally, the lower-elevation populations do not need to endure as long of an off-season as their montane conspecifics, so the overwintering nutrient reservoir need not be as voluminous.

#### *Ancillary Selection Hypothesis*

Berven (1981) hypothesized that selection in colder thermal regimes acts primarily upon egg size in aquatic animals, in that larger eggs use a smaller share of their energy for the act of hatching, thus increasing the energy content of the hatchlings and improving their fitness at low temperatures. He cites evidence of embryonic and hatchling vertebrates whose survival rate at low temperatures was correlated with the size of the eggs they came from. The eggs of wild-caught *A. herberti* are difficult to accurately measure because they change size over the course of development (Smith, 1974) and there is no accurate method to divine discrete stages of egg-age. Post-zygotic nymph size is unaffected by local temperature regime (Chapter 2).

Selection in colder regimes for larger early-instar nymphs, as predicted by larger eggs, would give these insects a greater hedge against fluctuating conditions at the beginning of the growth season. Additionally, *Abedus herberti* nymphs are eager cannibals (Pelegriin, personal observation; Velasco and Millan, 1998) and larger size confers undisputable advantage in a struggle to the death between conspecifics.

Selection for large size at lower elevations is likely balanced by selection for numerically large broods. Since male back-space is a limiting factor in the fecundity of these insects (Smith, 1979; Kraus, 1985), a smaller diameter per egg will result in greater number of offspring.

### *Latitude*

The ferocious waterbugs in this study are confined to a relatively thin band of latitude, from approximately 31.3° N to 34.9°N. Latitude correlates only weakly with body size among these insects; the association with elevation is much stronger. The known distribution is approximately 27.7°N to 37.2°N but the species may extend much further south, possibly as far as 20.4°N (Menke, 1960.) Curiously, the northernmost known populations of *Abedus herberti* belonging to the subspecies *A. herberti utahensis* (*ibid.*) are not the largest; in fact they are smaller than the mean size of all populations considered in this study. Other ecological factors such as thermal stability may be driving selection for smaller size in *A. h. utahensis* (Chapter 2). Because the southern limit of *A. herberti* is unknown, it is not yet possible to say whether these insects follow the positive association between latitude and size as predicted by Bergmann.

The northern region of *Abedus herberti* (*sensu stricto*) is dominated by the Mogollon Rim (Fig. 3.1), an arc of mountains at the limit of a trend of northward elevation. Population morphometry in this region is likely to be responding to a conflation of altitude and latitude. Within the study area covered in this work,

altitude does not correlate significantly with latitude ( $R^2 = 0.1178$ ,  $df = 12$ ,  $P = 0.2296$ .)

### *Ecotype differences*

Results of regression analyses were consistent with or without the inclusion of individuals from *ciénaga*-type environments. While the slope of the regressions became somewhat shallower, the significance of altitude correlating with width and length, and latitude correlating with length but not width, endured the subtraction of the bugs native to *ciénagas*. The correlation between body size and altitude is robust.

### *Sexual differences*

Males bearing eggs were much more easily seen by humans than unencumbered bugs, and consequently it is reasonable to assume they are more likely to be spotted by terrestrial predators, which may account for the disproportionate number of females captured in our surveys. In all cases, the mean size of females exceeded the mean size of males, but the differences were only significant in four populations. Female insects are typically larger than males, in that they produce eggs, and larger size equals more eggs, ergo higher fitness, whereas the same selective pressure does not typically apply to male insects. However, since this particular insect assigns egg-brooding duties to the male, selection for larger females may be constrained by the physical limitations of the male. Specifically, the dimensions of the male hemelytra will limit how many

eggs a female can posit (Smith, 1979; Kraus, 1985). Some males collected in the field had eggs covering their entire hemelytra and pronota, almost up to their eyes. Within populations, smaller males will be able to brood fewer eggs, and thus will suffer reduced fecundity. Evolution will favor larger, broader males. This selective regime may explain why the differences in size between males and females of the species are so seldom significant.

### SUMMARY

While there is clearly a relationship between altitude and body size, the mechanism responsible for the relationship remains obscure. Whether the cells of *Abedus herberti* are getting bigger with elevation or the populations add on more cells as altitude increases, selection certainly plays an important role, such that bugs living at the same altitude in widely disparate mountain ranges converge on similar dimensions of length and width. There appears to be an optimal body size for a given elevation or temperature regime that balances a variety of selective forces.

The Thermal Response hypothesis, i.e. a physiological response to local thermal ecology seems to be the best-supported, in that corroborative evidence has been reported from many taxa. There appears to be, among a gradient of body sizes and thermal regimes, an optimal size that goes with a particular thermal environment. If, as in *Drosophila*, the cells of *A. herberti* are growing larger as a response to lower temperatures, then the salience of particular aspects of the

animal's ecology and life-history is diminished. However, this hypothesis does not explain why the condition exists; it merely shifts the investigatory onus to the cellular level.

