INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

The role of habitat quality in shaping evolutionary dynamics, population dynamics, and conservation planning.

by

Jonathan M. Hoekstra

A dissertation submitted in partial fulfillment of the requirements for the degree of

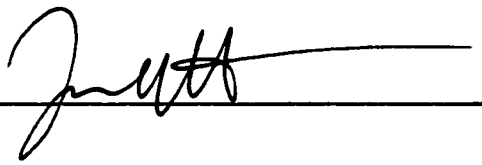
Doctor of Philosophy

University of Washington
2001

Program Authorized to Offer Degree: Zoology

Doctoral Dissertation

In presenting this dissertation in partial fulfillment of the requirements for the Doctoral degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of the dissertation is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for copying or reproduction of this dissertation may be referred to Bell and Howell Information and Learning, 300 North Zeeb Road, P.O. Box 1346, Ann Arbor, MI 48106-1346, to whom the author has granted "the right to reproduce and sell (a) copies of the manuscript in microform and/or (b) printed copies of the manuscript made from microform."

Signature 

Date 8/17/2001

University of Washington
Graduate School

This is to certify that I have examined this copy of a doctoral dissertation by

Jonathan M. Hoekstra

and have found that it is complete and satisfactory in all respects, and that any and all revisions required by the final examining committee have been made.

Chairs of Supervisory Committee:

Joel G. Kingsolver

Raymond B. Huey

Reading Committee:

Daniel Schindler

Date: 8/17/2001

University of Washington

Abstract

The role of habitat quality in shaping
evolutionary dynamics, population dynamics, and conservation planning.

by Jonathan M. Hoekstra

Co-chairpersons of the Supervisory Committee

Professor Joel G. Kingsolver
Professor Raymond B. Huey
Department of Zoology

Understanding ecological and evolutionary consequences of variation in habitat quality is increasingly important as biologists seek to address human-mediated environmental change. I investigated effects of natural temperature variation on individual fitness and population dynamics of *Drosophila melanogaster* inhabiting rotting apples in orchards. I also examined how critical habitat designations have influenced recovery plans for threatened and endangered species.

I exposed *D. melanogaster* to field temperatures in rotting apples and measured survivorship and development time. Extreme temperatures in sun-exposed apples reduced survivorship of *D. melanogaster* by more than 50% relative to that in shaded apples. This difference is comparable to that caused by seasonal changes in ambient temperature, and suggested that selection will target traits conferring greater heat resistance.

I next conducted a 3 x 2 factorial experiment that revealed an interaction effect of ethanol and temperature variation between shaded and sun-exposed apples on development time. Adverse effects of high ethanol concentrations and extreme temperatures in sun-exposed apples mitigated one another. Expected correlation between selection for ethanol and heat resistance depends on whether this result derived from cross-induction of physiological stress responses or abiotic interactions between temperature and ethanol.

To test an hypothesis that habitat heterogeneity reduces climate-induced population variability, I established populations of *D. melanogaster* during summer and autumn in large field cages that enclosed "landscapes" of rotting apples. I manipulated habitat heterogeneity with shade cloth to create homogeneous landscapes of either shaded or sun-exposed apples, and heterogeneous landscapes with both. Population density and growth rate varied less between summer and autumn in heterogeneous cages than in either homogeneous treatment. These results suggested that managing for habitat heterogeneity may reduce population variability and thus mitigate risks of stochastic extinction.

Lastly, I examined how critical habitat designations influenced recovery plans for threatened and endangered species. Plans for species with critical habitat were not more likely than others to prescribe habitat-based recovery actions or habitat-based recovery criteria. Findings suggested that recovery plans for endangered species have not benefited from critical habitat designations and raised questions about how policies might be amended so that future recovery plans will benefit.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

The Department of Zoology has provided an exceptionally stimulating, challenging, and fun intellectual environment for me during my graduate studies. I am grateful to so many people who, in one or many ways, influenced my thinking, my research, and my teaching.

Joel Kingsolver, my advisor, gave me broad latitude to pursue my own research interests, persistently encouraged me to address questions with intellectual and experimental rigor, and inspired me to seek innovative methods. Joel had a knack for asking cosmic questions that forced me to place my research in context and perspective, and then laser-sharp questions that forced me to bring it into a sharp focus.

Ray Huey made me a believer in the power and potential of *Drosophila* to reveal the mysteries of ecology and evolutionary biology – or at least to investigate the ecological and evolutionary effects of temperature. Ray's comments on both manuscripts and presentations helped improve my communication skills.

Daniel Schindler provided helpful advice for analyzing population dynamics and thoughtful comments on my dissertation.

Mike Beecher served as my Graduate School Representative.

Peter Kareiva opened a number of exciting doors into conservation research including the use of science in habitat conservation plans, and conservation planning for Pacific salmon. These "side projects" to my dissertation research reinvigorated my interests in conservation biology.

Dee Boersma pushed me through some of the doors that Peter Kareiva opened. In particular, she convinced me to be an active part of a comprehensive review of endangered species recovery plans that gave me invaluable experience with planning and organizing a large collaborative research project.

Martin Feder from the University of Chicago garners special acknowledgment because he unwittingly inspired my research into the temperature effects of *Drosophila melanogaster* after I read one of his manuscripts. Shortly after I struck up correspondence with Martin, he very generously provided field equipment and travel money for me to measure the temperature of apples at some orchards in California, and then hosted me in his lab for a week.

Tom Daniel, Gary Odell, Elizabeth Thompson and the rest of the folks from the infamous Math Bio seminar were instrumental in helping me develop my skills in mathematical

modeling and analysis. Math Bio was a truly unique forum. I especially appreciated Gary's (sometimes excessive) inquisitiveness and Tom's rotten puns.

Teaching was just as important for my graduate training as was research. I was thrilled by the training and mentorship I received from the all of the faculty members for whom I was a teaching assistant. Mary Pat Wenderoth stood out as the gold standard for engaging students both in the lecture hall and in the lab. John Edwards made me a colleague in teaching the entomology course. Jon Herron's course on teaching helped me develop tools and skills that I will apply in all of my future teaching efforts.

Janet Lane provided assistance in both the lab and field with the insert experiments described in Chapter 2 and the cage experiments in Chapter 3. The research in Chapter 3 would not have been possible without the assistance of Ben Strauss who helped devise the field protocol for estimating population densities in the field cages.

Of course, I could not have accomplished anything without the help of the Department's administrative staff – Kathryn Hahn, Judy Farrow, Brianna Divine, Susie Denison, Doug Young, Sue Bartroff, and my fellow Michigander, Belinda Sachs. I am especially grateful for their extraordinary patience with me as I time and again pestered them with questions and requests.

Finally, I wish to acknowledge lab mates, fellow grad students, and other colleagues for their assistance and feedback as well as their friendship and camaraderie over the years.

The extended King-Huey lab family (present and past) – Lisa Crozier, Brad Gaolach, Ben Strauss, Anhthu Hoang, Melanie Frazier, Michael Dillon, Gwen Schlicta, Sher Rehr, Margen Carlson, Art Woods, George Gilchrist, David Berrigan, Patricia Gibert.

And, in no particular order -- Gary Chang, Amanda Stanley, John Payne, Chris Harley, Jim Regetz, Alan Clark, Ned Place, Chris Hess, Jeff Bradley, Lynn Catlin, and Hopi "the other" Hoekstra.

I was supported at various times in my research by a Mathematical Biology Training Grant Fellowship, a research assistantship from the Joint Institute for the Study of the Atmosphere and Oceans, grants to Joel Kingsolver and Dee Boersma, Zoology Department Hardship Funds, and an NSF Dissertation Improvement Grant.

DEDICATION

I dedicate my dissertation to:

My parents, Donald Hoekstra and Barbara Armstrong, who encouraged my interests in biology and science and always supported my academic pursuits.

And to my wife, Jennifer Steele, who supported me through all of the challenges of graduate school.

INTRODUCTION

Habitat is more than just the backdrop against which biological processes unfold. It is a dynamic stage with which organisms interact and by which both individuals and populations are affected. Understanding these interactions and effects is increasingly important as human activity modifies the physical environment on which biotic communities ultimately depend. Elevated atmospheric carbon dioxide, global warming, and altered hydrological regimes are just a sample of environmental changes due to human activity (McCarthy et al. 2001). Predicting and mitigating for the ecological and evolutionary consequences of these changes is a paramount challenge now facing ecologists, evolutionary biologists, and conservation biologists.

In the first three chapters, I investigated the effects of one aspect of habitat quality – temperature – on individual fitness and population dynamics of *Drosophila melanogaster*. For this, as for so many aspects of biology, *D. melanogaster* proved to be an excellent model system that yielded some new insights into the implications of habitat quality for natural selection and population persistence. In the final chapter, I took a different tack and examined how what we already know about the effects of habitat quality on species has been incorporated into conservation plans for species at risk of extinction. Below, I highlight each chapter in turn.

Chapter 1 described the effects of natural temperature variation on two components of fitness – survivorship and development time. To experimentally measure these effects in the field, I developed a protocol by which I could expose *D. melanogaster*

to temperatures in one of its natural habitats – rotting apples – while controlling for the effects of food quality and larval density. The key finding of these experiments was that spatial variation of diurnal temperatures in sun-exposed and shaded apples within orchards had a substantial effect on survivorship of *D. melanogaster*. Survivorship was reduced by more than 50% in sun-exposed apples compared to shaded apples. This results suggested that natural selection will target traits that improve resistance to extreme diurnal temperatures and associate effects of heat stress.

In Chapter 2, I tested the effects of ethanol and temperature variation between shaded and sun-exposed apples. Both factors were known to independently affect fitness of *D. melanogaster*, but their interaction effect was poorly understood. I elaborated on the experimental methods from Chapter 1 to expose *D. melanogaster* to temperatures in shaded and sun-exposed apples while simultaneously manipulating the concentration of ethanol in the food medium. These experiments revealed a previously undescribed interaction effect by which the adverse effects on development time of high ethanol concentrations and extreme diurnal temperatures in sun-exposed apples mitigated one another. The implication of this effect for natural selection will depend on whether it reflected cross-induction of physiological stress responses or an abiotic interaction between temperature and ethanol in the apples. In the former case, selection for ethanol and heat resistance should be positively correlated, while in the former case, traits should be negatively correlated.

Chapter 3 reported an experimental test of whether habitat heterogeneity reduced population variability caused by seasonal climate variation. I established replicate

experimental populations of *D. melanogaster* during summer and autumn in large field cages that enclosed "landscapes" of rotting apples. I manipulated thermal heterogeneity in the cages with shade cloth to create homogeneous landscapes of either shaded or sun-exposed apples, and heterogeneous landscapes with both shaded and sun-exposed apples. The rank order of final population densities and maximum rates of population increase in shaded and sun-exposed cages reversed between summer and autumn, while densities and growth rates in heterogeneous cages were consistent between seasons. These results demonstrated that habitat heterogeneity can reduce population variability and suggested that managing for habitat heterogeneity may be an effective conservation strategy for hedging against stochastic extinction risks.

Lastly, in Chapter 4, I turned my attention to policy matters concerning the role of critical habitat designations played in conservation and recovery planning for species listed under the U. S. Endangered Species Act. The analyses presented in this chapter used data compiled by a comprehensive review of endangered species recovery plans that I helped to plan and conduct. We expected that critical habitat designations should promote more frequent prescriptions for habitat-based recovery actions and habitat-based criteria for assessing recovery of listed species. However, we found no significant differences between plans for species with and without critical habitat. Our findings suggested that recovery plans for endangered species have not benefited from critical habitat designations and raised challenging questions about how regulations concerning critical habitat and recovery planning might be changed so that the expected benefits will be realized in future recovery efforts.

CHAPTER 1: EFFECTS OF NATURAL TEMPERATURE VARIATION ON SURVIVORSHIP AND DEVELOPMENT TIME OF *DROSOPHILA MELANOGASTER.*

INTRODUCTION

The pronounced effect of temperature on survivorship and development time of *Drosophila melanogaster* has long been known from laboratory studies where flies were raised under a range of constant temperature conditions (Loeb and Northrop 1917; Bonnier 1926; Powsner 1935; David and Clavel 1967; Cohet et al. 1980). Survivorship is high over a broad range of temperatures and then falls sharply as lower and upper thermal tolerance limits are approached (Figure 1). Development time is more broadly sensitive to changes in temperature, decreasing rapidly as temperature increases toward an optimum development temperature above which development time increases again (Figure 1). How do these reaction norms translate into component measures of fitness (i.e., viability and generation time) under the variable temperature conditions that *D. melanogaster* and other ectotherms experience in nature?

Temperature in nature varies tremendously over many scales of time and space (Levins 1968). Seasonal and geographic variation in mean ambient temperature is superimposed by short-term weather patterns and diurnal temperature fluctuations. For *D. melanogaster* that develop in rotting fruit in the field, temperature also varies spatially depending on the degree to which fruit are exposed to the sun. Temperatures in rotting fruit are generally similar to those of the ambient environment. However, when fruit are

exposed to the sun, fruit temperature may heat above ambient air temperature by as many as 20°C (Feder et al. 1997a). In orchards, the orderly arrangement of trees creates regular patterns of sun and shade on the ground that determines the extent to which rotting fruit are sun-exposed or shaded. Fruit near the base of a tree are shaded most of the day such that fruit temperature tracks the diurnal fluctuations of ambient air temperature. Fruit near the center of orchard rows are sun-exposed during the day such that diurnal temperature fluctuations are exaggerated by elevated daily maximum temperatures (Feder et al. 1999). Temperature also varies on a small spatial scale due to temperature gradients of up to 5°C within individual fruits.

Natural temperature variation in rotting fruit has substantial physiological effects on indwelling larvae of *D. melanogaster* that, in turn, may affect components of fitness such as survivorship and development time. First, elevated diurnal temperatures in sun-exposed fruit are often high enough to cause heat stress and to trigger heat shock responses such as expression of heat shock proteins (Feder et al. 1997a). Second, diurnal temperature fluctuations span a large range, even in shaded fruit (Feder et al. 1999), Figure 2). Consequently, indwelling larvae could experience night-time temperatures cold enough to slow or even suspend development within hours of experiencing physiologically stressful daytime temperatures. This realized range of diurnal temperature fluctuation is considerably greater than any of the variable temperature regimes that have been investigated in the laboratory (e.g., Ludwig and Cable 1933; Siddiqui and Barlow 1972). Last, the consequences of diurnal temperature fluctuations may be modified by seasonal variation in mean ambient temperature. During summer,

diurnal temperatures in sun-exposed fruit are more likely to induce heat stress or even exceed upper thermal tolerance limits of indwelling larvae. During cooler seasons, daytime fruit temperatures are less likely to induce heat stress, but night-time temperatures are more likely to slow or suspend development.

So what are the consequences of natural temperature variation on fitness of *D. melanogaster*? Predicting the consequences by integrating traditional reaction norms is difficult because the extremes of diurnal temperature fluctuations often exceed apparent tolerance limits observed under constant temperature conditions (compare range of temperatures spanned by the reaction norms in Figure 1 and that spanned by diurnal temperature fluctuations in Figure 2). This problem might be resolved by measuring reaction norms over shorter time intervals to quantify performance during brief exposure to more extreme temperatures (Kingsolver 2000), but such measures are increasingly difficult over short time intervals. Furthermore, both empirical studies and theoretical analyses suggest that unpredictable, non-linear effects of fluctuating temperatures will still cause predictions and reality to diverge (Siddiqui and Barlow 1972; Hagstrum and Milliken 1991; Worner 1992; but see Liu et al. 1995).

Here I present an alternative approach to measuring and predicting the effects of natural temperature variation on fitness of *D. melanogaster*. I put fruit and flies into orchards and directly measured two components of fitness – survivorship and development time – under natural temperature conditions. I then compared these data to evaluate the effect of three gradients of natural temperature variation. First, I examined the effect of spatial variation in diurnal temperatures between shaded and sun-exposed

apples. Second, I assessed the effect of seasonal variation in mean ambient temperature by measuring fitness at different times during summer and autumn. Finally, I investigated the effect of small-scale spatial variation due to temperature gradients within individual fruit. The results of these analyses demonstrated that natural temperature variation has significant – and sometimes surprising – effects on survivorship and development time of *D. melanogaster*, and suggested that natural temperature variation could exert strong selection on thermo-tolerance traits.

METHODS

Measuring effects of natural temperature variation

I evaluated the effect of natural temperature variation on *D. melanogaster* by measuring two components of fitness: egg-to-adult survivorship and egg-to-adult development time. Survivorship to reproductive maturity is an obviously important component of any organism's fitness. Development time is also a key component of fitness because the rotting fruit in which larvae develop are ephemeral resources. Shorter development time increases the likelihood that an individual can complete development before the resource decays away. Shorter development time also increases fitness because it shortens generation time, and thus increases the intrinsic rate of increase (r) of a lineage (Fisher 1958). In habitats where the abundance and availability of rotting fruit changes seasonally, the capacity to exploit those resources by rapidly increasing the number of progeny would be a clear advantage.

To measure larval survivorship and development time under natural temperature conditions, I placed eggs of *D. melanogaster* into small "inserts" that were put into apples. The apples were then placed in orchard field sites to expose developing larvae to natural temperatures. Inserts were constructed from the upper ribbed portion of 1000 µl pipet tips (VWR brand for Gilson pipetman) that formed small rigid cylinders. A small swatch of 152 µm Nytex mesh was glued to the bottom of each insert to prevent larvae from crawling out or in, while still allowing gas and fluid exchange. Inserts were filled with a standard food medium made from cornmeal, molasses, yeast and agar. After eggs were transferred into an insert (see below), a piece of Parafilm was wrapped over the top to close the insert. The Parafilm was perforated with a pin to retard desiccation of the food while still allowing for sufficient gas exchange into the insert.

Inserts were pushed into pilot holes in apples until their tops were flush with the apple skin. A snug fit into the pilot hole minimized desiccation of the surrounding fruit and ensured that inserts were the same temperature as the surrounding fruit. During pilot testing, I placed thermocouples inside inserts and in surrounding fruit, and monitored temperatures throughout the day to verify that insert temperatures accurately tracked apple temperatures ($r^2 > 0.99$). The inserts effectively segregated experimental animals from any other larvae that free-ranging *Drosophila* might lay into an apple during field exposure. They also enabled me to control food quality and larval density. Standard food medium was used to avoid confounding complications that might be introduced by variation in the quality of rotting apples. A larval density of 10 eggs per insert was chosen to minimize adverse effects of crowding (Palabost 1972).

Eggs were collected from laboratory colonies of wild-type *D. melanogaster*. Colonies were maintained at large population sizes with overlapping generations in 1-gallon containers into which fresh food medium was regularly introduced. During early experiments, I collected eggs from a wild-type stock originally collected in California by Larry Harshman, and maintained at 25°C and a 12 hour light-dark cycle. During later experiments, eggs were collected from wild-type stock collected locally in Washington State by the author and maintained at ambient room temperature (ca. 20-22°C) and on a natural light-dark cycle. Survivorship and development time data for eggs collected from these stocks were pooled for analysis.

The basic experimental protocol involved transferring age-matched cohorts of ten *D. melanogaster* eggs each into inserts filled with standard food medium, putting inserts into apples, and placing apples on the ground in an orchard where developing larvae were exposed to natural temperature conditions. Eggs were collected by allowing flies in the laboratory colonies to oviposit on medium-filled petri dishes left in colony cages for 4 to 12 hours. Working under a dissecting microscope, I then transferred ten eggs into each insert using a small bristled brush. The following day, inserts were placed into apples and left in an orchard for one week. After the seven day field exposure, I collected the inserts and returned them to the laboratory, where I peeled off the Parafilm covers and placed inserts individually in test tubes stopped with cotton.

I monitored the test tubes daily for adult eclosion as larvae completed development under ambient laboratory conditions (20-22°C, natural light-dark). Each day, any adults that eclosed in an insert were counted and removed from the test tubes.

Survivorship for each adult fly was recorded (survivorship = 1) and development time was calculated as the difference between eclosion date and the date that eggs were collected. Any eggs placed into inserts that did not successfully develop into adults were assumed to have died (survivorship = 0). Development time could not be calculated for those individuals.

After all surviving adults had eclosed (usually within 2 to 3 weeks), I used the data to calculate estimates of survivorship and development time for each insert. Survivorship was number of adults that emerged from an insert divided by 10, the number of eggs initially put into each insert. Average development time in each insert was calculated as the geometric mean of observed development times of surviving adults. This admitted variation among observations, but limited disproportionate influence of outliers. No estimate of development time was made for inserts in which survivorship was 0. If more than ten flies eclosed from a single insert, survivorship was set to 1.0 and mean development time was estimated from observed development times for the first 10 individuals. "Extra" flies eclosed from approximately 10% of inserts. Some of the extra flies reflected counting errors made when eggs were first transferred into the inserts. Others were interlopers from the field that hitchhiked on the outside of an insert, or the progeny of experimental flies that successfully mated and reproduced between daily observations.

The effects of natural temperature variation were evaluated by comparing survivorship and development time across three axes of variation: spatial variation in diurnal temperatures (in sun-exposed versus shaded apples), seasonal variation in mean

ambient temperature, and within-apple temperature gradients. To test the effect of spatial variation in diurnal temperatures, I placed apples with inserts near the base of trees (shaded) and near the center of orchard rows (sun-exposed). These positions marked the extremes of sun-exposure within an orchard. Diurnal temperatures in sun-exposed fruit would be highest while those in shaded fruit would remain close to ambient air temperature throughout the day. During three experimental runs, I also positioned apples beneath the edge of tree canopies where they would be shaded for half the day and sun-exposed for the other ("partial"). Diurnal temperatures and the consequent effects on survivorship and development time were expected to be intermediate between those in shaded and sun-exposed fruit.

To assay the effect of seasonal variation in ambient temperature from summer through autumn, I repeated the basic experimental protocol 22 times between July and October 1998 (15 runs) and July and October 1999 (7 runs). I used daily mean air temperature reported by nearby weather stations as the index for seasonal variation in ambient temperature. Ambient temperature during each experimental run was calculated as the average of daily mean air temperatures during the seven days that apples were left in the field. Half of the experimental runs were conducted in an orchard near Wenatchee, Washington (inland), and the rest in an orchard near Bothell, Washington (coastal). I combined data from both sites to evaluate fitness effects over a broader range of ambient temperatures. Overlap in ambient temperatures at the two sites also enabled me to distinguish any random site effects. Comparison of monthly mean air temperatures reported at nearby weather stations indicated that ambient temperatures at the two field

sites generally differed by about 3-4°C. However, this difference was comparable to between-year variation at any single site, and was less than differences between seasons (Figure 3, NCDC 2001; WSU-PAWS 2001).

Finally, to test the effect of small-scale temperature gradients within individual apples, I positioned an insert on the top (T), south (S), west (W), east (E), north (N), and bottom (B) sides of each apple. Apples were secured with a wire landscaping flag during field exposures to prevent accidental reorientation. Inserts on the top and south sides were expected to experience the highest relative temperatures because they were more directly exposed to the sun. Conversely, inserts on the bottom and north sides were expected to experience relatively cooler temperatures since they were protected from direct sun-exposure.