The Quick Ripening hypothesis, i.e. the populations at lower elevations have longer developmental windows and can produce more generations per season by reducing developmental time and size at maturity, stands to reason in comparisons of very high altitude populations and very low altitude populations, but does not reasonably account for those in between. There is a gradient of body sizes that correlates with a gradient of elevation, forming two presumably continuous variables. Generations per season are not continuous variables; they must necessarily be whole numbers. While late-instar nymphs are occasionally found during the cold months (Lytle, unpublished data) they are not found frequently enough to be said to constitute a fractional generation between growing seasons.

The Ancillary Selection hypothesis, i.e. that selection is acting primarily upon egg and early nymph size rather than adult size, is reasonable considering the competitive and environmental pressures on nymphs in the early part of the growth season. Larger nymphs have greater advantages in colder conditions. On the other end of the gradient, females producing smaller eggs at lower elevations are able to fit more of these eggs on the males' back, and with the need for higher post-egg energy content ameliorated by a warmer environment, fitness is increased by sheer number of offspring. However, this hypothesis alone does not explain why the animals grow to such an extent, if selection is only acting on the

youngest stages. Growth of this magnitude is energetically costly and incurs many risks.

It is therefore likely that the adult size of any given population of *Abedus herberti* is determined by a multiplicity of factors, with Bergmann's rule and the Ancillary Selection hypothesis coming to the fore as the most evolutionarily viable explanations. The correlation between adult size and elevation is clear; the mechanism for this correlation must necessarily be relegated to the realm of speculation, until such investigations are designed that can conclusively determine the roles of physiological response and natural selection in the optimization of particular body sizes in specific thermal environments.

## CHAPTER 4

### Rearing Techniques for *Abedus herberti*

This work describes the short-term and long-term maintenance, husbandry and rearing of *Abedus herberti* in an artificial environment. While this animal resides in a variety of precipitation, flow, and temperature regimes in nature, laboratory conditions can be standardized to provide an optimal habitat for any individual of the species, regardless of its provenance. The laboratory longevity of these bugs is at least thirty months at the time of writing.

Adult bugs were gathered from the wild and transported to the lab in watertight plastic boxes containing damp leaves. There they were dried with a paper towel and marked with silver Sharpie<sup>®</sup> marker on the hemelytra. Each bug received three marks: one to distinguish its place of origin, one to tell male from female, and a unique number. The optimal place for marking was found to be between the clavus and connexivum; the bugs could reach nearly any other spot and scrape the paint off with their claws (Figure 4.1).





Figure 4.1: Silver markings on *Abedus herberti*. The two top symbols indicate sex and origin, the bottom is the number of the individual.

Wild adult *A. herberti* are generally gregarious, gathering in large numbers at the waterline of rock-walled pools. They breathe by way of extendable air-straps at the end of their abdomen, so they usually assume a head-down resting posture from which they can easily extend their abdomen upward to take a breath, and scuttle down along the wall in case of threat. To imitate familiar habitat, the bugs were placed in white plastic tubs (36cm x 29cm x 18cm) containing two red bricks and water up to the level of the brick. This mimicked their native rocky substrate while also preventing the bugs from wedging themselves under anything, a problem when trying to ensure each bug has eaten. When experimentally inactive, bugs were separated by sex.

All adults were kept at 20°C on a 14/10 light/dark regime, to mimic summertime in Arizona. Water was changed once a week. During inactive experimental periods, the bugs were moved to a 10°C room on a 12/12 light/dark regime. Their bricks were stacked to form a +, thereby creating overhanging shelf space which the bugs seek during the cold season. Water was kept to above the level of the bottom brick. In this state the bugs entered a low-metabolism hibernation condition which could persist for months. Every three months they were taken back into the warm room, allowed to return to 20°C, and fed. Individuals whose marks had faded were re-tattooed.

Bugs were fed twice a week on crickets and mealworms raised on Total<sup>®</sup> cereal to ensure nutritional repletion. Nymphs and adults assume a hunting pose in which they grip an emergent substrate such as a stone or plant with their meso- and metatarsi, head down and the tip of the abdomen near the surface. The raptorial forelegs are left free for grappling. Prey is either snatched when it comes into range or, in the case of terrestrial insects struggling on the water surface, seized from underneath. If the prey is equal or greater in size to the bug, it will extend its metathoracic legs such that the tarsi form a subequilateral triangle with the breathing straws at the surface of the water. This forms a stable inverted tripod with the head of the bug and the prey item at the bottom, and seems to hasten the subjugation of the prey animal through oxygen deprivation and denial of leverage. The bugs strongly preferred live, struggling prey. In early feeding trials, bugs attacked and ate fish, tadpoles, crustaceans, gastropods, annelids, arachnids, newborn mice, and insects.

### *Mating*

A male and a gravid female were placed into a white plastic tub with a single emergent rock with a gentle slope on at least one side. With only a single emergence location, the bugs were sure to become aware of each other. Smith (1979) observed a complex of courtship behaviors that preceded mating, including sparring, grasping, inverted embraces, and male drumming. The latter is the only behavior I regularly witnessed, though in two cases I saw the inverted embrace. Males solicit female sexual attention with a stereotyped combination of drumming their abdomen against the substrate and emitting a low grunting stridulation. The mechanism of this noise is unknown. Males drummed most frequently after water-changes in their tubs, regardless of presence of females. Some males perpetually refused to initiate congress with any partner.

The females deposited a single layer of eggs on the males' hemelytra, anchoring the eggs with a gelatinous adhesive mortar (Fig. 4.2). An excellent account of *A. herberti*'s mating and life cycle can be found in Smith (1974).



Figure 4.2: *Abedus herberti* oviposition.

Brooding males were isolated in clear plastic obstetric tubs (8cm tall, 13cm basal diameter, 1 liter) with a fist-sized river rock in the middle. This allowed the males to sit comfortably with the eggs partially submerged. Egg-encumbered males continued to take food for two weeks after oviposition, but thereafter they would lunge at prey and grasp momentarily before letting go of it. When the eggs hatched, the lunging behavior abated until after the egg-mass dropped off.

### *Culture*

The eggs hatched 24-28 days after deposition. Nymphs emerged honey-colored and clustered around the father for an hour or two, until they darkened to

a dull subfusc. Some of the nymphs bore a dorsal median stripe (described in Smith, 1976) which was determined by Schnack et al (1994) to be heritable via a simple autosomal dominant allele.

Nymphs in nature tend to cling to emergent sticks and stems, just below the surface. They are extremely territorial and will not suffer another nymph to share a hunting locus or emergent substrate. Disputes are resolved through violence, with the loser either fleeing into open water or becoming the winner's meal. Older nymphs actively hunt younger nymphs, even from their own brood. After hatching, nymphs were sequestered in 4-ounce (118.292mL) portion cups. Emergent vegetation was standardized by placing an upside-down steel hex bolt (3cm long, .9cm wide, 7 threads/cm) in the center of the cup. The threads made easy grasping-substrate for the nymphs and also provided an excellent anchor for molting (Fig. 4.3). Each nymph was assigned a patronymic number and an individual letter, written on its cup (Fig. 4.4).





Figure 4.3: Second-instar nymph and exuvia, shortly after molting.



Figure 4.4: Portion cups with bolts for nymph segregation.

First-instar nymphs were typically fed *Drosophila melanogaster*. The *Drosophila* were narcotized by refrigeration, and then scattered onto a dish of ice-

water. They could then be plucked from the surface of the ice-water and offered to the nymphs. As a fly warmed up, it began to struggle on the surface, thereby triggering an attack-lunge from the nymph. Most nymphs quickly coupled the approach of forceps with an imminent meal, and would lunge long before the food was delivered. Many mistook the forceps for a larger prey item, and attempted to pierce the steel tines with their stylets. Young nymphs also thrived on early instar corixids, oligochaete worms, mosquito larvae, and *Formica* ants. Later instar nymphs received crickets, mealworms, houseflies and carpenter ants.

After reaching the fourth instar, nymphs were transferred to 16-ounce (473.168mL) round clear plastic tubs with 5mm holes drilled into the base. These were weighted with a small rock, which also served as substrate for the nymph, and placed in a larger tub filled with water to just below the level of the rock. This way several nymphs could share the same reservoir of water (Fig. 4.5).

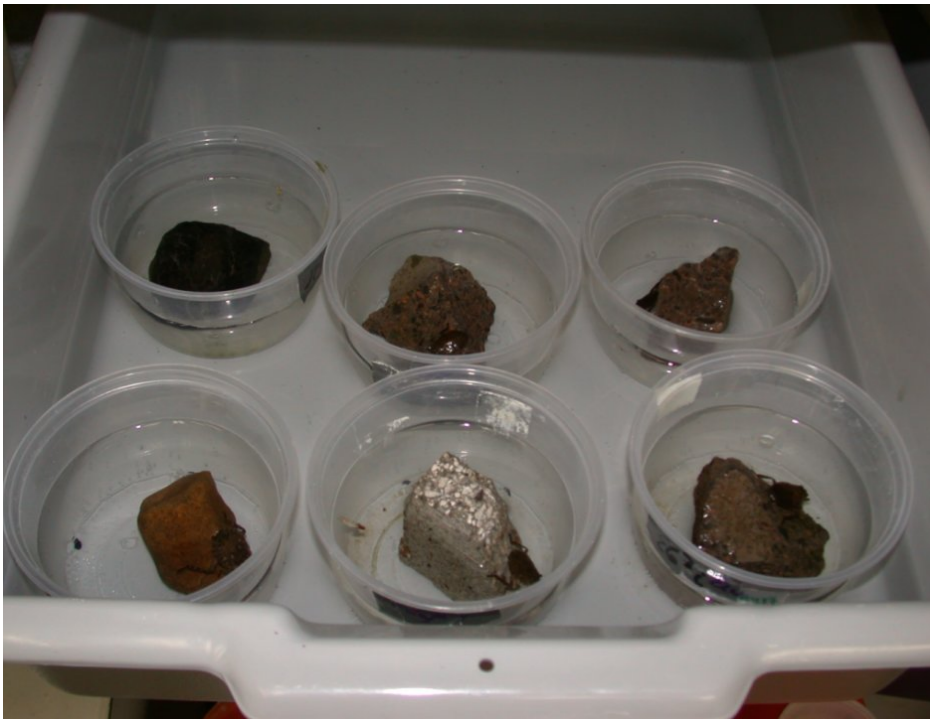


Figure 4.5: Porous tubs allow nymphs to share water reservoir.