Data analysis

The effects of spatial, seasonal, and within-fruit temperature variation were evaluated by fitting generalized linear models (GLMs) to the estimates of survivorship and development time in the inserts. The effect of spatial variation in diurnal apple temperatures was parameterized as a categorical factor defined by the degree of sun-exposure (i.e., shaded or sun-exposed). I excluded data for apples placed under the edge of tree canopies because they were collected during only 3 of the 22 experimental runs. After analysis of all data for shaded and sun-exposed apples, I extracted the subset of data from those runs that included the "partial" exposure position, and repeated the statistical analysis to assess whether the effects were indeed intermediate between those of shaded and sun-exposed treatments.

The effect of seasonal temperature was parameterized as a polynomial regression of survivorship or development time on the mean ambient temperature during each experimental run. Cubic splines fit to the data suggested that the relationship between survivorship and ambient temperature could be characterized by a fourth-order polynomial. The relationship between development time and ambient temperature appeared to be quadratic.

The effect of the six within-apple positions was parameterized by unordered categorical factors, although an approximate ordering from coolest to warmest – B, N, E, W, S, T – was postulated *a priori* for reasons noted above.

Survivorship was modeled using a logistic link function because the data derived from a binomial process (McCullugh and Nelder 1989). Development times were log-transformed for model fitting. The basic form of the GLMs fit to the survivorship and development time data was:

$$g(Y_{iTjk}) = \mu + \alpha_{Si} + (\beta_0 + \beta_1 T_a + \ldots + \beta_m T_a^m) + \alpha_{Wj} + (\gamma_{Si,0} + \gamma_{Si,1} T_a + \ldots + \gamma_{Si,m} T_a^m) + \alpha_{Si,Wj} + (\gamma_{Wj,0} + \gamma_{Wj,1} T_a + \gamma_{Wj,m} T_a^m) + \varepsilon_k \quad (1)$$

where

Y_{iTjk} = observation k of response (survivorship or development time) under sun-exposure i, mean ambient temperature, T_a, and at position j within an apple,

$g(Y)$ = link function transformation of response, Y (logistic transformation for survivorship, log transformation for development time),

μ = mean response estimated over all sun-exposures, ambient temperatures and within-

fruit positions,

α_{Si} = additive effect of spatial variation in sun-exposure (i = shaded or sun-exposed),

β_m = coefficients for m-order polynomial regression on mean ambient temperature T_a,

α_{Wj} = additive effect of temperature gradients within apples (j =B, N, E, W, S, or T),

$\gamma_{Si,m}$ = coefficients for m-order interaction effect between sun-exposure i and mean ambient temperature T_a,

$\alpha_{Si,Wj}$ = interaction effect of sun-exposure i and within-apple position j,

$\gamma_{Ij,x}$ = coefficients for m-order interaction effect between within-apple position j and mean ambient temperature T_a, and

ε_k = random error associated with observation k (assumed independent and identically distributed among observations).

Maximum likelihood estimates of model coefficients were determined by iterated re-weighted least squares ("glm" procedure, S-Plus2000, Insightful). Best-fit models of survivorship and development time that included only significant effects were determined by step-wise reduction of the full model above ("step" procedure, S-plus2000, Insightful). Step-wise additions to a null model were also run to validate the terms in the best-fit models. Marginal effects of spatial, seasonal and within-fruit temperature variation were estimated from the linear predictor coefficients of the best-fit GLMs. The significance of effects in the GLMs was assessed by analysis of deviance. The difference in residual deviance between nested models with and without an effect (d.f. = p and d.f. = q,

respectively) was compared to a Chi-square distribution with (p-q) degrees of freedom (McCullugh and Nelder 1989).

RESULTS

Effects on survivorship

Survivorship of *D. melanogaster* was significantly affected by natural temperature variation due to spatial variation in sun-exposure within orchards, seasonal changes in mean ambient air temperature, and small-scale temperature gradients within individual apples (Table 1). Survivorship in sun-exposed apples (17.9%) was only about one-third of that in shaded apples (54.7%) (Figure 4). Subsequent analysis of data from three experimental runs that included apples placed under the edge of tree canopies showed that survivorship in the partial exposure position was intermediate between that in shaded and sun-exposed fruit (Figure 4). This latter result suggested that the effect of spatial variation in sun-exposure changes monotonically between the shaded and sun-exposed extremes.

Survivorship exhibited a curvilinear norm of reaction on daily mean air temperature, the index for seasonal variation in ambient temperature (Figure 5, Table 1). Survivorship was maximum when mean ambient air temperature was about 15°C. Survivorship decreased to about 25% when ambient temperature fell below 8°C, and was reduced to about 10% when ambient temperature exceeded 25°C. Seasonal variation in mean ambient temperature from summer to autumn caused survivorship to first increase and then decrease as ambient temperatures cooled. The specific trajectory and range of

seasonal variation in survivorship will depend on the range over which ambient temperature varies in a given year at a given site (e.g., Figure 3).

Survivorship was also affected by a significant interaction effect between sun-exposure and seasonal variation in mean ambient temperature (Table 1). The reduction in survivorship in sun-exposed apples compared to shaded apples was more pronounced under high ambient temperature than it was under cooler ambient conditions (Figure 6).

Survivorship was even affected by small-scale temperature gradients within apples (Table 1). Survivorship was relatively lower in inserts placed on the south and top sides of apples (Figure 7). This effect was dependent on an interaction effect of within-apple position and sun-exposure (Table 1). The reduction of survivorship on the top and south sides of apples was especially pronounced in sun-exposed apples, but not detected in shaded fruit (Figure 8).

Maximum likelihood estimates of the linear coefficients for the GLM of survivorship are summarized in Table 2. These coefficients could be substituted into Equation 1 to predict survivorship under any combination of sun-exposure, mean ambient temperature, and within-apple position.

Effects on development time

Unlike survivorship, egg-to-adult development time was surprisingly insensitive to natural temperature variation – only seasonal variation in mean ambient temperature had a significant effect on development time (Table 3). Development time varied inversely with mean ambient temperature though variance was greater when ambient temperatures were very cool or very warm (Figure 5). Average development time

appeared slightly longer in sun-exposed apples (15.8 days) than in shaded apples (15 days), but the difference was not significant (Figure 4). Temperature variation within apples had no effect on development time (Figure 7).

Development time could be predicted as a function of mean ambient temperature according to the following coefficients estimated by the best-fit GLM:

$$\ln(t_{dev}) = 2.724 + (0.776 - 0.060\overline{T}_a + 0.001(\overline{T}_a)^2) \quad (2)$$

where $\ln(t_{dev})$ = natural log-transformation of development time, t_{dev}, and

$\overline{T}_a$ = mean ambient air temperature.

DISCUSSION

Fitness effects of natural temperature variation

Survivorship of *D. melanogaster* larvae was significantly affected by spatial variation in sun-exposure, seasonal variation in mean ambient temperature, and even within-apple temperature gradients. Development time, on the other hand, was only affected by seasonal changes in mean ambient temperature. These effects of natural temperature variation were surprisingly different from what one would expect based on laboratory studies (cf. Figure 1). Under constant temperature conditions in the laboratory, survivorship of *D. melanogaster* is only sensitive to changes in temperature near upper and lower tolerance limits while development time changes continuously with temperature.

Differences in diurnal temperatures due to spatial variation in sun-exposure of apples resulted in substantially lower survivorship in sun-exposed apples than in shaded

apples (Figure 4). This effect was more pronounced when mean ambient temperature was warmer (Figure 6). I attributed reduced survivorship to the acute effect of extreme diurnal temperatures in sun-exposed apples that can induce heat stress and elevate mortality (Feder et al. 1997a). When mean ambient temperatures were warm, maximum diurnal temperatures were especially high, and thus the adverse effect on survivorship was more pronounced. Extreme diurnal temperatures were further implicated in reducing survivorship by the significant effect of within-apple position (Figure 7). When the sun heats apples, temperature on the sunny side of the fruit can be 5°C warmer than temperature on the shady side (Feder et al. 1997a). The consequence of such within-apple temperature variation was manifested in significantly reduced survivorship on the top and south sides of sun-exposed apples (Figure 8). No such effect was observed in shaded apples where sun-induced temperature gradients would not be expected.

Surprisingly, development time was not significantly affected by spatial variation in apple temperatures (Figure 4 and Figure 7). The apparent insensitivity of development time may have stemmed from the fact that for most of each day, temperatures in shaded and sun-exposed apples are very similar (e.g., Figure 2). Extreme diurnal temperature might have affected development rate, but exposure to such temperatures may have been too brief to cause significant differences in total development time. The magnitude of observed differences may also have been limited by the experimental protocol that exposed larvae to field temperatures for only part of the life stage.

Seasonal variation in mean ambient temperature significantly affected both survivorship and development time. Survivorship was highest at intermediate

temperatures and declined when mean ambient temperature was much colder or much warmer (Figure 5). This reaction norm exhibited the curvilinear shape that characterizes many performance curves (Huey and Stevenson 1979), but differed in two important aspects from survivorship norms reported from laboratory studies (David and Clavel 1967; Cohet et al. 1980). First, survivorship was sensitive to changes in temperature over a much broader range of temperatures. This was reflected by a more peaked reaction norm with sloped sides compared to a more step-like norm observed under constant temperatures (cf. Figure 1). Second, the range of mean ambient temperatures over which survivorship was highest was substantially cooler than the constant temperatures generally maintained in the laboratory. Most notably, mean ambient temperature of 25°C appeared to mark an upper thermal limit in the field, whereas that same temperature is generally considered optimal in the lab (David et al. 1984). I attribute these differences to the consequences of diurnal temperature fluctuations about the mean ambient temperature. When mean ambient temperature was warm, larvae were exposed to higher diurnal temperatures that were more like to cause heat stress or death. When ambient temperature was cool, night-time low temperatures exposed larvae to adverse effects of cold (McKenzie 1975). The intermediate optimum of the reaction norm reflects the narrow range of mean ambient temperatures under which survivorship remained high because neither daytime high or night-time low temperatures were too extreme.

Development time generally decreased as mean ambient temperature increased (Figure 5). This reaction norm was similar to those from laboratory studies (e.g., Powsner 1935; David and Clavel 1967), but also exhibited some notable differences.

Development times under cool ambient conditions were much shorter than those measured under comparable constant temperatures in the lab, while those under warm ambient conditions were about three days longer than under comparable constant temperatures (compare Figure 1 and Figure 5). Both differences likely reflect effects of diurnally fluctuating temperatures in the field. Development often accelerates when temperatures fluctuate about a cool mean that exposes larvae to cold temperatures at night but decelerates when temperatures fluctuate about warmer means that expose larvae to high daytime temperatures (Hagstrum and Milliken 1991). The latter effect may reflect a non-lethal consequence of mild heat stress under high daytime temperatures. The net result in these experiments was that development time showed less sensitivity to changes in mean ambient temperature than would be predicted from constant temperature reaction norms.

Implications for natural selection

Differential survivorship and development time induced by natural temperature variation create opportunity for natural selection to act on populations of *D. melanogaster*. The results of these experiments suggest how such selection may shape the evolutionary landscape for *D. melanogaster*. First, spatial variation in sun-exposure will result in differential survivorship in shaded and sun-exposed apples. Such differences in relative fitness will exert strong selection for lineages that are better able to cope with the extreme diurnal temperatures in sun-exposed fruit.

One potential evolutionary outcome is for female flies to selectively oviposit in shaded apples. This would obviously be advantageous for the survivorship of a female's

offspring, but evidence for such behavior is equivocal. *D. melanogaster* females will discriminate among oviposition sites along a temperature gradient in the laboratory (Fogleman 1979). Some selectivity for oviposition site temperature in the field was inferred from a recapture study using a temperature-sensitive eye-color mutant (Jones et al. 1987). However, wild *Drosophila* readily oviposited in fruit that had heated to nearly 50°C earlier in the day (Feder et al. 1997b). The lack of clear oviposition site preferences by female *Drosophila* despite the substantial difference in offspring survivorship between shaded and sun-exposed apples suggests that females may be hedging trade-offs between extreme diurnal temperatures in sun-exposed fruit and other factors influencing offspring survivorship (i.e., bet-hedging, Philippi and Seger 1989). For example, females might oviposit in sun-exposed fruit to avoid adverse effects of crowding in shaded fruit (R. Huey, personal communication), or perhaps to evade parasitoids or pathogens that may have lower tolerances for high temperature (e.g., *Wolbachia*, Feder et al. 1999).

Another evolutionary outcome of selection for coping with extreme diurnal temperatures is improved physiological mechanisms for resistance to heat stress. Heat-shock proteins (HSP's) are a likely target of selection. HSP's are a highly conserved family of proteins that help organisms survive exposure to high temperature and other physiological stressors by acting as "molecular chaperones" that prevent denaturing and agglutenation of proteins (Parsell and Lindquist 1994). Expression of HSP's is triggered by exposure to diurnal temperatures in sun-exposed fruit (Feder et al. 1997a), and heritable variation in HSP copy number has been shown to confer differential resistance

to natural heat shocks (Feder et al. 1996; Feder and Krebs 1998). Thus, lineages with greater HSP copy numbers may be selected in a population.

Seasonal variation in mean ambient temperature may influence the strength of selection induced by spatial variation in sun-exposure. Selection for coping with extreme diurnal temperatures should be strongest under warm ambient conditions when the difference in survivorship between shaded and sun-exposed fruit is greatest (Figure 6). Seasonal temperature variation may also induce selection that acts across generations to favor lineages with phenotypic norms of reaction that are better adapted to the local patterns of seasonal climate change (Via and Lande 1985; Stearns and Koella 1986). By similar argument, geographic variation in mean ambient temperatures may cause isolated populations to diverge as a consequence of selection for adaptation to local climate.

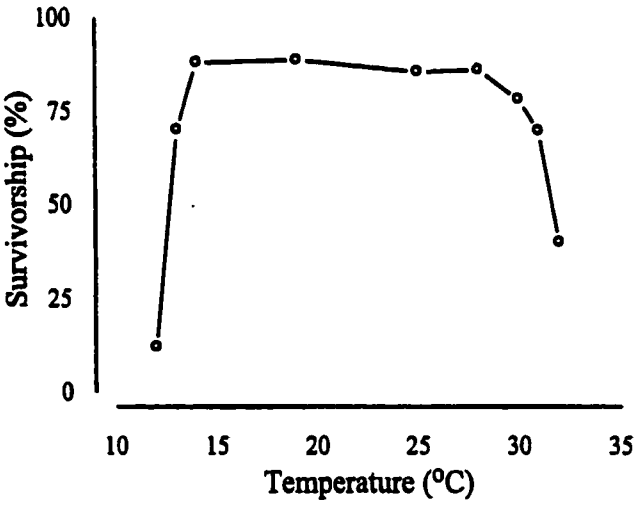
The expectations discussed above differ markedly from those one might predict on the basis of reaction norms measured under constant temperatures. Most notably, the large effect on survivorship of diurnal temperature variation between shaded and sun-exposed fruit would be entirely unpredicted since survivorship is generally high over a broad range of constant temperatures. One might naively predict that development time would be shorter in warmer sun-exposed apples than in cooler shaded apples. Instead, the opposite trend was suggested in my field experiments where average development time in sun-exposed fruit (15.8 days) appeared slightly longer than in shaded fruit (15.0 days), though the difference was not statistically significant (Table 3).

Understanding how natural temperature variation affects components of fitness such as survivorship and development time is a necessary step toward identifying the

ecological determinants of natural selection and predicting the evolutionary consequences for ectotherms such as *D. melanogaster*. My experiments demonstrated that survivorship was significantly affected by spatial variation in diurnal temperatures due to patterns of shade and sun-exposure, by seasonal variation in mean ambient temperature, and even by within-apple temperature gradients. Development time, on the other hand, was only affected by seasonal variation in mean ambient temperature. Based on my findings, I expect that natural selection will target traits that improve survivorship under the extreme diurnal temperature fluctuations experienced in nature.

Figure 1. Reaction norms of survivorship and development time of *Drosophila melanogaster* measured under constant temperatures.

Data were adapted from (David and Clavel 1967).


100
75
50
25
0
Survivorship (%)
10
15
20
25
30
35
Temperature (°C)

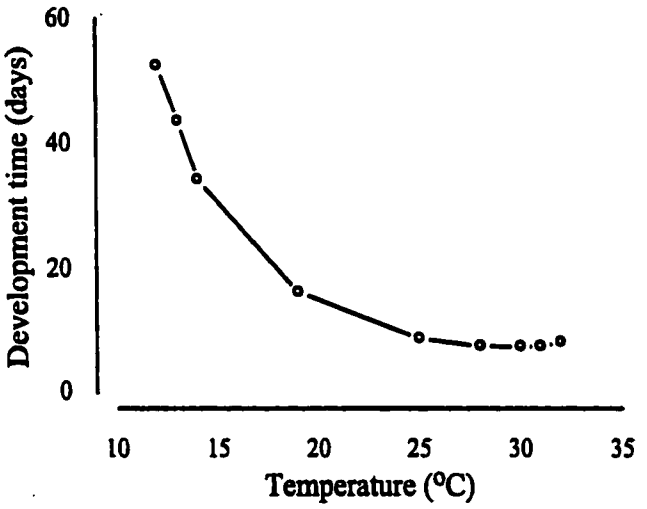

60
40
20
0
Development time (days)
10
15
20
25
30
35
Temperature (°C)

Figure 2. Diurnal temperature profiles from shaded and sun-exposed apples.

Each panel shows a 48-hour trace of temperatures from pairs of shaded and sun-exposed apples recorded on different dates. Temperature was measured with a copper-constantan thermocouple imbedded into each apple. Measurements were recorded every 15 minutes by a Campbell 21X datalogger.

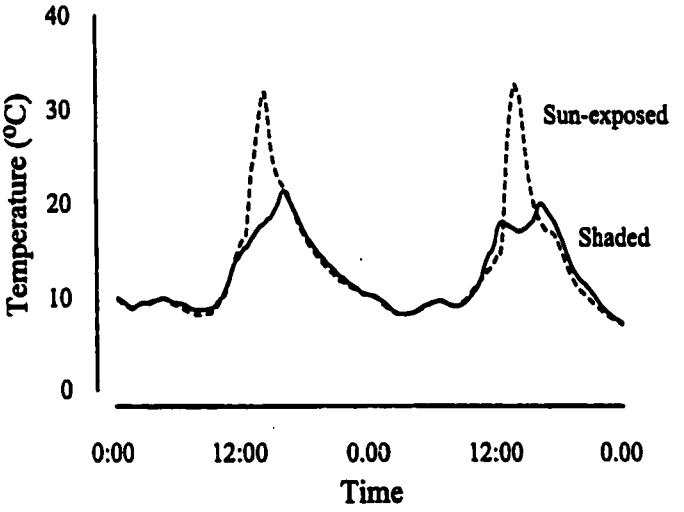

40
30
20
10
0
Temperature (°C)
Sun-exposed
Shaded
0:00
12:00
0.00
12:00
0.00
Time

Figure 3. Monthly mean temperatures reported near orchard field sites during 1998 and 1999.

Weather data for the field site near Bothell, Washington were reported by the Monroe weather station, about 10km distant but at similar altitude (NCDC 2001). Data for the field site in Wenatchee, Washington were recorded on site (NCDC 2001; WSU-PAWS 2001).

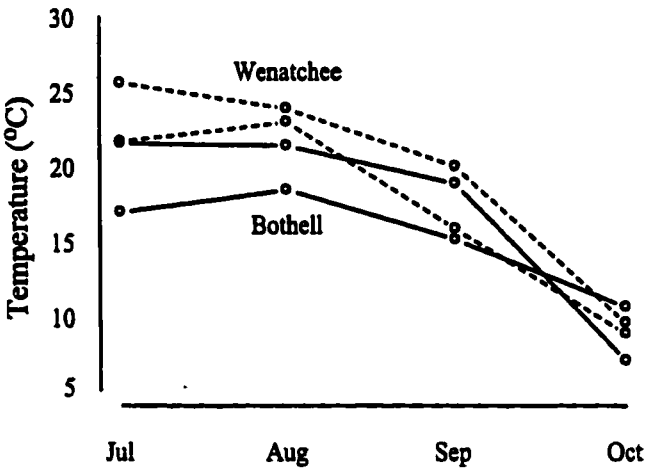
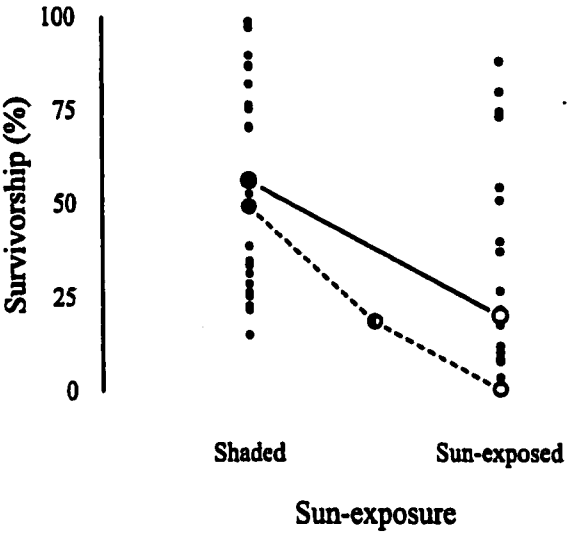

30
25
20
15
10
5
Temperature (°C)
Wenatchee
Bothell
Jul
Aug
Sep
Oct

Figure 4. Survivorship and development time of *Drosophila melanogaster* in shaded and sun-exposed apples.

Average survivorship was calculated from estimated coefficients of the best-fit GLM (Table 2). Average development time was calculated from the estimated coefficients of a GLM that included the sun-exposure factor even though the effect was not statistically significant.

The second set of points in each panel mark survivorship and development time in shaded, sun-exposed and partially exposed ("partial") apples measured in a subset of experimental trials.


100
75
50
25
0
Survivorship (%)
Shaded
Sun-exposed
Sun-exposure

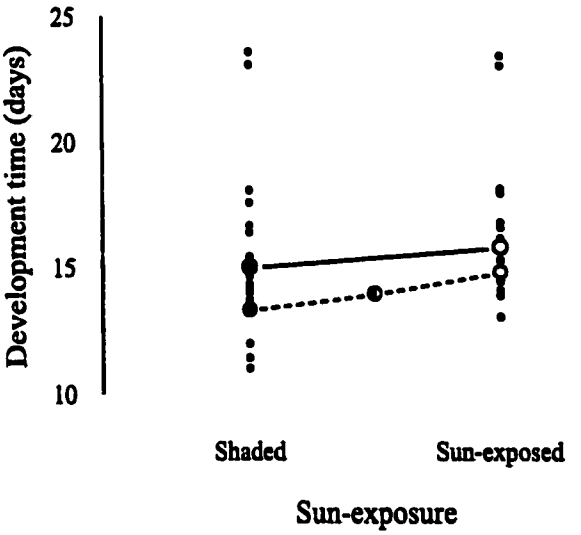
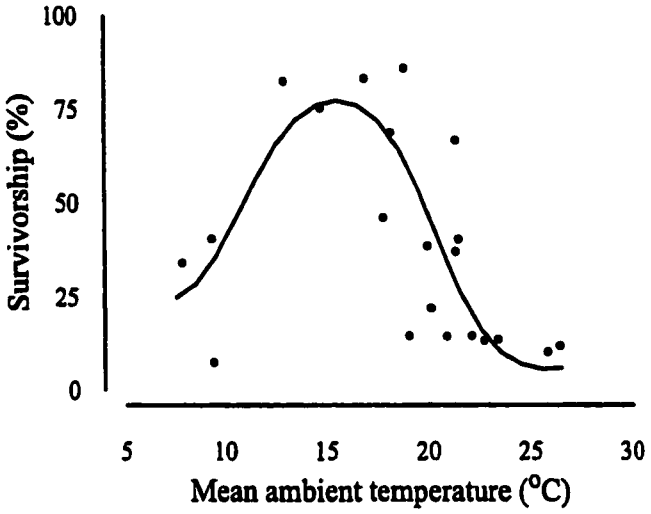

25
20
15
10
Development time (days)
Shaded
Sun-exposed
Sun-exposure

Figure 5. Survivorship and development time of *Drosophila melanogaster* under different mean ambient air temperatures.