Fifth instar bugs showed signs of molting when their developing wingpads turned black (Fig. 4.6). They usually molted within 48 hours of this occurrence. The fifth-instar-to-imago molt was often difficult and unsuccessful. Fifth instar individuals who showed the impending molt stigma were placed in 1 liter obstetric tubs described above, with a medium-sized rock in the center. The use of de-ionized water caused a low success rate, possibly due to hypoosmosis. Normal tap water proved the best medium for the final molt.



Figure 4.6: Darkened wingpads of fifth instar indicate imminent molt.

The typical fraction of a single brood that reached adulthood was around one in ten. Some crossbred broods (See Appendix 1) fared much more poorly. Mortality was highest in the second and fifth instars. It was never clear what the



second instars were dying of. Some portion of them generally stopped feeding and crawled out of the water on top of their bolt. Efforts to keep them moist were futile: they had simply given up the will to live. Fifth instars usually died of failure to molt.

Teneral imago bugs were allowed two days to harden before they were marked. They were kept sequestered from other adults for at least three weeks while their shells hardened. Individuals who were integrated too quickly were cannibalized.

### *Pests and Mortality*

White bacterial film grew readily on the water surface, forming a membrane that interfered with nymph respiration and necessitating frequent water changes. However, three other microorganisms eventually established themselves in the water supply: a desmid alga, an unidentified diatom, and an oomycete, *Legniperdiales*. The oomycete attacked the remains of insect prey and occasionally formed clumps on egg masses, but did not grow on living *A. herberti*, nor did it harm the viability of eggs. Bacterial film did not grow on water containing these other organisms. While the sheets of diatoms forming on the insides of the cups did not look handsome, the bugs did not suffer any ill effects.

Some wild-caught adult individuals died within the first week, but subsequent survivors have been in our laboratory for as long as thirty months.

When an adult died in the collective tubs, others would quickly eat it. However, I never saw an adult actually capturing and killing another adult. Moribund individuals of all ages often crawled up out of the water onto the rock or brick and await death by desiccation.

### *Filth*

*Abedus herberti* can, as most belostomatids, eject a stream of liquid excrement in a high-pressure geyser. This is often employed as a defense mechanism when the bugs are handled, but fortunately in this species the substance is not extremely offensive. However, nymphs living in cups will spray their waste as high and as far away from themselves as possible, perhaps to keep from fouling their habitat. This can lead to unsightly streaks on white walls. I raised five nymphs in yogurt cups on the back of a toilet in Costa Rica, and the nymphs liberally applied their spray to the wall-tiles with an almost artistic vigor. Fortunately the tiles were easily cleaned, but later the nymphs were moved unbeknownst to me to a small side-table, from which vantage they had access to white paint. This proved considerably harder to clean and left smudges despite our best efforts.

### *Escape*

This organism is an expert escape artist. A rock placed too close to the wall of a tub can serve as a fulcrum for a stretched-out metathoracic leg. The calibration marks on a hospital surgical tub can act as a tiny ladder for an ambitious fugitive insect. Nymphs occasionally scaled the sheer walls of their portion cups or used the drilled pore-holes in their tubs as footholds.

Typically, with constant supplies of food and water, the bugs did not try to escape. Under the care of inconstant, distracted undergraduate lab technicians, however, the bugs left the confines of their containers to try their luck in the hallway.

### *Bites*

Wild *Abedus herberti* can deliver a painful bite with their stout beak. It is impossible to ignore but rarely lasts more than ninety seconds. After generations of welfare in the laboratory, the bugs are quite tame and very rarely attempt to bite human hands. In two years of raising and handling them, over a thousand individuals, I have only been bitten twice. The impending bite can be recognized by the extension of the beak and the dorsal flexion of the head. Wild individuals also secrete a bright blue fluid from glands in their head when infuriated; this is followed by a bite-attempt.

### *Heat*

The placement of a submersible heater in the water reservoir of the nymphs, set to 30°C, shortened overall development time, from hatch to adult, from over four months to approximately three months. These animals evidently conform to a degree-day regime; much work remains, however, to determine the exact number of degree-days needed for development.

*Abedus herberti* is a hardy, robust insect that can easily be kept in culture. It is flightless and breathes air, and requires only a tub of water with a rock in it to thrive. While keeping live food may seem onerous, a refrigerated container of mealworms can sustain these bugs for weeks. The large size and agreeable disposition of these insects make them excellent candidates for aquatic culture and study.

### *Caveat custos*

I tried this technique to raise a single brood of a similar species, *Abedus indentatus*. Although there was little difficulty persuading the adults to mate, the nymphs grew extremely slowly and seemed to lose all hope in the fourth instar. They ceased taking food and crept as far under their rocks as they could go, until eventually succumbing to their megrims and perishing.

I also tried the technique on an unidentified Costa Rican species of *Belostoma*, which I captured as first-instars in a swamp in La Selva Biological Reserve near Sarapiquí. The regime answered very well for this species, and I was able to raise all five of my charges to fourth- or fifth-instars before I had to leave the country.

Dr Noriraka Ichikawa has written on the rearing of *Lethocerus indicus*, but his work has not yet been translated from the Japanese and remains cryptic to the monoglot reader.

*Abedus herberti* is an excellent candidate for a model organism to be kept in laboratory culture. Its large size and easy domestication lend themselves well to entomological instruction. It can be kept in simple tap water, and it does not have a discriminating palate. It reproduces readily, and complex brooding and molting behaviors are easily observed. This work should provide the basis for care and rearing of *Abedus herberti* for scientists, educators, and amateur enthusiasts.

## CHAPTER 5

### Summary and Conclusions

The ferocious waterbug *Abedus herberti* is a remarkable model for the study of evolution because its native habitat in southeastern Arizona and northern Sonora, Mexico essentially consists of a series of replicated treatments across gradients of elevation and latitude, that have apparently been isolated for thousands of generations. Body size variation within populations is small, which makes hypotheses about factors influencing body size easier to support.

Among known populations, *A. herberti* subdivides into two types based on local ecology: those found in ciénaga outflows (*C*-type) and those found in mountain runoff streams (*M*-type). The *C*-type bugs are significantly smaller and attain maturity faster than the *M*-type bugs. The two types belong to the same biological species and are capable of crossbreeding to produce fertile offspring. Mitochondrial DNA analysis suggests that *C*-type bugs have arisen independently in each ciénaga, and that they are not more closely related to other *C*-type bugs than they are to *M*-type bugs. This supports the hypothesis that adult body size is a trait that converges on similar phenotypes in similar environments. Raising offspring of both *M*-type and *C*-type bugs under identical conditions in a common-garden experiment showed that parental phenotype persisted in offspring, suggesting that the characters in question were heritable rather than a

result of phenotypic plasticity. The common-garden experiment also showed that *C*-type nymphs developed more quickly than *M*-type nymphs.

The *ciénaga* outflows that house the *C*-type bugs tend to be warmer and more thermally stable through the year. Mountain creeks tend to dry down in the summer before the monsoons come, persisting only in deeper pools, which grow hot and stagnant. In the wintertime these creeks can ice over, and can become torrential in the early spring when snow at higher elevations begins to melt. These two very different regimes of temperature and seasonality may have contributed to the evolution of two different types of the same species.

The Thermal Response Hypothesis, predicting selection for larger body size at higher altitudes, and by association lower temperatures, is a biological tendency that has been established for many taxa. *Abedus herberti* also follows this pattern, with individuals from higher populations tending to be larger than those from lower populations, notwithstanding different ecotypes. The mechanism for this thermal response is unknown in *A. herberti*; other workers report cell-size increase in cold-reared *Drosophila* lines. What particular benefits are conferred on an ectotherm like *A. herberti* strictly by greater size at lower temperatures remain unclear. However, taking other factors into consideration, such as life history and seasonality, sheds some light on the possibilities of adaptive advantage as conferred by variable body size.

The Quick Ripening Hypothesis, supported by the observed shorter developmental time of *C*-type nymphs, suggests that smaller size correlates with warmer temperatures because the seasonal conditions favorable to nymph

development extend through a greater portion of the year, and quicker development means more offspring and consequentially higher fitness. At lower temperatures there may be a minimum size requirement for successful overwintering, such that a quick maturation time would only lead to frivolous broods of offspring who could not withstand the winter.

The Ancillary Selection Hypothesis and Tough Nymph Hypothesis posit that selection may be acting primarily on eggs or first-instar nymphs. Larger eggs in colder thermal regimes confer more energy-content into nymphs, which may help them survive fluctuating, unpredictable springtime conditions. Additionally, the pre-monsoon summer streams favor large air-breathing predators, mostly Coleoptera and Hemiptera, and larger size would be a definite advantage in an eat-or-be-eaten system. On the other end of the thermal regime, the warmer temperatures may favor smaller egg size because in the absence of springtime cold snaps and high concentrations of hungry predators, the nymphs do not need the added energy content, and the smaller each egg is, the more eggs can fit onto a male's back in a single brood.

While these hypotheses are not mutually exclusive, the one that draws the most support from the evidence provided from this research is the Thermal Response Hypothesis, which predicts larger individuals from populations living under colder temperature regimes. The correlation between body size and altitude in Chapter 3 suggests that there is an optimal body size for these insects, as predicted by the mean annual temperature of their home reach. The multiple correlations in other aquatic invertebrate taxa corroborate a link between



temperature and body size. However, this does nothing to undermine support for the other hypotheses. It may simply be that the size differences began to evolve as a strict response to temperature, but that they conveyed adaptive advantages to *Abedus herberti* in a variety of other arenas and were reinforced by other selective regimes.

A useful addendum to this research would be a survey of *Abedus herberti* across its range, to map the morphological variation of the species across greater gradients of latitude, altitude and ecotype. In a theoretical sense, the distribution of a species among such a variety of local conditions seems ripe for a speciation event. It is possible that some remote offshoot population of *A. herberti* has adapted so extensively to its home reach that it could constitute a separate species. Continued study of *Abedus herberti* can bring valuable insight into the process of ecological speciation and the nature of divergence between isolated populations.