Polynomial regressions of survivorship and development time on mean ambient temperature were parameterized from estimated coefficients of the best-fit GLMs (Table 2). Circles mark mean survivorship and development time observed during each experimental trial.


100
75
50
25
0
Survivorship (%)
5
10
15
20
25
30
Mean ambient temperature (°C)

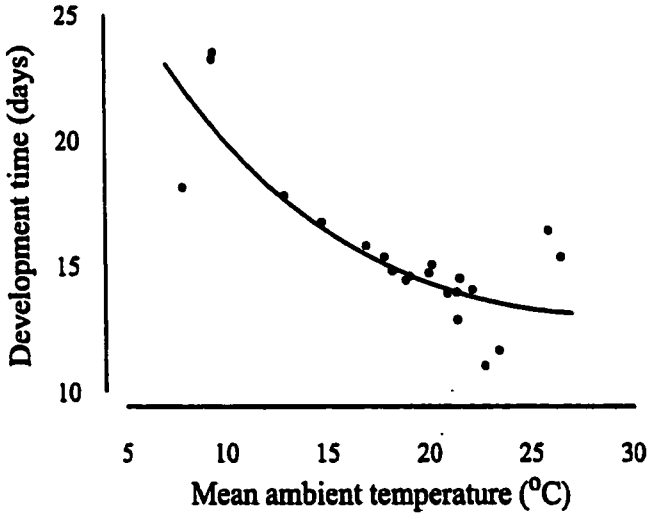

25
20
15
10
Development time (days)
5
10
15
20
25
30
Mean ambient temperature (°C)

Figure 6. Interaction effect of sun-exposure and mean ambient temperature on survivorship of *Drosophila melanogaster*.

Polynomial regressions of survivorship in shaded and sun-exposed apples on mean ambient temperature were parameterized from estimated coefficients of the best-fit GLM (Table 2). Filled and open circles mark mean survivorship observed in shaded and sun-exposed apples, respectively, during each experimental trial.

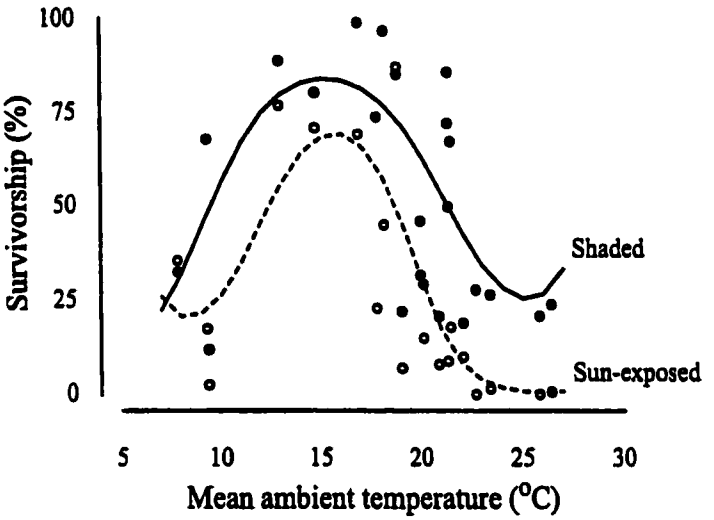
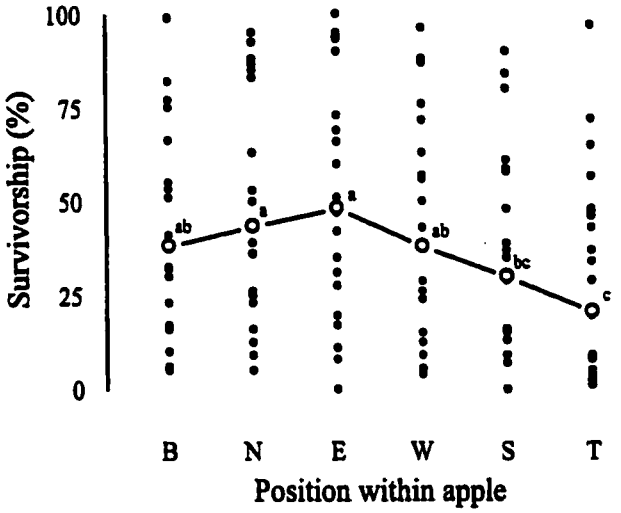

100
75
50
25
0
Survivorship (%)
Shaded
Sun-exposed
5
10
15
20
25
30
Mean ambient temperature (°C)

Figure 7. Survivorship and development time of *Drosophila melanogaster* at different positions within an apple.

Survivorship was calculated from estimated coefficients of the best-fit GLM (Table 2). Development time was calculated from estimated coefficients of a GLM that included a position factor even though the effect was not statistically significant. Different superscript letters indicate significant pair-wise differences ($p<0.05$) in survivorship between within-apple positions.


100
75
50
25
0
Survivorship (%)
ab
a
a
ab
bc
c
B
N
E
W
S
T
Position within apple

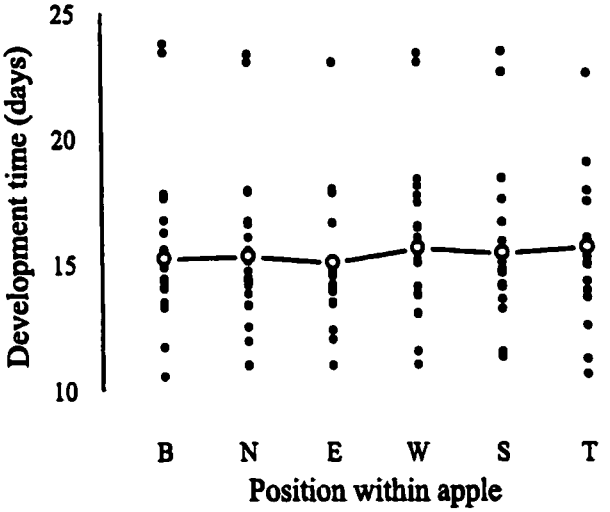

25
20
15
10
Development time (days)
B
N
E
W
S
T
Position within apple

Figure 8. Interaction effect of sun-exposure and position within an apple on survivorship of *Drosophila melanogaster*.

Survivorship at different positions within shaded apples (filled circles) and sun-exposed apples (open circles) were calculated from estimated coefficients of the best-fit GLM (Table 2). Different superscript letters indicate significant pair-wise differences ($p<0.05$) between within-apple positions in shaded or sun-exposed apples.

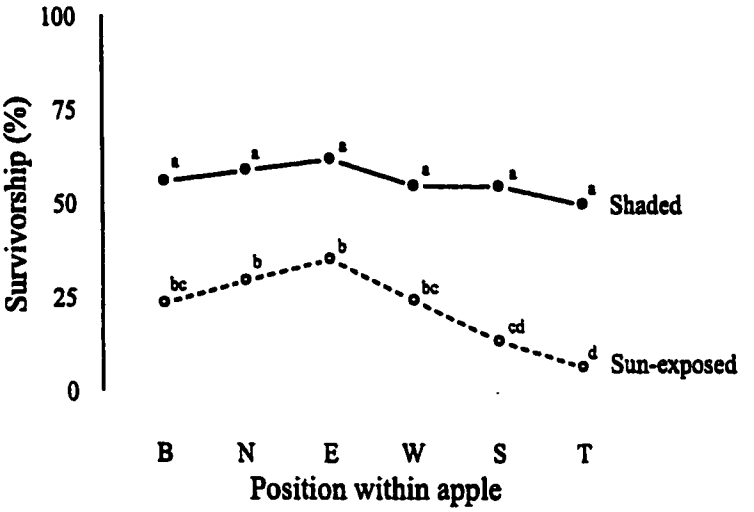

100
75
50
25
0
Survivorship (%)
a
a
a
a
a
a
Shaded
bc
b
b
bc
cd
d
Sun-exposed
B
N
E
W
S
T
Position within apple

Table 1. Analysis of deviance table
for nested generalized linear models of survivorship
of *Drosophila melanogaster*.

Table includes all main and pair-wise interaction effects of experimental factors. Significance of main and interaction effects was determined by comparing difference in residual deviance between nested models with and without an effect to Chi-squared distributions with appropriate degrees of freedom (McCullugh and Nelder 1989).

Dependent variable: survivorship	Deviance	df	Residual deviance	Residual df	p
NULL			1167.83	1199	
Mean ambient temperature	195.53	4	972.31	1195	0
Sun-exposure	102.36	1	869.95	1194	0
Within-apple position	26.05	5	843.91	1189	<<0.001
Ambient temperature x Sun-exposure	15.65	4	828.26	1185	<0.004
Within-apple position x Sun-exposure	14.05	5	814.21	1180	0.015
Ambient T x Within-apple position	12.62	20	801.58	1160	0.893

Table 2. Linear predictor coefficients
from best-fit generalized linear model of survivorship
of *Drosophila melanogaster.*

Predictors are presented on a logistic scale to preserve additivity. Estimated standard errors are given in parentheses. Linear predictors were estimated by multiplying maximum likelihood estimates of design variable coefficients by the model matrix. Standard errors of the linear predictors were estimated conservatively by multiplying standard errors of the design variable coefficients by the model matrix.

Mean survivorship:	μ =	-0.580 (0.068)
Sun-exposure:	α_{shaded} =	0.817 (0.068)
	$\alpha_{sun-exposed}$ =	-0.817 (0.068)
Ambient temperature:	β_0 =	12.325 (5.326)
	β_1 =	-4.675 (1.545)
	β_2 =	0.571 (0.157)
	β_3 =	-2.66e-2 (0.68e-2)
	β_4 =	4.12e-4 (1.05e-4)
Within-apple position:	α_B =	0.102 (0.236)
	α_N =	0.319 (0.056)
	α_E =	0.511 (0.009)
	α_W =	0.098 (0.053)
	α_S =	-0.268 (0.093)
	α_T =	-0.763 (0.137)

Ambient temperature x Sun-exposure:	Shaded	Sun-exposed
$\gamma_{0,i}$ =	-12.222 (5.326)	12.222 (5.326)
$\gamma_{1,i}$ =	3.218 (1.545)	-3.218 (1.545)
$\gamma_{2,i}$ =	-0.305 (0.157)	0.305 (0.157)
$\gamma_{3,i}$ =	0.012 (0.007)	-0.012 (0.007)
$\gamma_{4,i}$ =	-1.66e-4 (1.05e-4)	1.66e-4 (1.05e-4)

Within-apple position x Sun-exposure:	Shaded	Sun-exposed
$\alpha_{i,B}$ =	-0.107 (0.236)	0.107 (0.236)
$\alpha_{i,N}$ =	-0.202 (0.056)	0.202 (0.056)
$\alpha_{i,E}$ =	-0.271 (0.009)	0.271 (0.009)
$\alpha_{i,W}$ =	-0.149 (0.053)	0.149 (0.053)
$\alpha_{i,S}$ =	0.212 (0.093)	-0.212 (0.093)
$\alpha_{i,T}$ =	0.517 (0.137)	-0.517 (0.137)

Table 3. Analysis of deviance table
for nested generalized linear models of development time
of *Drosophila melanogaster*.

Table includes all main and pair-wise interaction effects of experimental variables. Only the effect of mean ambient temperature was significant. Significance of main and interaction effects was determined by comparing difference in residual deviance between nested models with and without an effect to Chi-squared distributions with appropriate degrees of freedom (McCullugh and Nelder 1989).

Dependent variable: development time	Deviance	df	Residual deviance	Residual df	p
NULL			22.943	710	
Ambient temperature	13.834	2	9.109	708	0.001
Sun-exposure	0.415	1	8.694	707	0.519
Within-apple position	0.138	5	8.556	702	1.000
Ambient temperature x Sun-exposure	0.511	2	8.046	700	0.775
Within-apple position x Sun-exposure	0.026	5	8.020	695	1.000
Ambient T x Within-apple position	0.244	10	7.776	685	1.000

CHAPTER 2:

INTERACTIVE EFFECTS OF TEMPERATURE AND ETHANOL ON SURVIVORSHIP AND DEVELOPMENT TIME OF *DROSOPHILA MELANOGASTER*

INTRODUCTION

Organisms in nature must cope with multiple environmental factors that affect physiological performance and fitness (Feder and Hofmann 1999). Understanding how the combined effects of such factors translate into natural selection that shapes evolutionary dynamics in populations is an exciting challenge for evolutionary biologists. If environmental factors have independent effects on fitness, natural selection for traits that confer fitness advantages will be imposed independently by each factor. On the other hand, if environmental factors have interactive effects on fitness, the strength and pattern of natural selection will depend on how environmental factors and their effects covary. For example, natural selection could be strengthened if two environmental factors have complementary effects that accentuate fitness differentials among individuals. Alternatively, natural selection could be weakened if environmental effects counteract one another and diminish fitness differentials.

Temperature and ethanol are two key environmental factors that affect fitness of *Drosophila melanogaster* (David et al. 1984). The independent effects of each on components of fitness for *D. melanogaster* have been well documented, but the interaction effect of these factors is poorly understood, especially in a natural ecological

setting such an orchard where *D. melanogaster* inhabit rotting fruit. After reviewing what is known about the independent effects of temperature and ethanol on fitness, I present the results of laboratory and field experiments designed to test for any significant interaction effect between the two factors. While my laboratory experiment corroborated previous investigations regarding the independent fitness effects of temperature and ethanol, the field experiments suggested that the two factors have a compensatory interaction effect in nature. The interaction was not anticipated from laboratory studies, but has important implications for expected patterns of natural selection on traits that confer heat and ethanol tolerance.

Seasonal variation in temperature experienced by *D. melanogaster* in nature has significant fitness consequences. Survivorship is highest under intermediate temperatures but decreases as ambient temperatures become too warm or cool (see Figure 1 in Chapter 1). Development time increases as mean ambient temperature cools. Different constant temperatures in the laboratory have qualitatively similar effects (Loeb and Northrop 1917; Powsner 1935; David and Clavel 1967). Seasonal variation in mean ambient temperature also influences fecundity, time to sexual maturity and longevity of adult *Drosophila* (Avelar et al. 1987; Junge-Berberovic 1996).

A more immediate cause of fitness differentials among individuals within a population is spatial variation in diurnal fruit temperatures due to patterns of shade and sun-exposure in orchards. *D. melanogaster* larvae in rotting fruit experience temperatures that fluctuate diurnally with ambient air temperature, but can rise to 15-20°C above ambient when exposed to sunlight (Feder 1997; Feder et al. 1997a). Such

heating episodes expose indwelling larvae to high temperatures that can trigger heat shock responses or even cause death (Feder and Krebs 1998). Exposure to such extreme diurnal temperatures varies spatially within orchard habitats depending on whether fruits are shaded by the tree canopy during the day or are sun-exposed in the center of a row (more extreme) (Chapter 1, Feder et al. 1999). The probability of larval survivorship under the more extreme temperature conditions in sun-exposed fruit is reduced by more than 50% relative to that in shaded fruit where diurnal temperatures are less extreme (Chapter 1).

Drosophila melanogaster using rotting fruit in nature must also cope with concentration-dependent effects of ethanol. Ethanol occurs naturally in decaying fruit as yeasts oxidize fruit sugars. Naturally occurring concentrations of ethanol in fruit generally range from trace amounts to 5% (by volume) (Gibson et al. 1981; Gibson and Wilks 1988), though concentrations greater than 10% have been measured in grape residues in which *D. melanogaster* were present (McKenzie and McKechnie 1979). At low concentrations (e.g., ~ 3%), larval survivorship and adult longevity increase, and larval development time decreases (Parsons et al. 1979; Parsons and Spence 1981). At higher concentrations, especially those above 10%, larval survivorship and adult longevity decrease and development time lengthened (VanDelden et al. 1978; Parsons et al. 1979; Chakir et al. 1993). The concentration-dependent effect of ethanol has been attributed to the alcohol dehydrogenase (ADH) system that metabolizes ethanol at low concentrations, but becomes saturated, and thus ineffective, at higher concentrations

(Deltombe-Lietaert et al. 1979; Geer et al. 1985; Heinstra et al. 1987; Chakir et al. 1993; Geer et al. 1993; Chakir et al. 1996).

It is logically plausible that temperature and ethanol may interact to affect fitness of *D. melanogaster* in nature, but evidence for such an interaction is equivocal. Coincident latitudinal clines of temperature, ethanol tolerance and the frequency of ADH allozymes in *Drosophila* populations suggest that temperature may mediate selection for ethanol tolerance (David et al. 1986). That these clines have been documented in Europe, North America and Australia (Parsons and Stanley 1981; Oakeshott et al. 1982; Cohan and Graf 1985; David et al. 1986; Berry and Kreitman 1993) would seem to strengthen a selectionist interpretation that these prominent geographic patterns were shaped by natural selection (Endler 1977). However, demonstration of expected covariance between ADH allele frequencies and ethanol tolerance was equivocal in laboratory selection experiments (VanDelden et al. 1978; Kohane and Parsons 1986) and within local field populations (McKenzie and McKechnie 1978; Hickey and McLean 1980; VanDelden 1982; McKechnie and McKenzie 1983; Kohane and Parsons 1986; Gibson and Wilks 1988). Other evidence suggests that ethanol may influence selection for tolerance of high diurnal temperatures. Heat shock responses can be induced by ethanol as well as by high temperature (Cornelius 1996; Feder and Hofmann 1999), but ethanol may inhibit the heat shock response at higher concentrations (Munks and Turner 1994).

I sought to measure the main and interaction effects of temperature and ethanol on two components of fitness for *D. melanogaster* – larval survivorship and development time. Survivorship to reproductive maturity is a critical component of fitness for any

organism. Development time is also important because it determines the likelihood that larvae can complete development in an ephemeral resource like rotting fruit. Short development times also reduce generation times, and thus increase a population's intrinsic rate of increase (Cole 1954). I expected larvae to be especially sensitive to the effects of temperature and ethanol because larvae have limited dispersal ability for escaping unfavorable temperature conditions (Feder 1997), and because they are the life stage during which most feeding – and thus ethanol consumption – occurs (Parsons 1977).

First, following traditional approaches of laboratory studies, I evaluated the interaction effect of constant temperature and ethanol in a 3 x 3 factorial design. I reared *D. melanogaster* under three different constant temperatures (17, 23, and 29°C) on standard food medium supplemented with three concentrations of ethanol (0, 3 and 9%) and measured survivorship and development time of larvae. I then conducted field experiments to evaluate the interaction between ethanol and temperature in sun-exposed and shaded apples in a 2 x 3 factorial design. For the field experiments, I reared *D. melanogaster* in either sun-exposed or shaded apples in an orchard where larvae would experience more extreme or less extreme diurnal temperature fluctuations, respectively. As in the laboratory experiment, food medium was supplemented with 3 levels of ethanol (0, 3 and 9%). Temperature and ethanol had independent effects on survivorship in both lab and field experiments. However, the two factors had a significant interaction effect such that the adverse effect of ethanol on development time disappeared when larvae

were also exposed to more extreme temperatures in sun-exposed apples. This latter effect was not observed in the laboratory experiment.

METHODS

The basic protocol for both laboratory and field experiments involved rearing age-matched cohorts of ten *D. melanogaster* eggs in several small "inserts" that were filled with standard food medium supplemented with three concentrations of ethanol (0%, 3%, 9%). After exposing larvae to different temperature treatments (described below), I allowed larvae to complete development under common laboratory conditions where I monitored adult emergence daily until no more adults emerged. From those data, I estimated survivorship and average development time in each insert.

Inserts were constructed from small rigid cylinders cut from the top of 1000 µl pipet tips (VWR brand for Gilson pipetman). The bottom of each insert was closed with a swatch of fine Nytex mesh (152 µm). After inserts were filled with food medium and eggs were transferred (see below), the tops were sealed with a piece of Parafilm wrapped over the top. The Nytex mesh bottom prevented larvae from crawling out of or into the insert while still allowing for gas and fluid exchange to the food medium inside the insert. I also perforated the Parafilm on top of each insert with a pin to provide for some gas exchange while still retarding desiccation of the food medium inside. The inserts effectively segregated experimental animals from other larvae that wild *Drosophila* might lay into an apple during field experiments, and from parasitoids or predators that might

prey on them. The inserts also allowed me to fix larval density at 10 per insert to minimize adverse effects of crowding (Scheiring et al. 1984).

Each insert was filled with a standard food medium made from cornmeal, molasses, agar and yeast and supplemented with one of three ethanol concentrations (0%, 3% and 9%). To prevent growth of mold and fungus in the food media, a 10 ml aliquot of tegosept (10% solution of methyl paraben in ethanol) was added to each 500 ml of food. The 3% and 9% supplements were prepared by adding an additional 6 ml and 18 ml of 95% ethanol, respectively, to 100 ml of standard food medium just before the agar congealed (after Hageman et al. 1990). These proportions were expected to yield target ethanol concentrations after allowing for some evaporation of ethanol from warm food medium.

Eggs for all of the experiments were collected from a laboratory colony of local-stock wild-type *D. melanogaster* collected near Bothell, Washington. The colony was maintained at a large population size with overlapping generations in 1-gallon containers into which fresh food medium was regularly introduced. The colony was kept at ambient room temperature (ca. 20-22°C) and on a natural light-dark cycle. Age-matched cohorts of eggs were collected for each experiment by allowing flies to oviposit on medium-filled petri dishes left in the colony cage for 4 to 12 hours. Working under a dissecting microscope, I then used a small bristled brush to transfer ten eggs into each insert.

For the laboratory experiment, I transferred eggs into 108 inserts and exposed larvae to one of three constant temperatures (17, 23, 29°C) for 7 days (36 inserts per temperature). Inserts with each of the three ethanol supplements were evenly distributed

within the temperature treatments to create a 3 x 3 factorial design (12 inserts for each temperature-ethanol combination). The constant temperature treatments were chosen to span the range of thermal tolerance reported in previous laboratory investigations (David et al. 1984).

For field experiments, I transferred ten eggs into each of 180 inserts filled with ethanol-supplemented food medium (60 inserts/ethanol treatment). Within 24 hours, I put inserts into apples (6 inserts/apple x 30 apples) and placed the apples in sun-exposed or shaded positions in orchard field sites where diurnal temperatures were more and less extreme, respectively. Inserts were pushed into pilot holes in apples until their tops were flush with the apple skin. A snug fit ensured that insert temperatures matched those in the surrounding fruit, and minimized desiccation. All inserts in an apple were filled with food medium with the same ethanol concentration. Inserts were evenly distributed on all sides of each apples.