In conclusion, I would like to mention that working with *Abedus herberti* in the field and in the laboratory has been tremendously exciting and rewarding. This animal is easy to find because of its large size, easy to care for because of its catholic diet, and possesses a remarkable suite of behaviors and habits that we have only begun to explore.

### Acknowledgements

This study was funded by NSF Grant 0445366, NSF Grant IIS-0326052, and the Rice Endowment. Research was conducted at the American Museum of Natural History's Southwest Research Station and at Oregon State University. Thanks to Dr Andrew V.Z. Brower for useful criticism on earlier drafts and Michael T. Bogan for extensive field assistance.

## BIBLIOGRAPHY

Alpatov, W.W. (1930) Phenotypical variation in body and cell size of *Drosophila melanogaster*. *Biological Bulletin of the Marine Biological Laboratory of Wood's Hole* 58, 85-103.

Arnett, Amy E. and Nicholas J. Gotelli (1999) 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.

Atkinson, D. (1994) Temperature and organism size – a biological law for ectotherms? *Advances in Ecological Research* 25, 1-58.

Bayoh, M.N. and S.W. Lindsay (2003): Effect of temperature on the development of aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of Entomological Research* 93, 375-381.

Bergmann, C. (1847) Über die Verhältnisse der Wärmökonomie der Thiere zu ihrer Grösse. *Goett. Stud.* 1, 595-708.

Berven, Keith (1982): The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. *Evolution* 36, 962-983.

Blanckenhorn, Wolf U. (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology* 75 (4), 385-407.

Bogan, Michael T. (2005) Diversity and Community Structure of Aquatic Insects in Isolated Montane Desert Streams. Master's Thesis, Oregon State University.

Bogan, Michael T. and David A. Lytle (2007) Seasonal flow variation allows 'time-sharing' by disparate aquatic insect communities in montane desert streams. *Freshwater Biology* 52, 290-304

Bradford, M.J. and D.A. Roff (1995) Genetic and phenotypic sources of life history variation along a cline in voltinism in the cricket *Allonemobius socius*. *Oecologia* 103, 319-326.

Brown, James H. and C. Robert Feldmeth (1971) Evolution in Constant and Fluctuating Thermal Environments: Thermal Tolerances of Desert Pupfish (*Cyprinodon*). *Evolution* 25 (2), 390-398.

Cushman, J. Hall, John H. Lawton, and Brian F.J. Manly (1993) Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia* 95, 30-37.

David, J.R. and P. Cappy (1982) Genetics and origin of a *Drosophila melanogaster* population recently introduced to the Seychelles. *Genetical Research* 40, 295-303.

Davidson, J. (1944) On the relationship between temperature and rate of development of insects at constant temperatures. *Journal of Animal Ecology* 13, 26-38.

Dyar, H.G. (1890) The number of moults of lepidopterous larvae. *Psyche, Camb.* 5, 420-422.

Hall, T.A. (1999) Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows. *Nucleic Acids Symp Ser* 41, 95-98.

Hynes, H.B.N. (1970): The ecology of stream insects. *Annual Review of Entomology* 15: 25-42.

Imai, Takeo (1937): Influence of temperature on the growth of *Drosophila melanogaster*. *Sci. Rep. Tohoku Imp. Univ.* 11, 403-417.

Kaplan, R. H. (1980): Ontogenetic energetics in *Ambystoma*. *Physiological Ecology* 53, 43-56.

Keffer, S.L. and J.E. McPherson (1988): Descriptions of nymphal instars of *Abedus breviceps* (Hemiptera: Belostomatidae). *The Great Lakes Entomologist* 26 (4), 169-174.

Kondoh, M. (1977): On the difference of vitality among worker ants under starvation. *Proceedings of the eighth international congress of the international union for the study of social insects (Wageningen)*, pp. 69-70. Centre for Agricultural Publishing and Documentation, The Netherlands.

Kraus, Bill (1985) Oviposition on the backs of female giant water bugs, *Abedus indentatus*: the consequence of a shortage in male back space? (Hemiptera, Belostomatidae). *Pan-Pacific Entomologist*, 61(1), 54-57.

Lewontin, R.C. (1965) Selection for colonizing ability. In *The Genetics of Colonizing Species* (H.G. Baker and G.L. Stebbings, eds.) pp. 77-91. Academic Press, New York.

Long, Austin A. (1966) Late Pleistocene and recent chronologies of Playa lakes in Arizona and New Mexico. PhD Dissertation, University of Arizona.

Lytle, D. A. (1999) Use of rainfall cues by *Abedus herberti* (Hemiptera: Belostomatidae): a mechanism for avoiding flash floods. *Journal of Insect Behavior* 12, 1-12.

Lytle, D.A., Michael T. Bogan and Debra S. Finn (in review): Evolution across a gradient of disturbance predictability.

Markarian, Ralph K. (1980) A study of the relationship between aquatic insect growth and water temperature in a small stream. *Hydrobiologia* 75, 81-95.

Martin, Paul S. (1963) *The Last 10000 Years: A Fossil Record of the American Southwest*. The University of Arizona Press.

Masaki, S. (1967) Geographic variation and climatic adaptations in the life cycles of crickets. *Evolution of insect migration and diapause* (H. Dingle, ed.) pp. 72-100. Springer-Verlag, New York.