One half of the apples were positioned in the center of orchard rows where they would be sun-exposed through much of the day. Larvae in these apples would be exposed to more extreme diurnal temperatures as a consequence of solar heating, and would thus be at greater risk of heat stress. The other apples were positioned around the base of apple trees and were thus shaded through most of the day. Larvae in these apples would experience less extreme diurnal temperature fluctuations and thus be less susceptible to natural heat stress. Apples were randomly assigned to shaded or sun-exposed positions in the orchard with the constraint that inserts containing each of the three ethanol treatments were evenly distributed between them. I repeated this 2 x 3

factorial design three times – on September 3, 1999 at an orchard near Bothell, Washington, and on September 8 and 15, 1999 at an orchard near Wenatchee, Washington.

Larvae were exposed to constant temperature treatments (17, 23, 29°C) in the laboratory or to natural diurnal temperature treatments (in shaded vs. sun-exposed apples) in orchard field sites for 7 days. Then inserts were collected and returned to the laboratory where Parafilm covers were removed and inserts were placed individually into test tubes stopped with cotton. Larvae completed development under common conditions of ambient room temperature and natural light dark cycle. Inserts were monitored daily for adult eclosion until no more adults emerged. Any adults that eclosed from an insert on a given day were counted and removed from the test tube. Survivorship for each fly was recorded (survivorship = 1), and development time was calculated as the difference between the date of egg collection and the observed date of eclosion. Any of the ten eggs originally transferred into an insert that did not eclose as adults were assumed to have died (survivorship = 0). Development times could not be calculated for those individuals.

After all surviving adults had eclosed (usually within 2 to 3 weeks of egg collection), estimates of survivorship and development time (conditioned on survival) were calculated for each insert. Survivorship was simply the total number of adults that eclosed from an insert divided by ten (the number of eggs originally transferred into the insert). Average development time in each insert was estimated as the geometric mean of observed development times. I used the geometric mean to limit the disproportionate influence of outliers. No estimates of average development time were made for inserts in

which survivorship was 0. If more than 10 flies eclosed from a single insert, survivorship was set to 1.0, and only development time data for the first 10 flies to eclose were used to calculate average development time. "Extra" flies eclosed from about 10% of inserts. Many were probably the result of counting errors made when eggs were originally transferred into the inserts. Others may have been interlopers from the field that hitchhiked into the lab on the outside of an insert, and some appeared to have exceptionally long development times that suggested they were progeny of experimental flies that successfully mated and reproduced between daily observations.

The main and interaction effects of constant temperature and ethanol concentration in both the laboratory and field experiments were evaluated by analysis of variance (ANOVA). Analyses of data from field experiments also included estimation of a random block effect of the three experimental replicates. Survivorship estimates were arcsine-square root transformed and development time estimates were log-transformed prior to analysis. Because sample sizes for development time were unbalanced – no estimates could be made for inserts in which survivorship was zero – the significance of effects was assessed using type III sums of squares (Cochran and Cox 1957). Multiple comparisons were performed to evaluate the significance of pair-wise differences between treatment means at the $p<0.05$ level (procedure "multicomp", S-plus 2000, Insightful, Inc.).

RESULTS

Effects of constant temperature and ethanol in the laboratory

High constant temperature conditions (i.e., 29°C) significantly reduced survivorship of *D. melanogaster* larvae compared to survivorship of larvae reared at 17°C or 23°C (Figure 9, Table 4). Survivorship was similar under the latter two constant temperature treatments. Increased temperature also significantly decreased development times (Figure 9, Table 5). All pair-wise differences between 17, 23, and 29°C treatment means were significant ($p<0.05$).

The effect of ethanol on survivorship of *D. melanogaster* larvae was not significant (Table 4), despite a potential trend toward higher survivorship under the intermediate ethanol concentration of 3% (Figure 10). Development time increased significantly with higher ethanol concentration, though the change was less than one-half day between the 0% and 9% ethanol treatments (Figure 10, Table 5).

Last, neither the interaction effect of constant temperature and ethanol treatments on survivorship nor that on development time were significant (Table 4 and Table 5, respectively). These latter results suggest that constant temperature and ethanol have independent effects on survivorship and development time of *D. melanogaster.*

Effects of diurnally fluctuating temperature and ethanol in the field

Exposure to more extreme diurnal temperatures in sun-exposed apples significantly reduced survivorship by more than half relative to that in shaded apples (Figure 11, Table 6). Average development time was about one-half day longer in sun-exposed apples (Figure 11, Table 7).

Addition of ethanol to the food medium significantly affected survivorship of *D. melanogaster* larvae (Figure 12, Table 6). Pair-wise comparisons indicated that an intermediate concentration (3%) of ethanol significantly increased survivorship of relative to that in unsupplemented (0%) or high ethanol concentration (9%) treatments ($p<0.05$). Survivorship in unsupplemented food and with high ethanol concentration was similar. Development time increased monotonically with increasing ethanol concentration (Figure 12, Table 7), though only the pair-wise difference of about 1 day between the unsupplemented and 9% ethanol treatments was significant ($p<0.05$).

The interaction effect on survivorship of spatial variation in diurnal temperatures and ethanol concentration in food was not significant (Figure 13, Table 6). This suggested that temperature and ethanol have independent effects on survivorship in the field. However, development was significantly affected by an interaction between spatial variation in diurnal temperatures and ethanol concentration (Figure 13, Table 7). The pair-wise difference between development times in sun-exposed apples and shaded apples without ethanol was significant ($p<0.05$) but diminished when the food medium was supplemented with a low concentration of ethanol (3%) and disappeared altogether when a high concentration of ethanol (9%) was added. The magnitude of the effect of ethanol on development time was greater in shaded apples than in sun-exposed apples.

Block effect in field experiments

The block effect of the three replicate runs of the field experiment was significant for both analyses of survivorship and development time data (Table 6 and Table 7, respectively). Though not a focus of this paper, the observation warrants a brief

comment. I attribute the block effect to differences in daily mean ambient air temperature during the three experimental runs that ranged from 17.8°C to 21.5°C (NCDC 2001; WSU-PAWS 2001). Both survivorship and development time are sensitive to changes in mean ambient temperature over that range (Chapter 1).

DISCUSSION

The effects of temperature and ethanol on survivorship in both the laboratory and field experiments were qualitatively similar. Survivorship was reduced under high constant temperature (Figure 9) and in sun-exposed apples where larvae experienced higher diurnal temperatures than in shaded apples (Figure 11). Survivorship in the field was improved with an intermediate concentration of ethanol, but not with higher ethanol concentrations (Figure 12). A similar trend was observed in the laboratory experiment (Figure 10), but it was not statistically significant. All of these results were consistent with those previously reported studies of the effect of temperature (e.g., David and Clavel 1967), Chapter 1) and ethanol (Parsons 1977).

In contrast, the effects on development time differed markedly between the laboratory and field experiments. Under constant temperature conditions in the laboratory, higher temperatures reduced development time (Figure 9). This effect has long been known (Loeb and Northrop 1917; Powsner 1935) and is considered a general life history feature (David et al. 1984). However, in the field experiments, development time appeared slightly longer in warmer sun-exposed apples (Figure 11). I attribute this surprising result to an adverse effect of extreme diurnal temperatures that may retard

development (e.g., heat stress). This effect could not be detected using traditional constant temperature treatments because they do not include natural diurnal fluctuations, and thus illustrates how important fitness effects could be missed if experimental treatments do not address ecologically relevant variation such as that of temperatures in shaded and sun-exposed apples.

The main effect of ethanol was qualitatively similar in the laboratory and field experiments (compare Figure 10 and Figure 12). However, the magnitude of differences between development time in unsupplemented and high ethanol concentration food medium was about twice as great under fluctuating field temperatures than under constant temperature conditions in the laboratory (one day versus one-half day, respectively).

The most striking and important difference between the results of laboratory and field experiments was the significant interaction effect on development time of temperature and ethanol that was revealed by the field experiments (Figure 13). The adverse effects of extreme diurnal temperature in sun-exposed apples and of high ethanol concentrations appeared to be mitigated when both factors were applied simultaneously. Exposure to more extreme diurnal temperatures in sun-exposed apples had no effect on development time when food was supplemented with a high concentration of ethanol. Furthermore, development time was less sensitive to increased ethanol concentrations in sun-exposed apples that it was in shaded apples.

This interaction effect has important implications regarding expectations about targets for natural selection. From the laboratory experiment, where temperature and ethanol appeared to have additive, non-interactive effects on fitness of *D. melanogaster*,

one might naively predict that natural selection would target advantageous temperature tolerance and ethanol tolerance traits independently of the other. However, recognition that temperature and ethanol have an interactive effect on fitness suggests two qualitatively different and intriguing possibilities regarding the targets and consequences of natural selection for *D. melanogaster* in nature.

First, the interaction effect could reflect an interacting physiological mechanism. High ethanol concentrations in food may cross-induce the heat shock response in larvae such that they are better "prepared" to cope with diurnal heat stress in sun-exposed apples. Many environmental factors, including ethanol, have been shown to cross-induce heat shock responses, and mild pre-treatment by inductive factors can increase resistance to subsequent and more intense heat shock (Parsell and Lindquist 1994; Feder and Hofmann 1999). Thus, chronic exposure to the increased physiological stress if high ethanol concentrations may induce more robust responses to heat stress, and ameliorate the fitness effect of extreme diurnal temperatures. The plausibility of this hypothesis could potentially be tested by comparing heat resistance of ethanol-exposed and ethanol-free larvae, and by assaying levels of heat shock protein (HSP) expression before and after exposure to heat shocks.

If this hypothesis were true, one would expect selection for increased heat tolerance and ethanol tolerance to be positively correlated. As larvae became more tolerant of ethanol, cross-induction of heat shock responses would attenuate. Consequently, larvae would be more susceptible to heat stress such that selection for increase heat stress resistance should intensify and positive correlation between heat and

ethanol tolerance would emerge. Such correlations have been demonstrated in both comparative studies and selection experiments (Chakir et al. 1993; Hoffmann and Parsons 1993b). These predictions might be explicitly tested in the laboratory by measuring the correlation between traits before and after selection for resistance to periodic heat stress in populations for which food was supplemented with either no or high concentrations of ethanol.

Another hypothesis regarding the interaction effect of temperature and ethanol in the field is that extreme diurnal temperatures in sun-exposed apples volatilized ethanol such that the actual concentration in inserts in sun-exposed apples was less than that in shaded apples. Ethanol concentration in artificial food media does decline over time as ethanol evaporates or is converted to derivative compounds such as acetic acid or acetaldehyde (Hageman et al. 1990). However, were this the sole cause of the interaction, then ethanol should also have had a diminished effect on survivorship in sun-exposed apples. The significant effect of intermediate ethanol concentrations (Figure 12) and absence of any interaction effect on survivorship (Figure 13) demonstrate that sufficient ethanol must have remained in the food medium. Still, volatilization of ethanol is an unavoidable problem in this and other investigations of ethanol tolerance that use artificial food media (Parsons 1977; Mercot et al. 1994). In naturally fermenting fruit, ethanol is produced endogenously so that the concentration is likely to be sustained or even increase in nature. Consequently, the effects of ethanol observed in the present experiments might be more pronounced on natural foods, and volatilization of ethanol from sun-exposed fruit may be important in the field.

If the observed interaction effect of temperature and ethanol were a consequence of volatilization, then one might predict a qualitatively different selection regime than that following from the cross-induction hypothesis. If high diurnal temperatures volatilize and reduce the concentration of ethanol in naturally fermenting fruit, extreme diurnal temperatures and ethanol concentrations would negatively covary between sun-exposed and shaded fruit. Larvae in sun-exposed fruit would be exposed to higher temperatures but lower ethanol concentrations, while larvae in shaded fruit would be exposed to less extreme diurnal temperatures but higher ethanol concentrations. Thus, two distinct selection regimes could exist – one for heat tolerance in sun-exposed fruit and one for ethanol tolerance in shaded fruit. In an orchard habitat, both of these regimes would be maintained over a fine spatial scale of just 1 or 2 meters (i.e., the distance from the shaded base of a tree to the sun-exposed center of a row). Adult flies can easily disperse over such distances so a population could be well-mixed over both selection regimes.

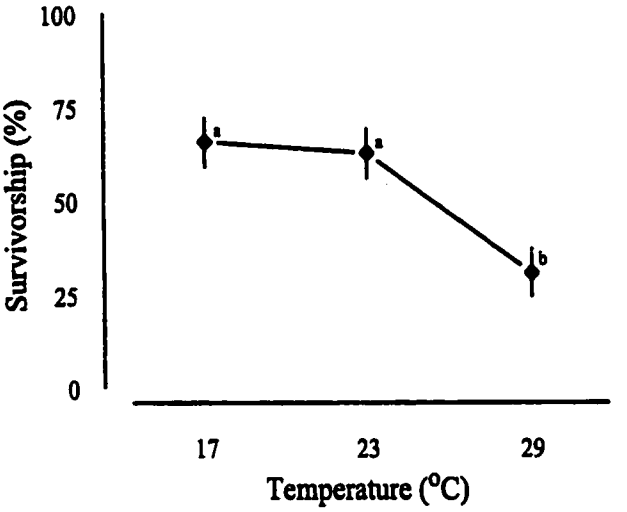
If flies oviposited in both shaded and sun-exposed apples, selection might favor a plastic phenotype whereby heat tolerance and ethanol tolerance negatively covary (assuming that there is some fitness cost to maintaining but not using physiological mechanisms for coping with various stressors). Alternatively, if flies preferentially oviposited in sun-exposed or shaded apples (but not both), a population could become subdivided with some lineages specialized for greater heat tolerance in sun-exposed fruit and other lineages specialized for greater ethanol tolerance in shaded fruit. This intriguing scenario seems less likely than the former, though, because flies in nature do

not seem to exhibit any oviposition preference with respect to sun-exposed versus shaded fruit (Feder et al. 1997b).

Studies such as the present one illustrate the exciting new insights that could be gained by investigating the simultaneous effects of multiple environmental factors on an organism's fitness. The interaction effect on development time of temperature and ethanol revealed by the field experiments was unanticipated from laboratory studies. Furthermore, the results provide the basis for posing and testing hypotheses about the physiological mechanisms mediating tolerance of ecological stressors. These insights may, in turn, reveal evolutionary dynamics underlying unexpected and yet unexplained patterns of divergence of populations under seemingly similar selection regimes (Kohane and Parsons 1986; Hoffmann and Parsons 1993a; McKechnie and Geer 1993).

Figure 9. Survivorship and development time of *Drosophila melanogaster* reared at constant 17, 23, and 29°C in the laboratory.

Data were pooled over all ethanol concentration treatments. Different superscripts denote significant pair-wise differences ($p<0.05$) between constant temperature treatments.


100
75
50
25
0
Survivorship (%)
a
a
b
17
23
29
Temperature (°C)

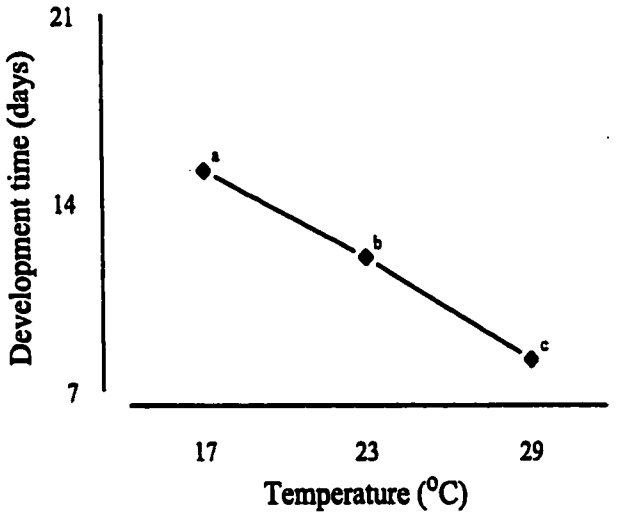
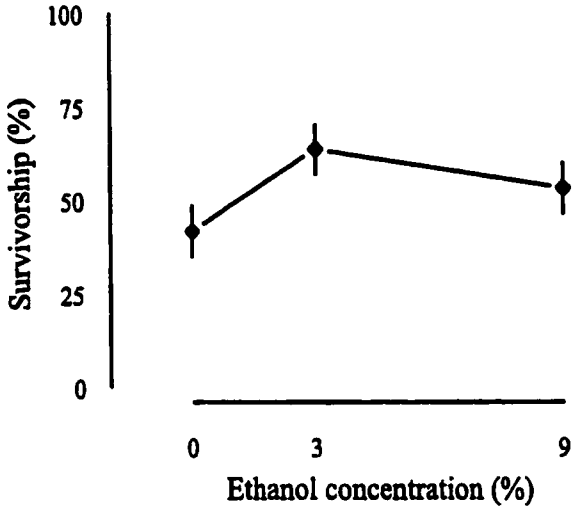

21
14
7
Development time (days)
a
b
c
17
23
29
Temperature (°C)

Figure 10. Survivorship and development time of *Drosophila melanogaster* reared in food medium supplemented with 0, 3, and 9% ethanol in the laboratory.

Data were pooled over all constant temperature treatments. Different superscript letters denote significant pair-wise differences ($p<0.05$) in development time between ethanol concentration treatments. Differences in survivorship were not statistically significant ($p=0.092$, Table 4).


100
75
50
25
0
Survivorship (%)
0
3
9
Ethanol concentration (%)

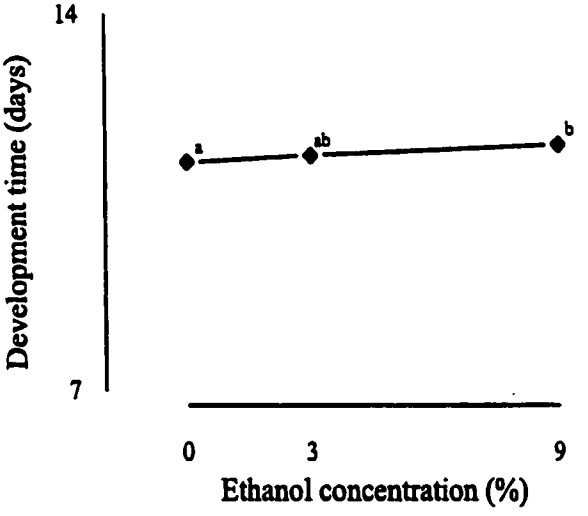
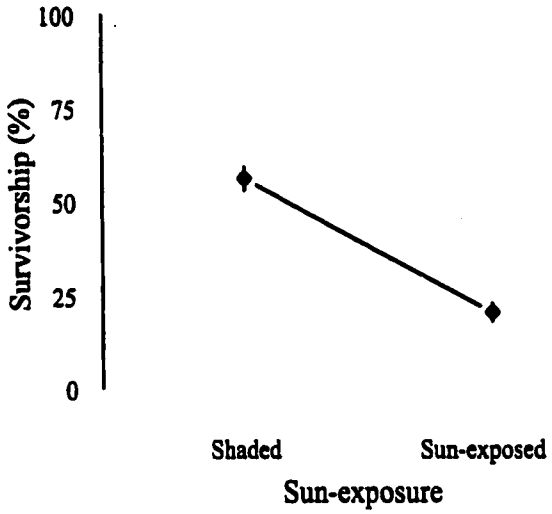

14
7
Development time (days)
a
ab
b
0
3
9
Ethanol concentration (%)

Figure 11. Survivorship and development time of *Drosophila melanogaster* reared in shaded and sun-exposed apples in the field.

Data were pooled over all ethanol concentration treatments.


100
75
50
25
0
Survivorship (%)
Shaded
Sun-exposed
Sun-exposure

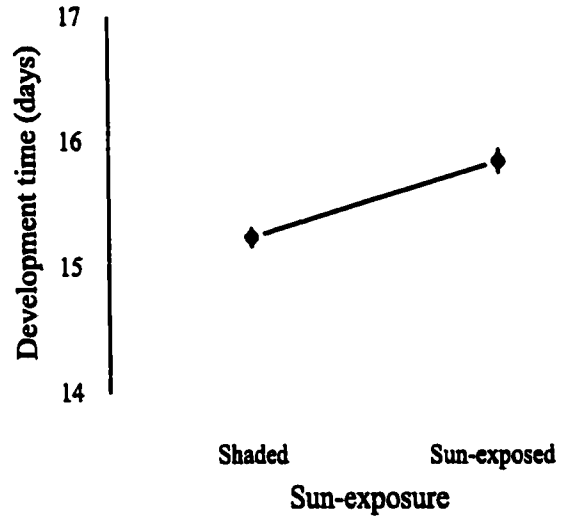
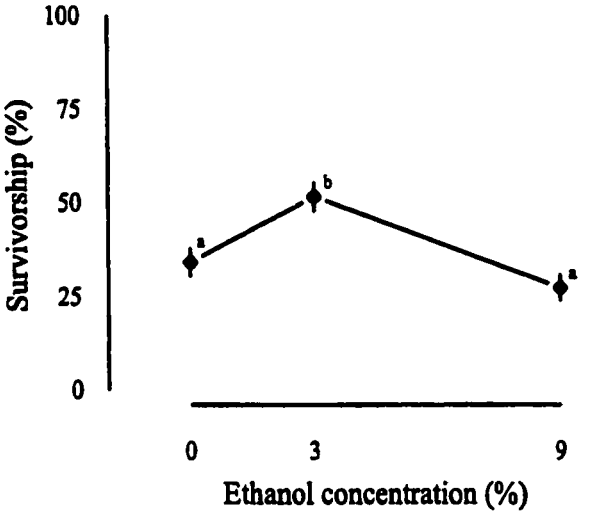

17
16
15
14
Development time (days)
Shaded
Sun-exposed
Sun-exposure

Figure 12. Survivorship and development time of *Drosophila melanogaster* reared in food medium supplemented with 0, 3, and 9% ethanol in the field.

Data were pooled over sun-exposure treatments. Different superscript letters denote significant pair-wise differences ($p<0.05$) between ethanol concentration treatments.


100
75
50
25
0
Survivorship (%)
a
b
a
0
3
9
Ethanol concentration (%)

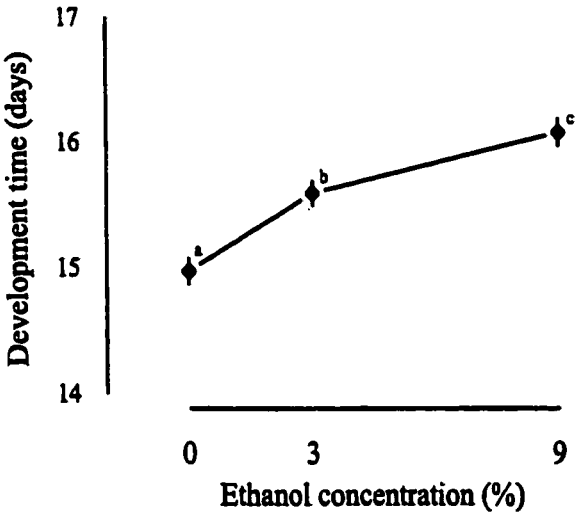
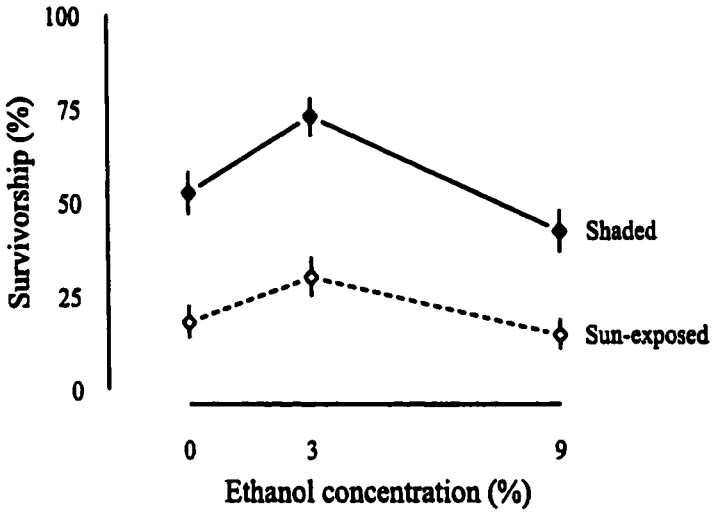

17
16
15
14
Development time (days)
a
b
c
0
3
9
Ethanol concentration (%)

Figure 13. Interaction effect of sun-exposure and ethanol concentration treatments on survivorship and development time of *Drosophila melanogaster* in the field.