Mayr, Ernst (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge.

Menke, Arnold S. (1960) A Taxonomic Study of the Genus *Abedus* Stål (Hemiptera, Belostomatidae). *University of California Publications in Entomology* 18(8), 393-440.

Miller, Robert Rush (1950) Speciation in Fishes of the Genera *Cyprinodon* and *Empetrichthys*, inhabiting the Death Valley Region. *Evolution* 4, 155-163.

Mitchell, R. (1974) The evolution of thermophily in hot springs. *Quarterly Review of Biology* 49, 229-42.

Pettus, D., and G.M. Angleton (1967) Comparative reproductive biology of montane and piedmont chorus frogs. *Evolution* 21, 500-507.

Purcell, M. and S.C. Welter (1990) Degree-day model for development of *Calocoris norvegicus* (Hemiptera: Miridae) and timing of management strategies. *Environmental Entomology* 19, 848-853.

Ray, Carleton (1960) The Application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology* 106, 85-108.

Schnack, Juan A, Robert L Smith and Eduardo A. Domizi (1994) Mode of inheritance of the phenotype "stripe" and survey of its frequency in a natural population of *Belostoma oxyurum* (Dufour) (Heteroptera: Belostomatidae). *Neotropica* 40 (103-104), 3-8.

Smith, Robert L. (1974) Life History of *Abedus herberti* in Central Arizona (Hemiptera: Belostomatidae). *Psyche* 81, 272-283.

- Smith, Robert L. (1975) Surface molting behavior and its possible respiratory significance for a giant water bug *Abedus herberti* Hidalgo (Hemiptera: Belostomatidae). *The Pan-Pacific Entomologist* 51 (4), 259-267.
- Smith, Robert L. (1979) Paternity Assurance and Altered Roles in the Mating Behaviour of a Giant Waterbug, *Abedus herberti* (Hemiptera: Belostomatidae). *Animal Behaviour* 27, 716-725.
- Smith, R.L. (1997) Evolution of paternal care in the giant waterbugs (Heteroptera: Belostomatidae). In *The Evolution of Social Behavior in Insects and Arachnids* (Choe, J.C. and Crespi, B.J. eds.) pp. 116-149. Cambridge University Press, Cambridge.
- Smith, Robert L and J.B. Smith (1976) Inheritance of a Naturally-Occurring Mutation in a Giant Waterbug. *Journal of Heredity* 67, 182-185.
- Swofford, D.L. (2002) PAUP\*: Phylogenetic Analysis Using Parsimony (\*and other methods.) Version 10, D.L. Swofford Associates, Sunderland, MA.
- Vannote, R.L. and B.W. Sweeney (1980) Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *American Naturalist* 115, 667-695.
- Velasco, J. and A. Millan (1998) Feeding Habits of Two Large Insects from a Desert Stream: *Abedus herberti* (Hemiptera: Belostomatidae) and *Thermonectus marmoratus* (Coleoptera: Dytiscidae). *Aquatic Insects* 20(2), 85-96.
- von Bertalanffy, L. (1960) Principles and theory of growth. In: *Fundamental Aspects of Normal and Malignant Growth* (W.N. Nowinski, Ed.). pp 137-259. Elsevier, Amsterdam.
- Ward, James V. and Jack A. Stanford (1982) Thermal Responses in the Evolutionary Ecology of Aquatic Insects. *Ann. Rev. Entomol.* 27, 97-117.
- Waters, M and C. Hayne (2001) Late Quaternary Arroyo Formation and Climate Change in the American Southwest. *Geology* 29, 399-402.
- Woosley, A. and M. Waters (1990) Reevaluations of Early Cochise Artifact Associations with Pleistocene Lake Cochise, Southeast Arizona. *American Antiquity* 55(2), 360-366.
- Zeh, D.W. and J. A. Zeh (1988) Condition dependent sex ornaments and field tests of sexual selection theory. *American Naturalist* 132, 454-459.

## APPENDICES

## APPENDIX 1

### A case against speciation

Both *Abedus herberti* and the desert pupfish of the former Lake Lahontan find themselves in a similar post-Pleistocene isolation, with disjunct species spread over a wide area (Miller, 1950). Many of these fish have segregated into extreme microhabitats like hot springs, and the original ancestral state has calved numerous new species that are particularly adapted to their own specific environment (Brown and Feldmeth, 1971). Morphological and physiological distinctions cleanly separate species.

*Abedus herberti*, an air-breathing insect subjected to similar post-Pleistocene habitat shrinkage, also shows considerable morphological variation. A case could be made based on antennal morphology for at least four subspecies of *A. herberti* (Menke, 1960); however, preliminary data suggest that populations separated by large distances or size disparities can still produce offspring.

For a quick assay of compatibility, I chose four populations of *A. herberti*, two large mountain types (type *M*, adult average length >35mm) and two small cienega types (type *C*, adult average length <32mm). I was constrained by the number of available *M1* virgin females; Smith (1979) demonstrated the potential for cuckoldry in females who stored sperm from previous matings. If there was to be a lack of compatibility between pairs, stored sperm from a previous mating with a compatible male would confuse the results.