Filled circles mark survivorship and development time in shaded apples. Open circles mark survivorship and development time in sun-exposed apples. Different superscript letters indicate significant pair-wise differences ($p<0.05$) between development times in shaded and sun-exposed apples and between ethanol concentration treatments. The interaction effect on survivorship was not significant ($p=0.538$, Table 6).


100
75
50
25
0
Survivorship (%)
Shaded
Sun-exposed
0
3
9
Ethanol concentration (%)

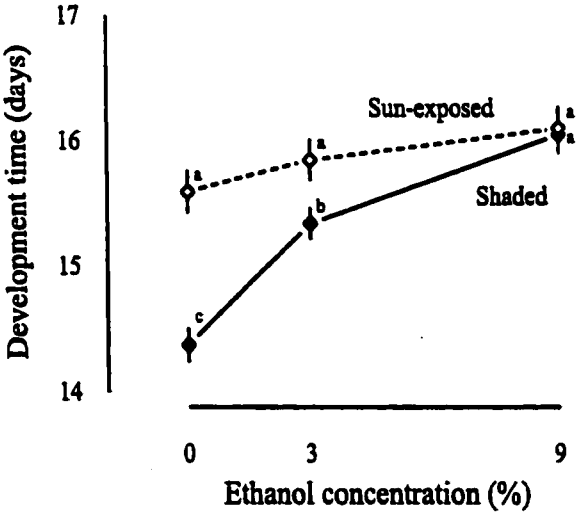

17
16
15
14
Development time (days)
Sun-exposed
Shaded
a
a
a
a
b
c
0
3
9
Ethanol concentration (%)

Table 4. Analysis of variance table
for effects of constant temperature and ethanol concentration treatments
on survivorship of *Drosophila melanogaster* in the laboratory.

Factorial design tested for main and interaction effects of constant temperature (17, 23, and 29°C) and ethanol concentration (0, 3, and 9%). Significance of effects was evaluated using Type III sums of squares.

Dependent variable: survivorship

	df	SS	MS	F	p
Constant temperature	2	2.834	1.417	7.767	<0.001
Ethanol concentration	2	0.893	0.447	2.449	0.092
[EtOH] x Temperature	4	0.188	0.047	0.257	0.905
Residuals	99	18.060	0.182		

Table 5. Analysis of variance table
for effects of constant temperature and ethanol concentration treatments
on development time of *Drosophila melanogaster* in the laboratory.

Factorial design tested for main and interaction effects of constant temperature (17, 23, and 29°C) and ethanol concentration (0, 3, and 9%). Significance of effects was evaluated using Type III sums of squares.

Dependent variable: development time

	df	SS	MS	F	p
Constant temperature	2	5.247	2.623	3278.7	0
Ethanol concentration	2	0.010	0.005	6.547	0.002
[EtOH] x Temperature	4	0.006	0.002	1.962	0.108
Residuals	81	0.065	0.0008		

Table 6. Analysis of variance table
for effects of sun-exposure and ethanol concentration treatments
on survivorship of *Drosophila melanogaster* in the field.

Factorial design tested for main and interaction effects of temperature differences between shaded and sun-exposed apples and ethanol concentration (0, 3, and 9%). Differences among replicate trials were treated as a random block effect. Significance of effects was evaluated over the within-block variance using Type III sums of squares.

Dependent variable: survivorship

	df	SS	MS	F	p
Sun-exposure	1	19.391	19.391	66.246	<<0.001
Ethanol concentration	2	6.046	3.023	10.327	<<0.001
[EtOH] x Sun-exposure	2	0.363	0.182	0.620	0.538
Block (replicate)	2	4.313	2.156	7.367	<0.001
Residuals	532	155.723	0.293		

Table 7. Analysis of variance table
for effects of sun-exposure and ethanol concentration treatments
on development time of *Drosophila melanogaster* in the field.

Factorial design tested for main and interaction effects of temperature differences between shaded and sun-exposed apples and ethanol concentration (0, 3, and 9%). Differences among replicate trials were treated as a random block effect. Significance of effects was evaluated over the within-block variance using Type III sums of squares.

Dependent variable: development time

	df	SS	MS	F	p
Sun-exposure	1	0.129	0.129	23.497	<<0.001
Ethanol concentration	2	0.274	0.137	24.874	<<0.001
[EtOH] x Sun-exposure	2	0.084	0.042	7.580	<0.001
Block (replicate)	2	0.235	0.117	21.281	<<0.001
Residuals	344	1.895	0.006		

CHAPTER 3:

AN EXPERIMENTAL DEMONSTRATION THAT HABITAT HETEROGENEITY CAN MITIGATE CLIMATE-INDUCED POPULATION VARIABILITY

INTRODUCTION

Populations are naturally susceptible to risks of stochastic extinction that stem from random variation in demographic rates (MacArthur and Wilson 1967), environmental fluctuations (Leigh 1981; Lande and Orzack 1988), or from catastrophic events (Mangel and Tier 1993; Mangel and Tier 1994). Extinction risks are generally predicted to increase with the magnitude of population variability, and decrease as population size increases. As human activity has reduced and fragmented populations of plants and animals, the risk of stochastic extinction has increased (Soule 1987).

To minimize extinction risk, conservation biologists and resource managers have focused primarily on efforts intended to increase population size (e.g., Shaffer 1981; Soule 1987). Toward that end, "rules of thumb" for habitat management have been derived from theories of island biogeography and metapopulation dynamics (Possingham et al. 2001). Habitat patches should be as large and contiguous as possible based on the inverse relationship between extinction risk and habitat area predicted by island biogeographic theory (MacArthur and Wilson 1967). According to metapopulation theory (Hanski 1999), when populations are subdivided over a fragmented landscape, dispersal among patches should be maximized to rescue or recolonize local populations and thus increase total population size. Attention to the role of population variability has

largely been limited to protocols for quantifying extinction risks (e.g., Dennis et al. 1991; Fagan et al. 1999).

Extinction risk might also be reduced if population variability were attenuated. To the extent that demographic variation is caused by climate variation (e.g., Birch and Andrewartha 1941; Davidson and Andrewartha 1948), population variability might be mitigated if habitat heterogeneity provides microenvironments that compensate for the effects of different climate conditions. For example, adverse effects of hot conditions could be ameliorated if at least a portion of a population could find refuge in a relatively cool microenvironment. In this way, habitat heterogeneity might hedge a population's ecological bets against the effects of unpredictable climate variation. This proposition is analogous to evolutionary bet-hedging concepts (Philippi and Seger 1989).

The mitigating effect of habitat heterogeneity on population variability was proposed to explain observed patterns of population variability, extinction and persistence in two insect metapopulations. First, during an extended drought in California, one population of Bay checkerspot butterfly (*Euphydryas editha bayensis*) went extinct while a second nearby population persisted (Hellmann et al. in review; McLaughlin et al. in review). Contrary to predictions of island biogeographic theory, the persistent population was that on the smaller patch of grassland habitat. The key factor determining population persistence in this case appeared to be topographic heterogeneity (Ehrlich and Murphy 1987). The smaller but topographically heterogeneous habitat provided cooler, more mesic microenvironments on north-facing slopes where larval

survivorship was sufficient to sustain the population through the drought (Weiss et al. 1987).

Additional evidence that habitat heterogeneity may reduce population variability was reported from studies of a metapopulation of bush cricket (*Metriopterus bicolor*) in Sweden. Normally, bush crickets were found in shortgrass habitat, but appeared to prefer more mesic tallgrass habitat during dry years (Kindvall 1995). During a 2-year drought, year-to-year variation in population size was negatively associated with a measured index of habitat heterogeneity, but was independent of population size, patch area, or inter-patch distances (Kindvall 1996). Significant relationships involving the latter factors would be predicted by theories on stochastic population dynamics (e.g., Tuljapurkar and Orzack 1980), island biogeography (MacArthur and Wilson 1967), and metapopulation dynamics (Hanski 1999), respectively. Kindvall (1996) also found habitat heterogeneity to be positively associated with population persistence through the drought, suggesting that habitat heterogeneity reduced extinction risk by reducing population variability.

The two case studies summarized above suggest that management actions aimed at maintaining or even increasing habitat heterogeneity could be effective for reducing population variability and thus mitigating extinction risk. However, the viability of this strategy depends on determining the extent to which the reported effects demonstrated a general ecological phenomenon or depended on a narrow set of contingencies. A conceptual model for the interactions between climate variation, habitat quality, and population dynamics (after Levins 1968) predicts conditions under which habitat

heterogeneity should or should not reduce population variability. The model also provides a framework for interpreting experimental results.

A conceptual model for how habitat heterogeneity may reduce population variability

Suppose that demographic rates in a population depend on survivorship, reproduction, or some other measure of individual fitness, and that fitness varies with temperature according to the relationship depicted in Figure 14 (Huey 1991). Fitness is highest under an intermediate temperature and declines as temperatures become too warm or too cool. Many components of fitness exhibit similar sensitivity to temperature (Huey and Berrigan 2001). In principle, other relevant environmental variables could be substituted for temperature. According to this model, variation in environmental temperature over time should cause fitness, and thus population dynamics to vary (Levins 1968).

Now suppose that the habitat occupied by a population were heterogeneous with respect to thermal microenvironment such that some habitat patches were relatively cool (S1) and others were relatively warm (S2) under a given environmental temperature (Figure 1a). Under a warm climate (E1), individuals would have higher fitness in the relatively cooler habitat patch (S1). In contrast, if environmental temperatures were more moderate (E2) or cool (E3), individuals in the warmer habitat patch (S2) would have higher fitness. Individual fitness in each habitat patch would still vary with changes in environmental temperature, but the net effect of environmental variation on population variability would depend on how the *average* fitness of individuals in the two habitats was affected.

To illustrate conditions under which habitat heterogeneity would be predicted to reduce population variability, suppose that environmental temperature alternated between warm (E1) and moderate (E2) states. Individual fitness in habitats S1 and S2 would be predicted to vary, but in opposite directions (Figure 14, middle panel). Consequently, average fitness taken over the two habitat types would be relatively invariable through time. Thus, habitat heterogeneity would compensate for the effect of environmental variation on individual fitness and reduce the magnitude of population variability through time.

In contrast, habitat heterogeneity would not reduce population variability if environment temperature alternated between moderate (E2) and cool (E3) states. Under this scenario, individual fitness in habitat S1 and S2 would vary synchronously such that average fitness would also vary with environmental temperature (Figure 14, bottom panel). In fact, since relative fitness in habitat S2 is always greater than that in habitat S1, habitat heterogeneity would reduce mean fitness compared to that in a population in habitat that was uniformly of type S2.

In sum, this model predicts that habitat heterogeneity can compensate for environmental variation to reduce population variability if the relative qualities of different habitat types (as measured by fitness or demographic rates) reverse under different environmental states. If the rank order of habitat qualities is invariant, habitat heterogeneity will not reduce population variability.

An experimental investigation of how habitat heterogeneity affects population variability

To experimentally test whether habitat heterogeneity reduces population variability caused by environmental variation, I co-opted one of biology's classic model organisms, *Drosophila melanogaster*, in one of its natural habitats – apple orchards. *D. melanogaster* uses rotting apples on an orchard floor as habitats on which adults feed and oviposit, and in which larvae develop. Temperature is a key determinant of fitness in these habitats because of its profound effect on numerous fitness-related traits (David et al. 1984). In the field, seasonal variation in ambient temperature has been shown to affect fecundity, time to sexual maturity, and longevity of adult *Drosophila* (Avelar et al. 1987; Junge-Berberovic 1996), and survivorship and development time of larvae (Chapter 1). Larvae are especially susceptible to the consequences of temperature in rotting apples because their limited mobility effectively restricts them to the fruit in which their mother laid them (Feder 1997; Feder et al. 1997b).

The landscape of rotting apples in an orchard is naturally heterogeneous with respect to temperature. Apples that fall near the center of orchard rows are relatively warm because they heat to 5-15°C above ambient air temperature when they are exposed to the sun during the day (Feder 1997; Feder et al. 1997a). Meanwhile, apples that fall near the base of the trees are relatively cool because they are shaded for most of the day and thus remain near ambient air temperature (Chapter 1). This heterogeneity between sun-exposed and shaded fruit, coupled with seasonal variation in ambient temperature, creates a scenario analogous to that supposed in the conceptual model above.

To test whether thermal heterogeneity between sun-exposed and shaded apples could reduce population variability, I established replicate populations of *D. melanogaster* in large field cages that enclosed standardized "landscapes" of rotting apples (cf. Dytham et al. 1997). Populations were established in summer and autumn to assay the effect of seasonal differences in ambient temperature, and assigned one of three habitat treatments in a 2 x 3 factorial design. I manipulated habitat heterogeneity in the cages with shade cloth to create homogeneous landscapes of either shaded (cool) or sun-exposed (warm) apples, and heterogeneous landscapes in which half of the apples were shaded and half were sun-exposed. I then monitored population density in each cage for 7 to 9 weeks. If habitat heterogeneity in orchards reduces population variability for *D. melanogaster*, I predicted that measures of population density and growth rate for populations in homogeneously shaded and sun-exposed cages should negatively covary between summer and autumn. Furthermore, population densities and growth rates for populations in heterogeneous cages should be intermediate between those for shaded and sun-exposed cages, and should vary less between seasons.

MATERIALS AND METHODS

A total of 36 experimental populations of *Drosophila melanogaster* were established in large field cages at two fallow agricultural sites near Woodinville, Washington and Carnation, Washington during summer and autumn of 2000. Each cage enclosed a landscape of 100 rotting apples. Three blocks of three experimental populations were established at each site during each season. Each block of cages

comprised three habitat treatments: a homogeneously *shaded* landscape of rotting apples, a homogeneously *sun-exposed* landscape, and a *heterogeneous* landscape with both shaded and sun-exposed apples. Populations were founded by releasing 500 wild-type adults into each cage. For two of the blocks of cages, populations were founded with 400 and 300 adults, respectively, because of limited supply from a source laboratory colony. Populations were maintained for 7 to 9 weeks during which time 3 to 5 generations of progeny developed and emerged.

Field cages were constructed of steel tube framing over which fine Lumite screening was fitted (commercially available at BioQuip Products). Screen mesh was sufficiently fine (32 x 32 mesh per inch) to prevent adult *Drosophila* from escaping or entering. Screens were staked flush to the ground to close the bottom of each cage. Some leakage was possible along the ground or possibly through a zippered opening, but pilot tests suggested that the number of trespassing flies was negligible compared to the size of founding populations. Stock cage dimensions were 1.8 x 1.8 x 1.8m. To maximize the incident angle of sunlight through cage roofs, cages were tilted to the south by cutting down or removing frame legs (Figure 15). During summer experiments, cages were tilted 30° by halving the height of the southerly legs. During autumn trials, cages were tilted 45° by removing the south legs altogether. Inside the cages, landscaping cloth was spread over the ground to prevent overgrowth and shading by field vegetation. Outside the cages, vegetation was regularly trimmed short.

Cages were set up in blocks of three within which the shaded, sun-exposed and heterogeneous habitat treatments were randomly assigned. The shaded cage was covered

by 85% shade cloth so that all of the apples inside were shaded through the day and were thus relatively cool. The sun-exposed cage was left uncovered to create a homogeneous, relatively warm landscape of sun-exposed apples. The heterogeneous treatment involved covering only part of a cage with 85% shade cloth so that one-half of the apples inside were shaded all day while the other half remained sun-exposed. To ensure that the boundary between shaded and sun-exposed apples was unambiguous, I clustered the apples into two groups separated by about 0.25 m.

A landscape of 100 rotting apples was arranged on the ground inside each cage. Unwaxed red delicious variety apples were purchased out of cold storage from a commercial fruit packing facility to ensure consistent fruit that should have been insect-free. Apples were placed into sealed plastic garbage bags inside of large plastic bins to avoid contamination by wild *Drosophila*. Fruit were left in the bins for at least two weeks prior to experiments so that apples would begin rotting. Natural variation among fruit ensured that individual apples were in different stages of rotting such that the landscape would continue to provide suitable *Drosophila* habitat for several weeks. To ensure consistency of the apple landscape among cages within blocks, apples from a given bin were mixed and then evenly distributed among the three cages in a block. Apple skins were broken by pricking them with a knife so that females could oviposit into the fruit.

Founding adults for each experimental population were collected from a large colony of wild-type *D. melanogaster* maintained in the laboratory. Initial stock for the laboratory colony was collected at an apple orchard near Bothell, Washington in autumn

1999. The colony was kept at room temperature (ca. 20-22°C) and under a natural light-dark cycle in 1-gallon containers into which fresh food medium was regularly supplied. To produce sufficient numbers of adults for founding experimental field populations, I allowed colony flies to oviposit on agar-filled petri dishes, and transferred the eggs into small medium-filled vials where flies developed under the temperature and light conditions described above. This also served to age-match flies by ensuring that all were from the same cohort of eggs. Once adults emerged in the vials, they were anesthetized on a CO_2 diffuser plate and counted into cohorts of 500. That same day, flies were released into the field cages.

I estimated population densities in each cage three times per week for 7 to 9 weeks to generate time series of density estimates. To estimate population density in a cage, I counted the number of flies in each of 10 sampling grids marked on the sides of each cage (cf. Catchpole and Shorrocks 1997). Each 20cm x 20cm sampling grid was subdivided into four 10cm x 10cm quadrants within which flies were more easily and consistently counted. Three sampling grids were marked on the east and west sides of each cage, and four on the north (Figure 15). No sampling grids were marked on the south side of the cages because of cage orientations. A similar protocol for large laboratory populations yielded reliable population density estimates at both very low and very high population densities (Catchpole and Shorrocks 1997). Calibration of density estimates to actual population sizes was not practical in my system, but repeated estimates of population densities during pilot tests suggested that estimation errors were small.

Flies generally roosted on or around the apples on the cage floors unless disturbed. Therefore, I initiated density estimates by tapping apples with a section of rigid tubing inserted through a small zippered opening at the bottom of the cage. Following this disturbance, flies were allowed to settle and alight on the cage walls for 30 seconds, after which time the number of flies in each of the 10 sampling grids was counted. To control for any biasing effects of phototaxis relative to the different shade cloth treatments, a large shade tent was temporarily positioned over the cage to standardize light conditions during observations.

Summer experiments were initiated by founding populations in blocks of cages on June 14, June 19, and July 10, 2000 at the Woodinville site, and on July 3, 7, and 12, 2000 at the Carnation site. Summer replicates were completed by September 5, 2000 when cages were dismantled and all remaining apples were removed from the field sites. Autumn experiments were initiated on September 1, 6, and 16, 2000 at both Woodinville and Carnation sites. These replicates were concluded by November 3, 2000. Cages for autumn replicates were set up on different spots within each field site to avoid capturing any flies from the summer experiments that may have pupated in the soil. The staggered start dates for blocks of cages within a season ensured that each block of populations at a site would experience a different series of weather conditions. Even with the considerable temporal overlap among blocks at a site, populations would be at different stages of development such that day-to-day weather effects would not be identical among blocks.

Data analysis

The experimental units for analysis were the time series of population density observations from each cage (n=36). Each time series included from 21 to 27 observations depending on how long a particular trial ran (7 to 9 weeks x 3 observations/week). Individual estimates of population density on each observation date were calculated as the arithmetic mean of the log(x+1)-transformed counts of flies in the 10 sampling grids.

Experimental treatments reflected a 2 x 3 factorial design with 2 seasons (summer and autumn) and 3 habitat treatments (shaded, sun-exposed, heterogeneous). Habitat treatments were replicated within blocks as in a split-plot experiment. Initial and final population density and several demographic parameters were estimated from each time series and compared using appropriate analysis of variance methods (procedures "anova" and "manova", S-plus 2000, Insightful Inc.). The basic statistical model used for these analyses was:

$$Y_{ijkl} = S_i + T_{j(k)} + (S:T)_{ij(k)} + B_k + \varepsilon_l \qquad (3)$$

where Y_{ijkl} = population density or demographic parameter estimated from cage l during season i and under habitat treatment j in block k,

S_i = effect of season i (summer, autumn),

$T_{j(k)}$ = effect of habitat treatment j (shaded, sun-exposed, heterogeneous) within block k,

$(S:T)_{ij(k)}$ = interaction effect of season i and habitat treatment j within block k,

B_k = random effect of block k, and

ε_l = random error associated with cage l (assumed ~$N(0,\sigma^2)$).

Significance of the season effect was evaluated over the between-block variance. Significance of habitat treatment and interaction effects was assessed over the within-block variance.

First, to verify that the experimental populations indeed started with similar densities, I calculated and compared the initial population density in each cage. I expected initial population densities to be similar since I founded populations with a fixed and consistent number of adults. Initial population density was calculated as the mean of observed population densities during the first two weeks after a population was founded. During this time, only founding adults and perhaps the very first progeny were present in the cages. This analysis provided a check that initial conditions were similar in all cages so that difference in final population densities or demographic parameters could be confidently attributed to the experimental treatments.

I next compared final population densities achieved by the end of each trial. Given similar initial densities, final population densities proved a coarse metric of population growth rate. Final population density was calculated as the mean of observed population densities after the sixth week of each trial. Populations were monitored for 7 to 9 weeks, so estimates were based on at least 3 observations (range 3 to 9). Sample size within a block were always balanced since the three populations were established simultaneously. Significant differences among final population densities would indicate that season or habitat treatments caused population growth rates to differ.

To examine the dynamics of experimental populations in more detail, several demographic parameters were estimated from each time series of population density observations. Each of the experimental populations was expected to exhibit qualitatively similar two-phase dynamics. During the first phase, only the founding adults would be present and their density would decrease as flies senesced and died. The second phase would be marked by a sharp increase in population density as progeny generations emerged. The lag time before progeny began emerging would be proportional to development time. After the initial increase, population density would asymptote as the population approached a density-dependent carrying capacity. These two phases of the population dynamics were modeled mathematically as an exponential decay function and a time-lagged saturating exponential function, respectively, as follows:

$$Y_t = \begin{Bmatrix} N_0 \cdot e^{-m \cdot t} \mid t \le g \\ K\left(1 - e^{-r(t-g)}\right) \mid t > g \end{Bmatrix} \tag{4}$$

where Y_t = population density at time t,

N_0 = initial population density at time 0,

m = daily mortality rate of founding adults,

g = lag time before progeny generations begin to emerge (proportional to development time),

K = carrying capacity for population, and

r = maximal rate of population increase (due to emergence of progeny generations)

These five variables represent quantitative measures of key demographic parameters underlying the population dynamics of experimental populations.

Best-fit parameter values for each experimental population were estimated by fitting the model in equation (2) to the time series of population density observations, and minimizing the negative log-likelihood of the parameters given the data (after Hilborn and Mangel 1997):

$$L[parameters \mid data] = \sum_t \left[\ln(\sigma) + \frac{(\ln(O_t) - \ln(E_t))^2}{2\sigma^2} \right] \quad (5)$$

where $L[parameters \mid data]$ = negative log-likelihood of parameters given data,

O_t = observed population density at time t,

E_t = expected population density at time t (predicted by equation 2),

σ = square-root of the mean squared deviation between observed and expected population densities.

Residuals in the likelihood equation (3) were calculated as the difference between log-transformed observations and expectations because deviations between observed and expected population densities appeared to be log-normally distributed. Model parameters were constrained to be greater than exp(-10) to facilitate estimation in cases where best-fit parameter values approached zero. Negative parameters were biologically implausible in the model.

Maximum likelihood parameter estimates for each experimental population were compared by applying the statistical model in equation (1) in a multivariate analysis of

variance (MANOVA). Estimates for all parameters but g were log-transformed prior to analysis to normalize standard errors. The Bartlett-Pillai trace test was used to assess the significance of season, habitat and interaction effects (Hand and Taylor 1987). Additive effects of experimental factors (e.g., season, habitat treatment) on each of the five demographic parameters were estimated from coefficients of the statistical model.

The significance of season and habitat treatment effects on individual demographic parameters was assessed by univariate analyses of variance. Estimates of demographic parameters were weighted by the inverse of the mean squared deviance between observed and predicted population densities. This effectively weighted estimates in proportion to their likelihood. Multiple comparisons were conducted as appropriate to pinpoint significant pair-wise differences between habitat treatments and between habitat-season combinations (procedure "multicomp", S-plus 2000, Insightful Inc.). Reported p-values for pair-wise comparisons were for family-wise errors.

Finally, the multivariate effects of season and habitat treatments on population dynamics were assessed qualitatively by plotting "average" population trajectories predicted by the MANOVA. Mean demographic parameters for each season-habitat combination were calculated by substituting appropriate coefficients from the MANOVA into equation (1) to yield a vector of predicted parameter values. These parameters were then substituted into the model in equation (2) to predict population density as a function of time.

Larval survivorship and development time measurements

Any effect of habitat heterogeneity on population variability presumably emerges from variation in individual fitness in sun-exposed and shaded apples. Therefore, I conducted complementary experiments to measure larval survivorship and development times during summer and autumn in the shaded and sun-exposed apples. The basic experimental protocol is detailed in Chapter 1. Briefly, ten *D. melanogaster* eggs were transferred into each of 60 small "inserts" that were put into 10 apples (6 inserts/apple) and then left either sun-exposed or shaded in a field cage (5 apples/habitat treatment). Cages used for these experiments did not contain experimental populations or a full landscape of rotting apples but were otherwise identical to those used for population trials. After one week, the inserts were collected, and placed into individual test tubes stopped with cotton, and maintained under ambient laboratory conditions. The test tubes were monitored daily for adult emergence as larvae completed development. Each day, any adults were counted and removed from each test tube.

Survivorship in each insert was estimated as the number of adults that emerged from an insert dived by 10 – the number eggs initially transferred into each insert. Development time for surviving flies was calculated as the difference between the date that eggs were collected and transferred into inserts and the date that adults emerged in a test tube. Survivorship during summer and autumn and in shaded and sun-exposed apples was compared using logistic regression (procedure "glm", S-plus 2000, Insightful Inc.). The significance of season and habitat effects was evaluated by analysis of deviance

(McCullugh and Nelder 1989). Habitat and season effects on mean development time in each insert were evaluated by ANOVA after log-transformation.

RESULTS

Qualitative inspection of time series of population density observations suggests that the dynamics of experimental populations differed between seasons and among habitat treatments. Figure 16 shows data from two blocks of cages – one from a summer trial and the other from an autumn trial – that illustrate these differences. Between seasons, population densities appeared lower during autumn than during summer. Importantly, the rank order of population densities in the three habitat treatments appeared to reverse between seasons. Among the time series in Figure 16, the shaded cage had the highest population density during summer while the sun-exposed cage had the highest density during autumn. During both seasons, population densities in the heterogeneous cage were intermediate relative to the other habitat treatments. Stochastic variation in population densities between observations reflects sampling error and the effects of day-to-day weather variation on adult activity. These qualitative comparisons were validated (below) by quantitative analyses of mean population densities and estimated demographic parameters.

Comparisons of initial population densities in the experimental cages indicated no significant differences with season or habitat treatment (Table 8) and thus verified that experimental populations were established at similar densities.

Final population densities achieved in the cages differed significantly between seasons and among habitat treatments, and also reflected a significant season-by-habitat interaction effect (Table 9). On average, final population densities were lower during autumn than during summer, presumably because of cooler climate conditions (Figure 17). Multiple pair-wise comparisons between habitat treatments indicated that, across seasons, heterogeneous and shaded cages supported similar densities ($p>0.9$) that were significantly greater than those in sun-exposed cages ($p<0.01$) (Figure 17).

The significant interaction effect of season and habitat treatment manifested patterns of variation among final population densities consistent with predictions if habitat heterogeneity reduced seasonal population variability (Figure 18). Within seasons, the rank order of habitat treatments reversed. During summer, the final population densities in sun-exposed cages were significantly lower than those in either shaded or heterogeneous cages ($p<0.001$). The latter two habitat treatments supported similar population densities ($p>0.9$). In contrast, during autumn, sun-exposed cages supported significantly greater population densities than shaded cages ($p<0.02$). Densities in heterogeneous cages were intermediate during both seasons, though pair-wise comparisons with sun-exposed and shaded cages indicated that those differences were not statistically significant ($p>0.2$). Between seasons, densities in shaded cages decreased from summer to autumn ($p<0.001$), while those in sun-exposed cages increased ($p<0.001$). Densities in heterogeneous cages decreased from summer to autumn as well ($p<0.05$), but the magnitude of change was less than that exhibited in either of the homogeneous habitat treatments.

Multivariate analysis of estimated demographic parameters revealed more detailed insights into how habitat treatments and seasonal climate change affected the dynamics of experimental populations. The MANOVA indicated a significant season effect as well as a significant interaction effect of season and habitat treatment (Table 10). Between summer and autumn, the average initial population density parameter ($n0$) decreased, the time lag before progeny generations began to emerge (g) increased, and the average carrying capacity of cages (K) decreased (Table 11). Seasonal differences in these parameters were considered significant ($p<0.05$) based on the results of univariate analyses of variance (Table 12). Founding adult mortality (m) and maximal rate of population increase (r) decreased from summer to autumn, but the differences were not significant in univariate ANOVAs. Significant univariate effects of habitat treatments on demographic parameters were ignored since the habitat effect was not significant in the multivariate analysis.

The interaction effect of season and habitat treatment stemmed primarily from significant differences among the model parameters representing maximum rate of population increase (r) and carrying capacity (K) in a cage (Table 12). As for final population densities, the interaction effect caused variation in r that was consistent with predictions if habitat heterogeneity reduced population variability (Figure 19). Univariate multiple comparisons between seasons within each habitat treatment indicated that the maximal rate of population increase decreased significantly in shaded cages from summer to autumn ($p<0.01$). In sun-exposed cages, r increased, though the difference was only marginally significant ($p=0.17$). There was no seasonal change in r in

heterogeneous cages ($p>0.9$). Carrying capacity, *K*, decreased significantly between summer and autumn in shaded cages ($p<0.01$), but was unchanged in sun-exposed and heterogeneous cages ($p>0.5$). Habitat-dependent seasonal differences in the other model parameters were also observed among the average values calculated from the MANOVA (Table 11), but none was significant in univariate analyses (Table 12).

The net consequences of the season and season-by-habitat treatment interaction effects on model parameters are illustrated in Figure 20, which shows population trajectories predicted by the MANOVA for each combination of habitat treatment and season. Model parameters were calculated by summing the appropriate additive effects for season, habitat treatment, and the interaction estimated by the MANOVA (see Table 11). Initial population dynamics were similar among habitat treatments with some quantitative difference in initial population density (*n0*) observed between seasons.

The key differences were manifested in the second phase of population dynamics as progeny generations emerged and population densities increased. The lag time before this second phase began differed by about 5 days between summer and autumn. During summer, populations in shaded cages increased more quickly and achieved higher density than those in sun-exposed cages. Population density in heterogeneous cages increased at a rate similar to that in shaded cages and achieved a similar carrying capacity, but lagged by a day or two. In contrast, during autumn, sun-exposed cages exhibited the most rapid rate of increase and the highest population density while populations in shaded cages merely sustained their founding density. Populations in heterogeneous cages increased at

a more modest rate than in sun-exposed cages, but again achieved similarly high carrying capacity.

Finally, survivorship in sun-exposed apples was only about 55% of that in shaded apples (25% versus 45%, respectively, p=0.023), but did not differ significantly between seasons (32% in summer versus 38% in autumn, p=0.48) or reflect a season by habitat interaction effect (Table 13). Development time was similar in shaded and sun-exposed apples (16.5 and 16.4 days, respectively). Development appeared to lengthen between summer (14.8 days) and autumn (17.8 days), but the difference was not significant (Table 14).

DISCUSSION

Experimental manipulation of the thermal heterogeneity of apple landscapes demonstrated that habitat heterogeneity reduced variability of *D. melanogaster* populations by compensating for the effects of seasonal climate change. Between summer and autumn, the rank orders of final population densities and maximum rates of population increase in shaded and sun-exposed cages reversed. Populations in heterogeneous cages exhibited intermediate, but consistent, dynamics across seasons (Figure 18 and Figure 19). Populations in homogeneously shaded cages grew more rapidly and achieved higher final densities during summer than did populations in sun-exposed cages. In contrast, during autumn, populations in sun-exposed cages grew more rapidly and achieved higher densities than those in shaded cages. Meanwhile, populations in heterogeneous cages capitalized on the seasonal advantages of shaded and

sun-exposed apples to maintain intermediate rates of population increase and to achieve relatively high population densities during both summer and autumn (Figure 20).

Interestingly, patterns of seasonal variation of individual fitness in shaded and sun-exposed apples contradicted the pattern of negative covariance predicted by the conceptual model. During both summer and autumn trials, survivorship was lower in sun-exposed apples and development time was similar. These results were consistent with those of experiments that compared survivorship and development time across a broad range of ambient temperatures (Chapter 1). Nonetheless, if fitness – as measured by survivorship and development time – were consistently greater in one habitat (e.g., shaded apples) than the other, the conceptual model would predict that habitat heterogeneity should *not* reduce population variability.

Clearly, some other factors must have modified or compensated for the effects on larval survivorship and development time. A number of plausible hypotheses may explain the discrepancy between the results of population trials and the measurements of individual fitness. Obvious candidate factors were food quality and larval density, both of which have been shown to affect population dynamics of *Drosophila* (Sang 1949). Experimental measurements of larval survivorship and development time necessarily controlled these variables by filling inserts with a standard food medium and fixing egg density at 10/insert. In contrast, the apple habitats provided for the experimental populations were initially consistent in terms of quantity and overall quality, but did not likely remain so. Warmer temperatures in sun-exposed fruit may have accelerated the

rates at which apples rotted or desiccated such that food quality in shaded and sun-exposed microhabitats differed.

The density of larvae in the rotting apples was also sure to vary. Larval density likely increased with adult density over the course of each trial. Furthermore, larval densities in shaded and sun-exposed apples may have differed because of differential survivorship (Chapter 1 and above), or perhaps as a consequence of preferential oviposition by females (but see Feder et al. 1997b). Temperature differences between sun-exposed and shaded fruit may also have indirectly affected adult survivorship or fecundity as a consequence of developmental temperature effects on adult body size, longevity, and fecundity (David et al. 1984).

Another intriguing possibility is that temperature differences among shaded and sun-exposed habitats modified interactions between *Drosophila* and other species in the cages such that the strength of the interactions varied with season and habitat treatment. While monitoring the experimental populations, I observed a diversity of other arthropods, including spiders, carabid beetles and braconid wasps that potentially preyed upon or parasitized *D. melanogaster*. Though not quantified, the abundance of these other taxa appeared to increase over the course of the trials. Furthermore, I witnessed successful predation by a spider and by a carabid beetle that provided positive – if anecdotal – evidence of direct species interactions. Experimental populations may also have competed with other *Drosophila* species[1] that were observed at low densities in

[1] Precise identification through the cage mesh was not possible, but other species likely included *D. subobscura* or *D. pseudoobscura* based on black body color.

some of the cages. Relevant examples of habitat-dependent species interactions include differential herbivory in sun-exposed and shaded habitats that determine the apparent shade distribution of a plant (Louda and Rodman 1996); and experimental demonstrations of temperature-dependent competitive exclusion of congeneric *Drosophila* (Davis et al. 1998) and *Tribolium* species (Park 1954; Park 1962). Consequences of parasitism in the *Drosophila* and *Tribolium* systems were also temperature-dependent.

The field experiments reported here demonstrate that habitat heterogeneity can reduce population variability by compensating for the effects of climate variation. Still, caution is warranted before this conclusion is applied as a general rule in the context of conservation management. The discrepancy between observed patterns of variation of population density and demographic parameters and variation in measures of individual fitness illustrates the inherent difficulty of scaling up individual-level effects to predict population dynamics, and highlights the importance of having detailed knowledge about a species' ecology to understand and predict the effects of habitat heterogeneity. The latter issue underscores an especially challenging and pervasive problem in conservation biology – basic biological information with which to make informed management decisions is too often limited or lacking (Kareiva et al. 1998; Harding et al. 2001).

To develop a more mechanistic and predictive understanding of how habitat heterogeneity can reduce population variability, the effects of other abiotic and biotic factors, such as food quality, larval density, and species interactions on both individuals and populations need to be explored. The experimental protocols used here could readily

be adapted to explicitly test alternative hypotheses, and thus make the *D. melanogaster* – orchard system ripe for investigating the interactions among climate, habitat quality, fitness and population dynamics.

Figure 14. Conceptual model for how habitat heterogeneity can reduce climate-induced population variability.

See text for explanation.

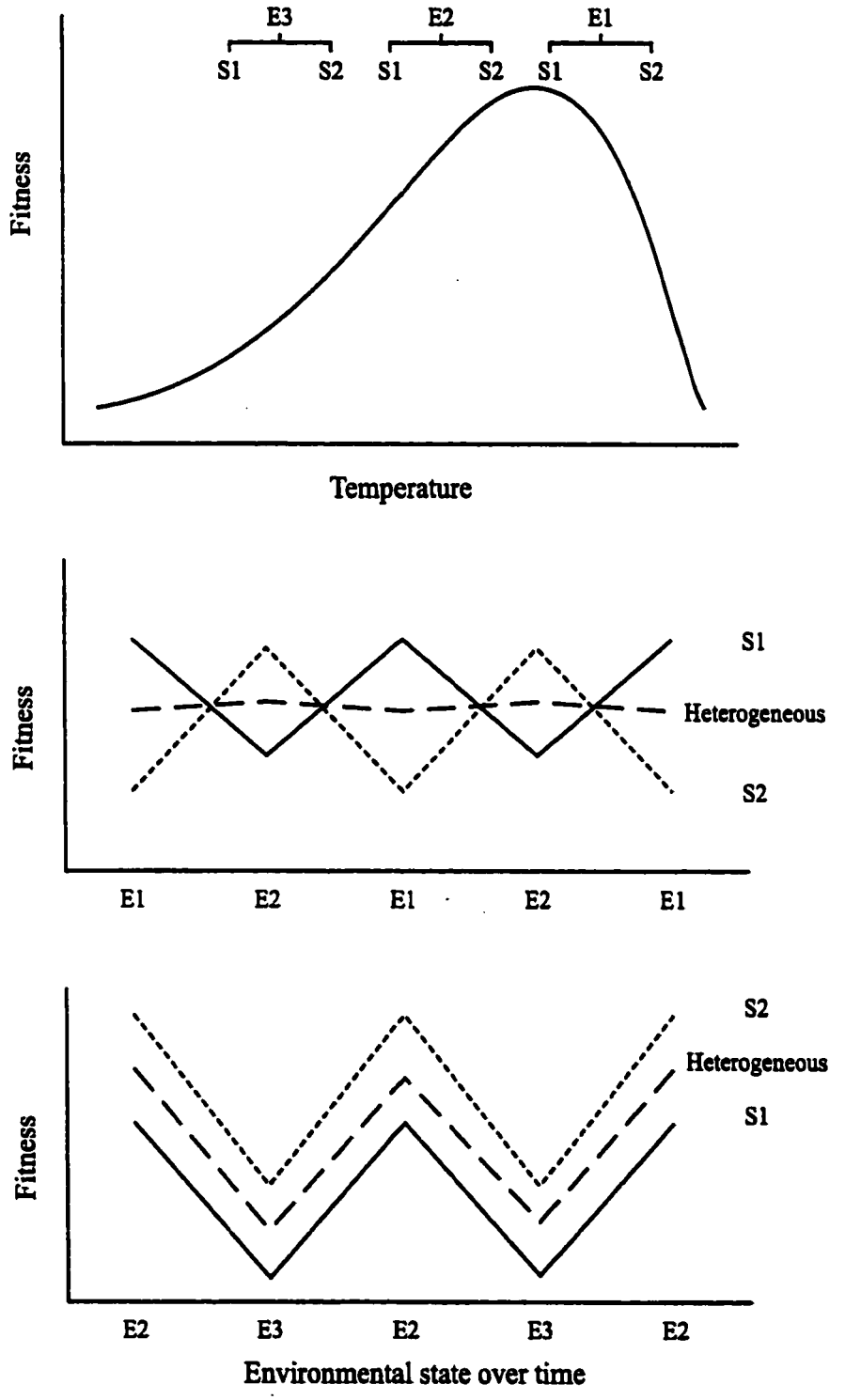
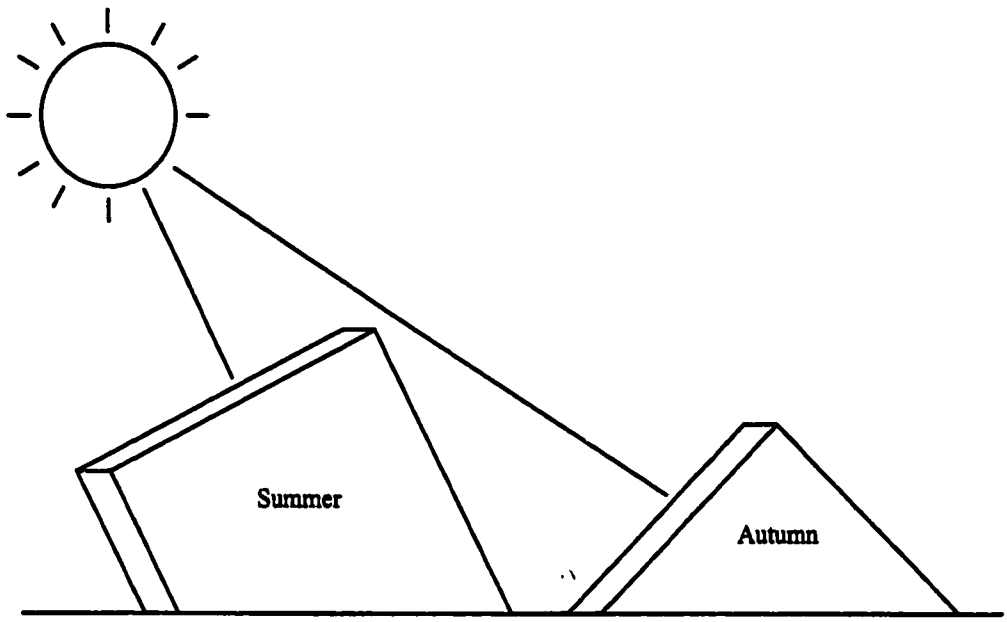

E3
E2
E1
S1
S2
S1
S2
S1
S2
Fitness
Temperature
Fitness
S1
Heterogeneous
S2
E1
E2
E1
E2
E1
Fitness
S2
Heterogeneous
S1
E2
E3
E2
E3
E2
Environmental state over time

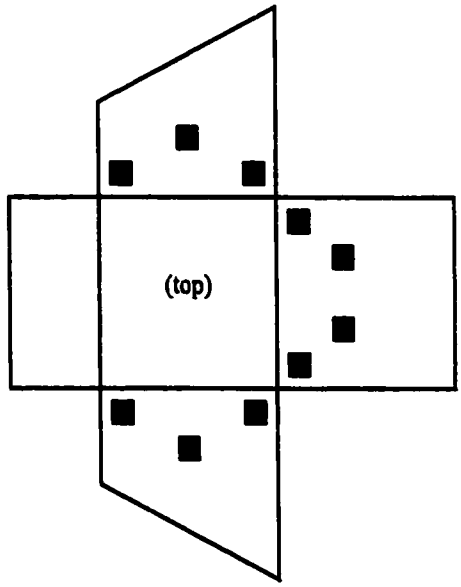
Figure 15. Schematic diagrams of field cages and sampling grids.

(Top panel) Frame legs on the south side of each cage were halved during summer trials and removed during autumn trials so that the angle of sunlight passing through the cage roofs would be maximized.

(Bottom panel) Exploded views of cage screening showing distribution of sampling grids for estimating population abundance during summer and autumn trials.



Summer



Autumn

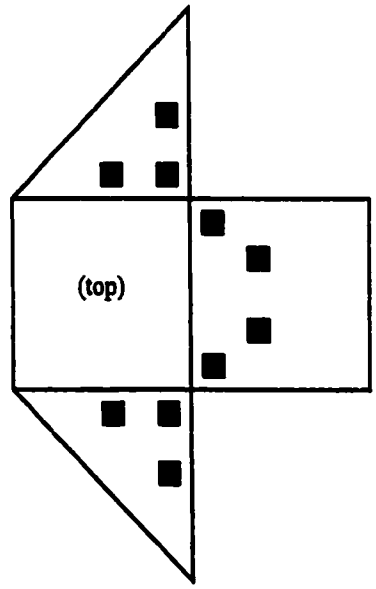


Figure 16. Examples of observed population density time series for populations of *Drosophila melanogaster* in field cages.

First column shows time series of population density from one block of 3 habitat treatments (shaded, heterogeneous, sun-exposed) during a summer trial. Second column shows time series of population density from one block of the 3 habitat treatments during an autumn trial. Each point represents the mean number of flies observed in the 10 sampling grids on a given day.