I arranged thirteen crosses as follows:

2 x C1♀ x M1♂  
 2 x C1♀ x C2♂  
 1 x M1♀ x C1♂  
 2 x M2♀ x C2♂  
 2 x M2♀ x M1♂  
 2 x C2♀ x M2♂  
 2 x C2♀ x C1♂

Table A1.1: Results of 13 crosses of *Abedus herberti* from different populations.

Cross	Mating	Oviposition	Hatch	% 3 <sup>rd</sup>	Adult
C1♀ x M1♂ <b>a</b>	Yes	Yes	Yes	16.7	No
C1♀ x M1♂ <b>b</b>	Yes	No	No	0	No
C1♀ x C2♂ <b>a</b>	Yes	Yes	Yes	16.7	No
C1♀ x C2♂ <b>b</b>	Yes	Yes	Yes	12.5	Yes
M1♀ x C1♂	Yes	Yes	Yes	20	Yes
M2♀ x C2♂ <b>a</b>	Yes	Yes	Yes	33.3	Yes
M2♀ x C2♂ <b>b</b>	Yes	Yes	Yes	29	No
M2♀ x M1♂ <b>a</b>	Yes	Yes	Yes	12.5	Yes
M2♀ x M1♂ <b>b</b>	Yes	Yes	Yes	29	No
C2♀ x M2♂ <b>a</b>	Yes	Yes	Yes	62.5	Yes
C2♀ x M2♂ <b>b</b>	Yes	Yes	Yes	42	Yes
C2♀ x C1♂ <b>a</b>	Yes	Yes	Yes	79	Yes
C2♀ x C1♂ <b>b</b>	Yes	Yes	Yes	42	Yes

Mating occurred in all crosses, suggesting these animals were able to recognize each other as conspecifics (Table 1). In only once case was there no oviposition: in that particular cross, eggs came out of the female but did not attach to the male. When there was a size disparity between male and female, the bugs had some minor positioning problems.

All eggs that were brooded yielded at least some hatchlings. I kept 24 of each hatch and raised them following the method described in Appendix 1. The nymphs with a C2 mother performed the best, enjoying the lowest mortality by the third instar.

These are preliminary results, and in the absence of a synchronized control cross and a balanced design, should be regarded with scientific wariness. The only meaningful conclusion I draw here is that hybridization across populations is possible and there are no intrinsic barriers to gene exchange within the animals themselves. Each type of cross ( $M1 \times C1$ ,  $M2 \times C2$ ,  $M1 \times M2$ ,  $C1 \times C2$ ) yielded at least one adult.

Whether all the hybrids are viable remains to be conclusively proven. So far one male hybrid and two female hybrids have successfully mated with wholeblooded individuals.

This early evidence suggests that separate populations of *Abedus herberti* are mere geographic polytypes. The repeated incidence of smaller adult body size concurring with lower elevations and spring-fed systems suggests the possibility of a causal correlation.

## APPENDIX 2

### Dyar's Rule and nymph morphometry

From Fall 2004 to Spring 2005 I raised *Abedus herberti* nymphs in the laboratory at 20°C on a 14/10 light/dark cycle. Approximately half of the first-instar nymphs were placed in a 10°C room; their length and width is reported here because they were brooded and hatched at 20°C. Those few nymphs who molted in the 10°C room are excluded from this table. Only a fraction of these nymphs were used for experimental analyses, but each one was measured as it molted. The mean sizes for each instar's length and width from each population are shown in Table 1.

Table A2.1: Number of broods, length and width of each instar in millimeters, and number of nymphs surviving at each instar ( $n$ ), grouped by population

POP	# broods	1 <sup>st</sup> instar			2 <sup>nd</sup> instar			3 <sup>rd</sup> instar			4 <sup>th</sup> instar			5 <sup>th</sup> instar		
		L	W	$n$	L	W	$n$	L	W	$n$	L	W	$n$	L	W	$n$
M1	4	7.1	4.2	75	9.8	5.8	34	14.4	8.0	21	19.9	11.2	12	27.5	15	6
M2	4	8.0	4.9	96	11	6.4	73	19.5	9.0	24	22.1	13.5	15	29.7	17	10
M3	3	7.8	4.7	72	10	6.3	34	18.5	8.9	7	-	-	0	-	-	0
C1	9	6.7	3.9	118	9.0	5.3	78	17.2	7.3	44	17.9	9.7	27	24.4	14	18
C2	2	6.6	4	67	9.1	5.3	35	17.3	7.5	11	18	11	2	-	-	0
C3	4	7.0	4.3	85	9.5	5.8	54	18.4	8.3	19	19	11	10	26	15	2

Dyar's Rule (1890) posits a maximal 1.4x size increase per molt for insects. I calculated a "Dyar Value" for length and width of each of these six populations with this formula:  $(\text{adult mean length}/1^{\text{st}} \text{ instar mean length})^{1/5}$

Table A3.2: Dyar's Values for length and width of six populations of *Abedus herberti* raised in the laboratory.

Population	Dyar's Value (Length)	Dyar's Value (Width)
M1	1.305	1.287
M2	1.286	1.263
M3	1.284	1.265
C1	1.281	1.264
C2	1.306	1.281
C3	1.291	1.259