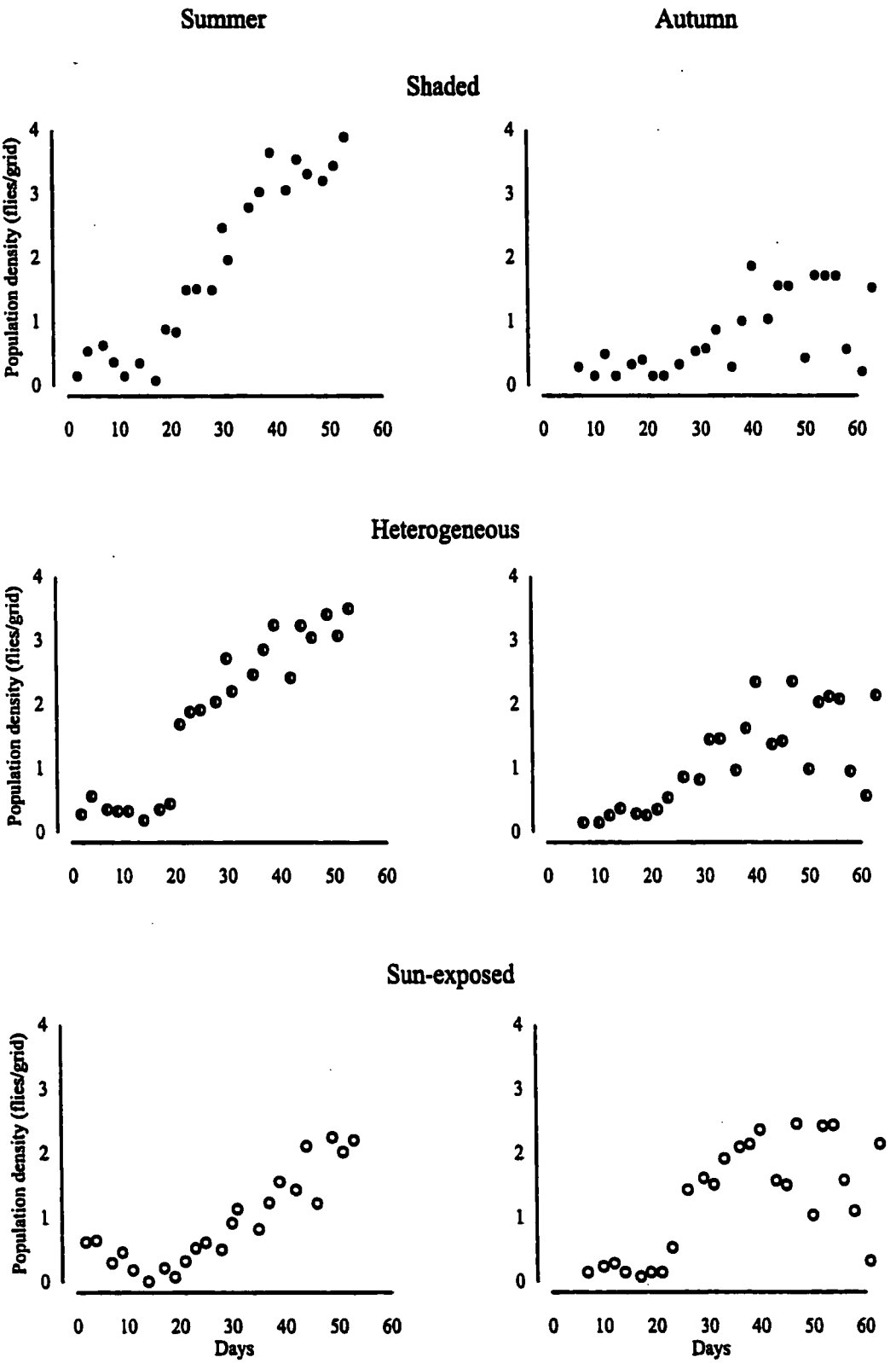

Summer
Autumn
Shaded
Heterogeneous
Sun-exposed
Population density (flies/grid)
0
1
2
3
4
0
10
20
30
40
50
60
Days

Figure 17. Main effects of season and habitat treatments on final density of *Drosophila melanogaster* populations in field cages.

Lines connecting average final densities between summer and autumn and among habitat treatments illustrate the relative magnitude of the season or habitat treatment effects. Different superscript letters denote significant pair-wise differences ($p<0.05$) between habitat treatments. The dotted line marks mean final population density taken over all experimental populations.

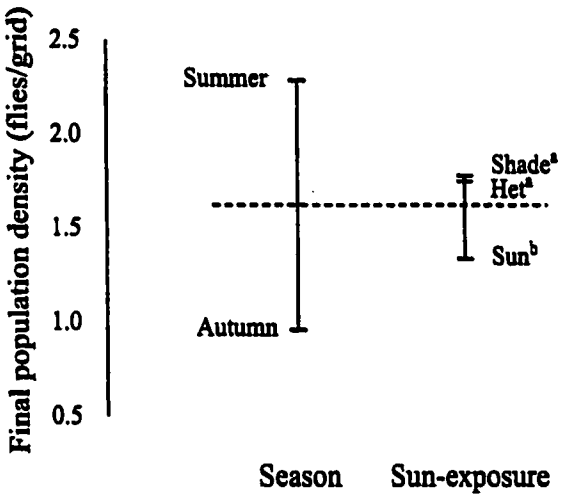

Final population density (flies/grid)
2.5
2.0
1.5
1.0
0.5
Summer
Autumn
Shade[a]
Het[a]
Sun[b]
Season
Sun-exposure

Figure 18. Interaction effect of season and habitat treatments on final density of *Drosophila melanogaster* populations in field cages.

Final densities were adjusted by removing the main effect of season to better illustrate the interaction effect. Different superscript letters denote significant pair-wise differences ($p<0.05$) between seasons and habitat treatments.

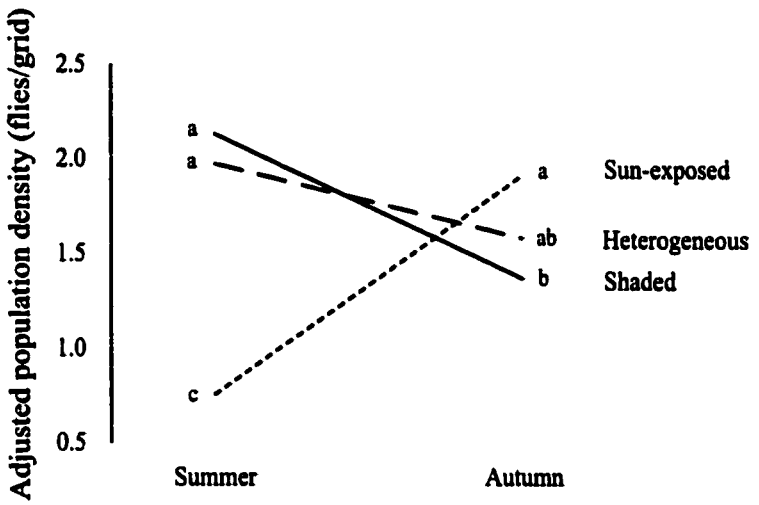

Adjusted population density (flies/grid)
2.5
2.0
1.5
1.0
0.5
a
a
c
a
ab
b
Sun-exposed
Heterogeneous
Shaded
Summer
Autumn

Figure 19. Interaction effect of season and habitat treatments on rates of population increase, *r*, for populations of *Drosophila melanogaster* in field cages.

See text for explanation of how rates of increase were estimated. Different superscript letters denote significant pair-wise differences ($p<0.05$) between seasons and habitat treatments from univariate analysis of variance for *r*.

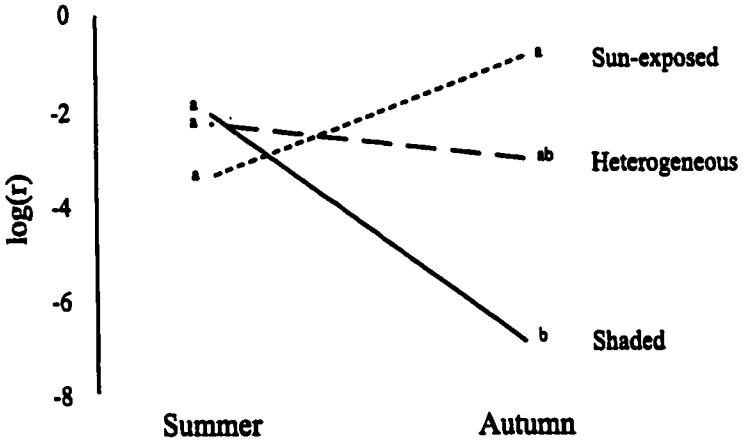

0
-2
-4
-6
-8
log(r)
a
a
a
ab
b
Sun-exposed
Heterogeneous
Shaded
Summer
Autumn

Figure 20. Population trajectories predicted by average demographic parameters for populations of *Drosophila melanogaster* in field cages with each combination of season and habitat treatments.

See text for explanation of parameter estimation. Average parameter values were calculated from multivariate analysis of variance (Table 11) and substituted into Equation 4 to predict population density as a function of time.

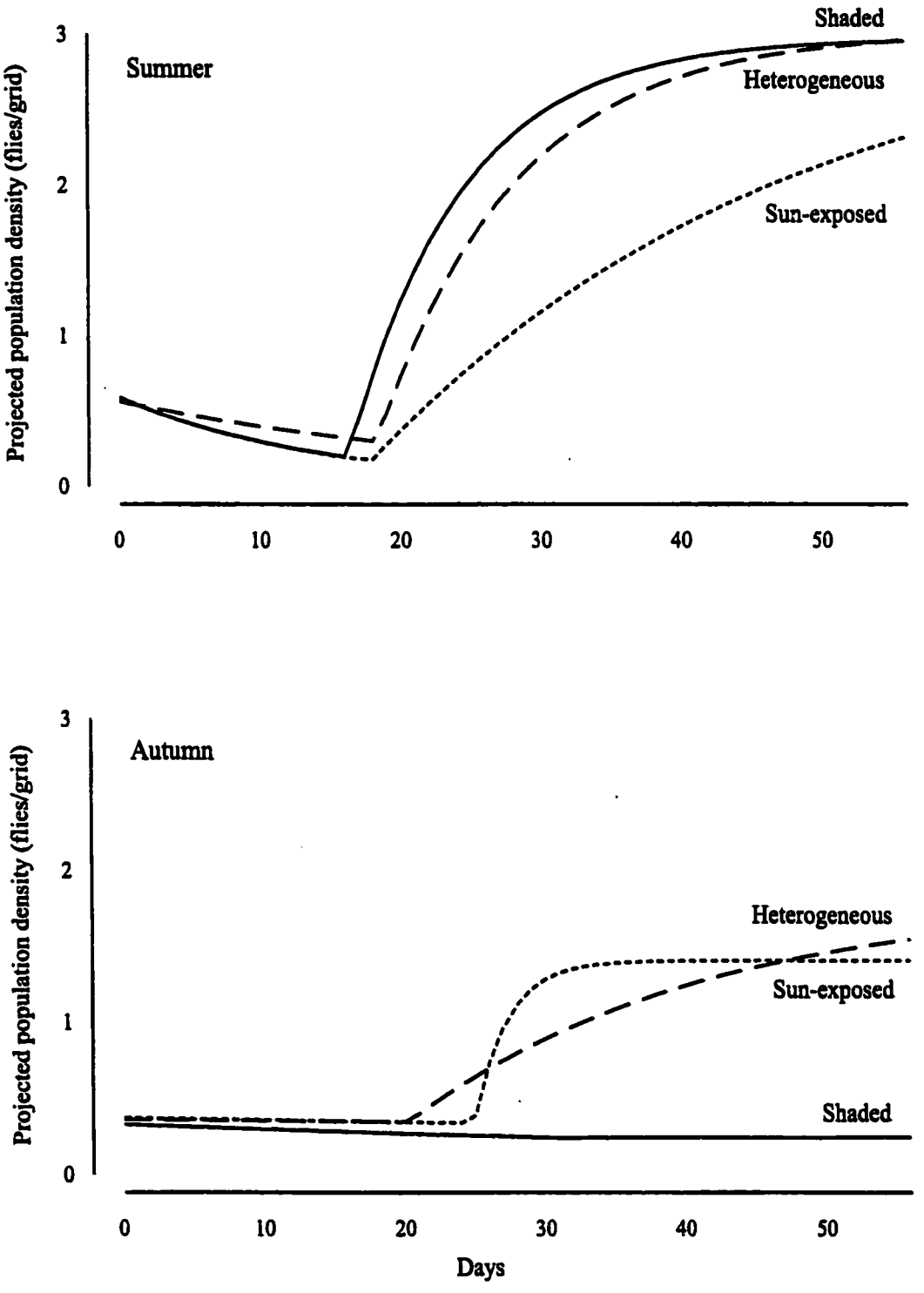

Summer
Shaded
Heterogeneous
Sun-exposed
Projected population density (flies/grid)
3
2
1
0
0
10
20
30
40
50
Autumn
Heterogeneous
Sun-exposed
Shaded
Projected population density (flies/grid)
3
2
1
0
0
10
20
30
40
50
Days

Table 8. Analysis of variance table
for effects of season and habitat treatments on initial density
of *Drosophila melanogaster* populations in field cages.

Factorial design tested for main and interaction effects of season (summer, autumn) and habitat treatment (shaded, sun-exposed, heterogeneous). Differences among blocks of cages were treated as a random effect. Significance of the season effect was evaluated over between-block variance. Significance of the habitat treatment and season-by-habitat interaction effects was evaluated over the within-block variance.

Dependent variable: initial density

	df	SS	MS	F	p
Season	1	0.025	0.025	0.988	0.344
Habitat treatment	2	0.012	0.006	1.483	0.251
Season x Habitat treatment	2	0.009	0.004	1.109	0.349
Residuals (between blocks)	10	0.252	0.025		
Residuals (within blocks)	20	0.079	0.004		

Table 9. Analysis of variance table
for effects of season and habitat treatments on final density
of *Drosophila melanogaster* populations in field cages.

Factorial design tested for main and interaction effects of season (summer, autumn) and habitat treatment (shaded, sun-exposed, heterogeneous). Differences among blocks of cages were treated as a random effect. Significance of the season effect was evaluated over between-block variance. Significance of the habitat treatment and season-by-habitat interaction effects was evaluated over the within-block variance.

Dependent variable: final density

	df	SS	MS	F	p
Season	1	15.910	15.910	27.060	<0.001
Habitat treatment	2	1.469	0.735	9.051	<0.002
Season x Habitat treatment	2	6.316	3.158	38.904	<<0.001
Residuals (between blocks)	10	5.880	0.588		
Residuals (within blocks)	20	1.623	0.081		

Table 10. Multivariate analysis of variance table
for effects of season and habitat treatments on demographic parameters
for *Drosophila melanogaster* populations in field cages.

See text for explanation of demographic parameters and estimation procedures. Estimated demographic parameters for each field cage population were treated as a single multivariate response variable.

Factorial design tested for main and interaction effects of season (summer, autumn) and habitat treatment (shaded, sun-exposed, heterogeneous). Differences among blocks of cages were treated as a random effect. Significance of effects was evaluated using the Bartlett-Pillai test (Hand and Taylor 1987). The season effect was evaluated over between-block residuals. The habitat treatment and season-by-habitat interaction effects were evaluated over the within-block residuals.

	df	Pillai's trace	F (approx.)	num df, den df	p
Season	1	0.836	8.468	5, 6	0.011
Habitat treatment	2	0.737	1.986	10, 34	0.067
Season x Habitat treatment	2	1.035	3.645	10, 34	0.002
Residuals (between blocks)	10				
Residuals (within blocks)	20				

Table 11. Average values of demographic parameters for populations of *Drosophila melanogaster* in field cages with each combination of season and habitat treatments.

Average values were calculated by summing the appropriate linear predictor coefficients estimated by the multivariate analysis of variance. These estimates exclude random block effects.

	n_0	m	g	r	K
Summer					
Shaded	0.585	0.068	16.28	0.124	2.78
Heterogeneous	0.561	0.345	18.31	0.101	2.72
Sun-exposed	0.587	0.069	17.91	0.034	3.01
Autumn					
Shaded	0.328	0.010	30.95	0.001	0.105
Heterogeneous	0.360	0.002	20.19	0.047	1.47
Sun-exposed	0.370	0.004	24.88	0.419	1.07

Table 12. Univariate analysis of variance tables
for effects of season and habitat treatment on demographic parameters
for *Drosophila melanogaster* populations in field cages.

Univariate comparisons of demographic parameters were performed to identify parameters most affected by multivariate season and habitat treatment effects. Any significant habitat treatment effects were ignored in univariate analyses because the multivariate effect was not significant (Table 10).

n0	df	SS	MS	F	p
Season	1	2.203	2.203	9.066	0.002
Habitat treatment	2	0.024	0.012	0.093	0.912
Season x Habitat treatment	2	0.033	0.016	0.127	0.881
Residuals (between blocks)	10	2.429	0.243		
Residuals (within blocks)	20	2.581	0.129		

m	df	SS	MS	F	p
Season	1	60.299	60.299	7.050	0.004
Habitat treatment	2	8.283	4.141	0.978	0.393
Season x Habitat treatment	2	2.004	1.002	0.237	0.791
Residuals (between blocks)	10	85.531	8.553		
Residuals (within blocks)	20	84.676	4.234		

g	df	SS	MS	F	p
Season	1	552.694	552.694	5.759	<<0.001
Habitat treatment	2	114.338	57.169	6.369	0.007
Season x Habitat treatment	2	248.660	124.330	13.850	<0.001
Residuals (between blocks)	10	959.670	95.967		
Residuals (within blocks)	20	179.532	8.977		

r	df	SS	MS	F	p
Season	1	9.348	9.348	2.047	0.164
Habitat treatment	2	37.007	18.504	4.462	0.025
Season x Habitat treatment	2	82.276	41.138	9.919	0.001
Residuals (between blocks)	10	45.656	4.566		
Residuals (within blocks)	20	82.946	4.147		

K	df	SS	MS	F	p
Season	1	24.262	24.262	5.006	0.010
Habitat treatment	2	12.652	6.326	2.641	0.096
Season x Habitat treatment	2	12.282	6.141	2.564	0.102
Residuals (between blocks)	10	48.473	4.847		
Residuals (within blocks)	20	47.899	2.395		

Table 13. Analysis of deviance table
for effects of season and habitat type on survivorship
of *Drosophila melanogaster* in field cages.

Survivorship during summer and autumn and in shaded and sun-exposed apples was compared by logistic regression. Significance of main and interaction effects of season and habitat type was determined by comparing the difference in residual deviance between nested models with and without an effect to a Chi-squared distribution with appropriate degrees of freedom (McCullugh and Nelder 1989).

	Deviance	df	Residual deviance	Residual df	p
Null			69.827	119	
Habitat type	5.176	1	64.651	118	0.023
Season	0.499	1	64.152	117	0.480
Season x Habitat type	0.048	1	64.104	116	0.827

Table 14. Analysis of deviance table
for effects of season and habitat type on development time
of *Drosophila melanogaster* in field cages.

Log-transformed development times during summer and autumn and in shaded and sun-exposed apples were compared using a generalized linear model that was numerically equivalent to an analysis of variance. Significance of main and interaction effects of season and habitat type was determined by comparing the difference in residual deviance between nested models with and without an effect to a Chi-squared distribution with appropriate degrees of freedom (McCullugh and Nelder 1989).

	Deviance	df	Residual deviance	Residual df	p
Null			0.901	79	
Habitat type	0.001	1	0.900	78	0.972
Season	0.696	1	0.204	77	0.404
Season x Habitat type	4.04e-4	1	0.204	76	0.984

CHAPTER 4:

A CRITICAL ROLE FOR CRITICAL HABITAT IN THE RECOVERY PLANNING PROCESS? NOT YET.[2]

INTRODUCTION

When a species is listed as threatened or endangered under the U. S. Endangered Species Act (ESA) (16 U.S.C. § 1531 et seq.), "critical habitat" is supposed to be designated as:

> "... the specific areas within the geographical area occupied by the species... on which are found those physical or biological features essential to the conservation of the species and which may require special management considerations or protection; and specific areas outside the geographical area occupied by the species... [that] are essential to the conservation of the species." (ESA sec. 3(5)(A))

Despite this legal mandate, critical habitat has been designated for fewer than 12% of the species listed under the ESA in the U. S. (145 of 1243 species as of April 2001) (USFWS 2001b; USFWS 2001a). For the great majority of species, the U. S. Fish and Wildlife Service (USFWS) or the National Marine Fisheries Service (NMFS), the two federal agencies responsible for administering the ESA, have made discretionary determinations that designation of critical habitat was either "not prudent" or "not

[2] William F. Fagan and Jeffrey E Bradley were collaborators on the research described in this chapter.

determinable". The former determination encompasses cases where vandalism may ensue, or where designation would not provide any additional protection or benefit for the listed species (50 CFR 424.12(a)(1)). The latter determination would be made if an agency did not have sufficient information about a species' distribution and habitat requirements to make a formal designation (50 CFR 424.12(a)(2)). Such discretion is allowed under the ESA by language stating that critical habitat be designated "to the maximum extent prudent and determinable" (ESA sec. 4(a)(3)(b)).

The USFWS' liberal exercise of discretion over the last quarter century has generated controversy over the role and importance of critical habitat designation under the ESA (see Patlis 2001 for a detailed history). Advocates for critical habitat designation argue that the statutory mandate for critical habitat designation is clear. To the extent that the ESA allows discretion with respect to designation, Congress intended decisions to not designate critical habitat to be exceptions rather than the norm (McDonald 1998; Darin 2000, but see Smith 1999). Furthermore, many conservationists maintain that the prohibition against adverse modification of critical habitat provides important legal protection for habitat that complements the prohibitions against "take" of listed species under section 9 of the ESA. These arguments have been made in numerous lawsuits that claim that the USFWS has neglected to protect listed species by not designating critical habitat (e.g., *Natural Resources Defense Council v. United States Department of the Interior* 1991; *Northern Spotted Owl v. Lujan* 1997).

The USFWS has defended its actions (or lack thereof) on the grounds that the legal protections for critical habitat are redundant to the "no jeopardy" provisions of

section 7 of the ESA (USFWS 1999b). USFWS argued that since critical habitat is essential to a species' conservation, destruction or adverse modification of critical habitat would necessarily jeopardize the species and thus would be prohibited. Furthermore, because prohibitions against adverse modification of critical habitat are limited to federal actions, USFWS asserted that critical habitat designations have only limited application to non-federal properties. Even when critical habitat designation could offer some benefits to a species, USFWS argued that high administrative costs outweigh the marginal benefits of designation. In particular, USFWS cited mandatory economic analyses that compare the biological benefits against socio-economic costs of designation for each property parcel that may be included (critical habitat designation is the only provision of the ESA under which economics are explicitly considered in the decision-making process).

Over the last decade, many of the USFWS' determinations to not designate critical habitat have been overturned by court orders (e.g., *Conservation Council v. Babbitt* 1998), in which USFWS was ordered to designate critical habitat for 245 plant species). In fact, USFWS was so inundated by court orders and settlement agreements requiring critical habitat designations that they have dedicated all budget resources for fiscal year 2001 to address a backlog of designations for about 300 species (USFWS 2000). This decision raises a fundamental question: How will these critical habitat designations affect efforts to recover threatened and endangered species?

In principle, it seems obvious that critical habitat designations *should* contribute positively toward recovery of listed species (Cheever 1996; Baldwin 1999). The primary

purpose of the ESA is "to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved"(ESA sec. 2(b)). Critical habitat, as defined in the ESA (see above), seems like it would be an important part of achieving that purpose. However, the extent to which such contributions have been realized in the past is probably limited because critical habitat was designated for so few species. Nonetheless, it is important that the contributions of previous critical habitat designations be assessed so that future designations can make the best possible contributions toward recovery efforts.

Here we examined the influence of critical habitat designations in the recovery planning process. Recovery plans are the central documents intended to inform, guide and coordinate recovery of listed species (USFWS 1995). As such, one would expect recovery plans to reflect the contributions made by each provision of the ESA. Thus, we investigated whether recovery plans for species with critical habitat were different than plans for species without critical habitat. Our objective was to identify meaningful characteristics of recovery plans that differed systematically as a function of critical habitat designation.

METHODS AND RESULTS

Our analyses relied on a large database developed by a project jointly funded by the Society for Conservation Biology (SCB), the National Center for Ecological Analysis and Synthesis (NCEAS), and USFWS. The database was compiled from reviews of ESA recovery plans for 181 listed species. This sample represented about 20% of all species

with approved recovery plans. The primary data and a key to those data are available at http://www.nceas.ucsb.edu/recovery. A complete description of the recovery plan review project and the compiled database is presented in Hoekstra et al. (in press). In describing specific analyses, we refer to questions (e.g., Q9) or columns of questions (e.g., col. EE) from that database so that the reader could consult the primary data to duplicate or build on our analyses.

Associations between critical habitat designation and species attributes

Before evaluating how critical habitat designations may have influenced specific aspects of the recovery planning process, we examined patterns of critical habitat designation among the 181 species whose recovery plans were included in the SCB database. The stratified random sample included about 20% of all listed species with approved recovery plans, so we expected it to be statistically representative. Among the sample, critical habitat had been designated for 32 species (18%). This proportion was greater than that observed in the entire population of listed species, but provided a better sample size for comparisons and analyses.

We began by comparing the frequency of critical habitat designation among species in different major taxonomic groups (vertebrates, invertebrates, plants) (Q66), and species with widespread versus localized distributions (Q69). The former comparison tested for potential taxonomic bias in how critical habitat had been designated in the past, while the latter tested whether the extent of a species' geographic range influenced decisions to designate critical habitat. We hypothesized that critical habitat might be easier to delineate and designate for species with more restricted

distributions. Motivated by USFWS assertions that critical habitat designations have minimal application on non-federal lands (USFWS 1999b), we also tested whether critical habitat was more likely designated for species found exclusively or primarily on federal lands versus non-federal lands (Q80). Finally, we compared the frequency of critical habitat designations among species facing high, moderate and low degrees of threat, and among species with high versus low recovery potential. These last comparisons were based on recovery priority assignments made by USFWS (Q62). High, moderate, and low degrees of threat were reflected in recovery priorities 1-6, 7-12, and 13-18, respectively. Species with high recovery potential were assigned priorities of 1-3, 7-9, or 13-15 depending on the associated degree of threat (see USFWS 1999c). The statistical significance of differences between groups of species was assessed with the G-test (Sokal and Rohlf 1981).

Critical habitat was disproportionately designated for vertebrate species, but did not differ with the extent of species' geographic range (Table 15). The latter result held regardless of whether we defined "localized" ranges as being <100 km^2, <10,000 km^2, or <50,000 km^2. Critical habitat did appear to be designated more often for species found exclusively or primarily on federal land, but the differences were not significant (Table 15). Finally, neither the degree of threat to a species nor the recovery potential of a species was associated with a higher frequency of critical habitat designation (Table 15).

Influence of critical habitat designation on recovery plan content

The extent to which critical habitat designations contributed toward recovery planning for threatened and endangered species should be manifest in differences

between recovery plans for species with and without critical habitat. We hypothesized that critical habitat designations could influence recovery plan contents in several specific ways: 1) critical habitat designation may increase the availability of information on a species' habitat requirements, 2) critical habitat designation may emphasize the seriousness of habitat threats facing a species, 3) critical habitat designation may underscore the importance of habitat management and monitoring actions, and 4) critical habitat designation may motivate definition of recovery criteria pertaining to a species' habitat. The causal relationships implied in these hypotheses depended on an assumption that critical habitat designation preceded recovery plan development. This is the chronology anticipated by the ESA, but is not always the case (e.g., spectacled eider, humpback chub, bonytail chub, western snowy plover). Among the 32 species in our sample for which critical habitat had been designated, designation preceded recovery plan approval in all cases. Differences between recovery plans for species with and without critical habitat were evaluated with the G-test or one-way analysis of variance (ANOVA), as appropriate for the data (Sokal and Rohlf 1981). The specific test used was identified for each analysis presented below.

To test whether critical habitat designation increased the availability of information on a species' habitat requirements, we compared the diversity of habitat requirements discussed in plans for species with and without critical habitat (Col. A: Q89-99). We expected that plans would discuss a greater diversity of topics regarding a species' habitat requirements if there were more information available. Between species

with and without designated critical habitat, there was no difference in the diversity of habitat requirements discussed (Table 16).

If critical habitat designation emphasized the seriousness of threats posed by habitat destruction, degradation or fragmentation, we expected that recovery plans for species with designated critical habitat would more consistently identify these habitat concerns among the most serious threats to a species. We compared the proportions of recovery plans for which reviewers ranked habitat destruction, degradation or fragmentation among the top three threats described in the plan (Q294-296). Habitat threats were highly ranked in all (100%) recovery plans for species with designated critical habitat, compared to 90% for species without critical habitat (Table 16). Additionally, of the 13 species for which habitat threats were not highly ranked, none had critical habitat designated.

Critical habitat designation may also underscore the importance of including habitat acquisition, management and monitoring among the recovery actions prescribed in a recovery plan. To test whether recovery plans for species with critical habitat were more likely to prescribe habitat acquisition or habitat management, we compared proportions of plans in which one or more habitat acquisition and habitat management tasks were proposed. Categories of habitat acquisition tasks in the database included securement of general habitat, breeding, feeding or sheltering habitat, and dispersal corridors (Col. EE: Q320, 324, 326, 328, 330). Categories of habitat management included restoration and maintenance of general habitat quality, breeding, feeding or

sheltering habitat, and dispersal corridors; and reduction of human disturbance (Col. EE: Q319, 321-323, 325, 327, 329, 331).

Recovery plans for species with critical habitat were no more likely to prescribe habitat acquisition (62%) than were plans for species without critical habitat (73%) (Table 16). Nor were plans for species with critical habitat more likely to prescribe habitat management (96 vs. 97%, respectively) (Table 16).

We similarly compared the proportions of plans in which one or more habitat monitoring tasks were prescribed. We also compared the diversity of monitoring tasks proposed. Categories of habitat monitoring tasks included habitat quantity, habitat quality, and trends in the two (Col. OO: Q366-370). Recovery plans for species with critical habitat were not significantly more likely to prescribe at least one habitat monitoring task (72% vs. 63%, respectively) (Table 16). However, on average, recovery plans for species with critical habitat prescribed a greater diversity of habitat monitoring tasks (2.0±0.3 SE) than did plans for species without critical habitat (1.4±0.1 SE) (Table 16).

Finally, we hypothesized that critical habitat designation may have motivated the authors of recovery plans to include considerations about a species' habitat among the criteria against which recovery would be measured. To test this, we compared the frequencies with which habitat status (quantity or quality) or securement of habitat or water rights was identified as a recovery metric (Col. EEE: Q412, 413, 417, 419). The proportion of plans in which such recovery criteria were defined did not differ with

critical habitat designation (18% vs. 20% for species with and without critical habitat, respectively) (Table 16).

DISCUSSION

Inspection of the associations between critical habitat designation and various attributes of listed species suggests that critical habitat designations have been biased toward vertebrate species (Table 15). This might be explained by the higher profile that vertebrates often command with the public (e.g. whooping crane, green sea turtle) that, in turn, could increase pressure for designation. Meanwhile, determinations to not designate critical habitat for invertebrate and plant species may have gone relatively uncontested, at least before recent court decisions that required designations for numerous plant species (e.g., *Conservation Council v. Babbitt* (1998) that addressed 245 Hawaiian plant species). Vertebrate species may also be better studied than invertebrates or plants such that the habitat requirements for those species were easier to identify and delineate for critical habitat designation.

The USFWS' assertion that critical habitat designations have little meaning on non-federal lands may explain the non-significant trend of more critical habitat designations for species found primarily on federal lands (Table 15). However, we were surprised to find that critical habitat designations did not vary with any of the other factors we examined, particularly the degree of threat faced by a species and a species' recovery potential. Both of these variables are key criteria that determine the recovery priority that USFWS assigns to species. If critical habitat offered even the slimmest

margin of additional protection and benefit to listed species, one would hope that it would be designated for those species most threatened or those with the greatest prospects for successful recovery.

Critical habitat designations did appear to influence the content of recovery plans in terms of the emphasis placed on habitat threats, and the diversity of habitat monitoring tasks prescribed (Table 16). In the latter case, critical habitat designation should have underscored the need for effective habitat monitoring since protection and maintenance of critical habitat is, by definition, essential for recovery of a species. Designation may also have facilitated incorporation of habitat monitoring into recovery plans by identifying specific habitats that should be monitored. Critical habitat designation may similarly have influenced the emphasis on habitat threats in recovery plans. However, since 90% of recovery plans for species without critical habitat also emphasized habitat threats, this is not a unique contribution of critical habitat designation.

Overall, we concluded that critical habitat designation has had, at best, a limited influence on recovery planning (Table 16). Designation of critical habitat did not appear to increase the availability of information on species' habitat requirements. Nor did it prompt more frequent prescriptions for habitat acquisition or habitat management. Finally, recovery plans for species with critical habitat were no more likely to include habitat-based recovery criteria. This was especially surprising since protection of critical habitat is supposed to be essential for species recovery.

There are several possible explanations for why critical habitat designations have not measurably affected the recovery planning process. First, our results may simply

document a self-fulfilling prophecy by the USFWS that critical habitat offers no real benefits for listed species (McDonald 1998). Alternatively, critical habitat designations may actually have made the hypothesized contributions, but the recovery planning process may compensate perfectly for those species without critical habitat. To the extent that this might be true, critical habitat designations would seem redundant to recovery planning efforts. Third, the potential contributions of critical habitat toward endangered species conservation are inherently handicapped by legislative and regulatory contradictions (Patlis 2001). For example, to the extent that extinction would not result, the ESA requires that critical habitat designations balance the biological needs of a species with the economic costs that designation may impose (ESA sec. 4(b)(2)). Furthermore, the ESA limits the scope of the prohibition against adverse modification of critical habitat to federal actions (ESA sec. 7(a)(2)). Together, these requirements effectively limit critical habitat designation to the subset of available or potential habitat (usually federally owned) in which the biological needs of a listed species do not substantially conflict with human economic interests. Finally, although designation of critical habitat as specific geographic areas may facilitate public notification and local conservation planning by mapping parcels with essential habitat, it also dichotomizes the landscape as critical or not critical habitat. Consequently, recovery efforts may become too narrowly focused such that actions outside designated critical habitat are discounted and regional conservation efforts are compromised (W. L. Minckley, pers. comm.).

If future critical habitat designations are to make more substantive contributions toward conservation and recovery of listed species, the policies and procedures governing

how critical habitat is designated need to change (Cheever 1996; USFWS 1999b). Patlis (2001) proposed that critical habitat should be designated as part of the recovery planning process, rather than prior to it. This is already true to the extent that recovery outlines are drawn up at the time a species is listed as threatened or endangered (USFWS 1995).

We suggest that critical habitat be designated biologically as a suite of habitat characters required by a species. These characters would establish clear and explicit standards regarding the biological and ecological requirements of listed species that, in turn, could guide conservation and recovery efforts on both local and regional scales. The geographic distribution of these habitat characters should still be delineated on maps as a complementary but secondary component of critical habitat designation. For example, rather than simply designating critical habitat for an endangered fish as specific segments of a river, critical habitat would be defined by necessary characteristics of the habitat such as flow rate, substrate, and water temperature. The geographic distribution of these characters could then be overlaid on maps to identify specific areas of critical habitat as well as areas with more or less potential for habitat restoration. These types of issues regarding critical habitat designation arise regularly as, for example, in the case of critical habitat designation for the Rio Grande silvery minnow (USFWS 1999a).

A standards-based system of critical habitat designation would refine and expand the role of critical habitat in endangered species conservation. Geographic areas that presently contain all elements of critical habitat could be delineated and protected as under the current system (e.g. by prohibition against adverse modification). In addition, though, the standards that define critical habitat would also establish criteria against

which the potential for, and success of, habitat restoration could be measured across all of a species' range. Standards-based designations would also facilitate economic analyses by providing a consistent system for estimating the present and potential biological value of any habitat patch. These values could be weighed against anticipated costs of habitat protection or restoration to better prioritize recovery actions. Finally, a standards-based system for critical habitat designation should improve public notification regarding potential impacts of a designation (cf. Baldwin 1999). In addition to providing maps of the distribution of critical habitat characters, the critical habitat standards would facilitate determinations of what specific changes or impacts to a habitat could be construed as adverse modification. To the extent that changes or modifications to critical habitat characters also constitute "harm" to a species (50 CFR 17.3), a standards-based designation would also clarify actions that may be construed as "take" of a listed species (as prohibited under section 9 of the ESA) (cf. Baldwin 1999).

The foundation for a standards-based system for designating critical habitat already exists within current USFWS procedures that require identification of "constituent elements" of critical habitat (50 CFR 424.12). Thus, our proposal could be realized by shifting the emphasis of critical habitat designation from the final delineation of lines on maps to explicit descriptions of the habitat characters used to define those map boundaries.

Whether or not our suggestion is adopted and implemented is ultimately up to policy-makers, and the USFWS and NMFS as responsible regulatory agencies. However, unless some affirmative changes are made, the many critical habitat designations

currently being made in response to court orders and settlement agreements are unlikely to promote recovery of those species. Meanwhile, listing decisions for other species have been suspended (USFWS 2000), leaving those species susceptible to extirpation. In the hopes that the USFWS' diversion of resources to critical habitat designations will do more than satisfy procedural demands, we propose that critical habitat be designated biologically as a suite of habitat requirements that will promote more effective contributions to recovery efforts for listed species.

Table 15. Associations between species attributes and the percentage of those species for which critical habitat has been designated.

Species distributions were categorized as "localized" if the species' range was less than 10,000km^2. The association did not change qualitatively when we redefined "localized" as ranges less than 100km^2 or less than 50,000km^2. Degrees of threat faced by species and recovery potentials of species were derived from recovery priorities assigned by USFWS (see USFWS 1999c).

Species attributes	% of species with critical habitat	N (total # of species)	G-statistic (d.f.)	p
Taxonomic group				
Vertebrate	34%	70	20.4	< 0.001
Invertebrate	15%	20	(1)	
Plant	6%	81		
Species distribution				
Widespread	25%	32	0.83	0.36
Localized	17.6%	108	(1)	
Property ownership				
Exclusively federal	33.3%	9	3.24	0.36
Primarily federal	27.6%	29	(3)	
Primarily non-federal	17.4%	46		
Exclusively non-federal	14.5%	62		
Degree of threat				
High (1-6)	19.1%	110	0.676	0.71
Moderate (7-12)	24.4%	41	(2)	
Low (13-18)	14.3%	7		
Recovery potential				
High (1-3, 7-9, 13-15)	20.6%	107	0.007	0.93
Low (4-6, 10-12, 16-18)	20.0%	50	(1)	

Table 16. Comparisons of recovery plan contents for species with and without critical habitat.

The diversity of habitat requirements discussed and the diversity of monitoring tasks prescribed were treated as continuous variables and compared with analysis of variance. Other values represented the percentage of recovery plans that ranked habitat threats highly, prescribed various habitat-based recovery tasks, or defined habitat-based recovery criteria.

Plan contents and characteristics	Critical habitat? Yes	No	Test statistic	p
Diversity of habitat requirements discussed	6.6	6.1	$F_{1,169}=2.22$	0.14
Habitat threats highly ranked	100%	90%	$G_1=5.6$	<0.02
Habitat acquisition prescribed	62%	73%	$G_1=1.45$	0.23
Habitat management prescribed	96%	97%	$G_1=0.10$	0.75
Habitat monitoring prescribed	72%	63%	$G_1=0.86$	0.35
Diversity of monitoring tasks prescribed	1.4	2.0	$F_{1,169}=4.71$	<0.03
Habitat-based recovery criteria	41%	43%	$G_1=0.07$	0.79

LIST OF REFERENCES

Avelar, T., Rocha-Pite, M. t. and Matos, M. 1987. Variation in some demographic parameters in *Drosophila simulans*, *D. subobscura* and *D. phaletara* (Diptera, Drosophilidae) throughout the year under semi-natural conditions in Portugal. *Acta Oecologica* 8(3): 347-356.

Baldwin, P. 1999. The role of designation of critical habitat under the Endangered Species Act. Washington, D. C., The National Council for Science and the Environment.

Berry, A. and Kreitman, M. 1993. Molecular analysis of an allozyme cline: alcohol-dehydrogenase in *Drosophila melanogaster* on the East Coast of North America. *Genetics* 134(3): 869-893.

Birch, L. C. and Andrewartha, H. G. 1941. The influence of weather on grasshopper plagues in South Australia. *Journal of the Department of Agriculture of Southern Australia* 45: 95-100.

Bonnier, G. 1926. Temperature and the time of development of the two sexes in *Drosophila*. *British Journal of Experimental Biology* 4: 186-195.

Catchpole, R. and Shorrocks, B. 1997. An alternative method for population estimation in large scale laboratory populations of *Drosophila*. *Drosophila Information Service* 80: 96-99.

Chakir, M., Capy, P., Genermont, J., Pla, E. and David, J. R. 1996. Adaptation to fermenting resources in *Drosophila melanogaster*: Ethanol and acetic acid tolerances share a common genetic basis. *Evolution* 50(2): 767-776.

Chakir, M., Peridy, O., Capy, P., Pla, E. and David, J. R. 1993. Adaptation to alcoholic fermentation in *Drosophila:* A parallel selection imposed by environmental ethanol and acetic acid. *Proceedings of the National Academy of Sciences of the United States of America* 90(8): 3621-3625.

Cheever, F. 1996. The road to recovery: a new way of thinking about the Endangered Species Act. *Ecology Law Quarterly* 23: 1-78.

Cochran, W. and Cox, G. 1957. Experimental Designs. New York, Wiley.

[50 CFR 424.12] United States Code of Federal Regulations. 2001a. Criteria for listing critical habitat (current through 4/2/2001). Available from *Congressional Universe* (Online service), Congressional Information Service, Bethesda, MD.

[50 CFR 17.3] United States Code of Federal Regulations. 2001b. Definition of "harm" (current through 4/2/2001). Available from *Congressional Universe* (Online service), Congressional Information Service, Bethesda, MD.

Cohan, F. M. and Graf, J. D. 1985. Latitudinal cline in *Drosophila melanogaster* for knockdown resistance to ethanol fumes and for rates of response to selection for further resistance. *Evolution* 39(2): 278-293.

Cohet, Y., Vouidibio, J. and David, J. R. 1980. Thermal Tolerance and Geographic-Distribution - a Comparison of Cosmopolitan and Tropical Endemic *Drosophila* Species. *Journal of Thermal Biology* 5(2): 69-74.

Cole, L. C. 1954. The population consequences of life history phenomena. *The Quarterly Review of Biology* 29: 103-137.

Conservation Council v. Babbitt. 1998. 2 F. Supp. 2d 1280, No. 97-00098 (U. S. District Court 1998).

Cornelius, G. 1996. Different stress factor signals converge at phosphoinositide turnover in *Drosophila* cells. *Journal of Thermal Biology* 21(2): 85-89.

Darin, T. F. 2000. Designating critical habitat under the Endangered Species Act: habitat protection versus agency discretion. *The Harvard Environmental Law Review* 24: 209-235.

David, J. R., Allemand, R., van Herrewege, J. and Cohet, Y. 1984. Ecophysiology: abiotic factors. The Genetics and Biology of *Drosophila*. M. Ashburner, H. L. Carson and J. N. Thompson, Jr. London, Academic Press. 3: 105-170.

David, J. R. and Clavel, M. F. 1967. Influence de la temperature d'elevage sur la mortalite larvo-nymphale et la duree de developpemente de la *Drosophile*. *Naturaliste Canadien* 94: 209-219.

David, J. R., Mercot, H., Capy, P., McEvey, S. F. and Vanherrewege, J. 1986. Alcohol tolerance and *Adh* gene-frequencies in European and African populations of *Drosophila melanogaster*. *Genetics Selection Evolution* 18(4): 405-416.

Davidson, J. and Andrewartha, H. G. 1948. The influence of rainfall, evaporation and atmospheric temperature on fluctuations in the size of a natural population of *Thrips imaginis* (Thysanoptera). *Journal of Animal Ecology* 17: 200-222.

Davis, A. J., Jenkinson, L. S., Lawton, J. H., Shorrocks, B. and Wood, S. 1998. Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391: 783-786.

Deltombe-Lietaert, M. C., Delcour, J. D., Lenelle-Monfort, N. and Elens, A. 1979. Ethanol metabolism in *Drosophila melanogaster*. *Experientia* 35: 579-580.

Dennis, B., Munholland, P. L. and Scott, J. M. 1991. Estimation of Growth and Extinction Parameters for Endangered Species. *Ecological Monographs* 61(2): 115-143.

Dobkin, D. S., Olivieri, I. and Ehrlich, P. R. 1987. Rainfall and the interaction of microclimate with larval resources in the population dynamics of checkerspot butterflies (*Euphydryas editha*) inhabiting serpentine grassland. *Oecologia* 71(2): 161-166.

Dytham, C., Shorrocks, B. and Cooper, R. 1997. Coexistence in large caged populations of *Drosophila* using a microcosm of a fruit market. *Drosophila Information Service* 71: 253.

Ehrlich, P. R. and Murphy, D. D. 1987. Conservation lessons from long-term studies of checkerspot butterflies. *Conservation Biology* 1: 122-131.

Endler, J. A. 1977. Geographic variation, speciation and clines. Princeton, Princeton University Press.

Fagan, W. F., Meir, E. and Moore, J. L. 1999. Variation thresholds for extinction and their implications for conservation strategies. *American Naturalist* 154(5): 510-520.

Feder, M. E. 1997. Necrotic fruit: A novel model system for thermal ecologists. *Journal of Thermal Biology* 22(1): 1-9.

Feder, M. E., Blair, N. and Figueras, H. 1997a. Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Functional Ecology* 11(1): 90-100.

Feder, M. E., Blair, N. and Figueras, H. 1997b. Oviposition site selection: Unresponsiveness of *Drosophila* to cues of potential thermal stress. *Animal Behaviour* 53: 585-588.

Feder, M. E., Cartano, N. V., Milos, L., Krebs, R. A. and Lindquist, S. L. 1996. Effect of engineering Hsp70 copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. *Journal of Experimental Biology* 199(8): 1837-1844.

Feder, M. E. and Hofmann, G. E. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243-282.

Feder, M. E., Karr, T. L., Yang, W., Hoekstra, J. M. and James, A. C. 1999. Interaction of *Drosophila* and its endosymbiont *Wolbachia*: Natural heat shock and the overcoming of sexual incompatibility. *American Zoologist* 39(2): 363-373.

Feder, M. E. and Krebs, R. A. 1998. Natural and genetic engineering of the heat-shock protein hsp70 in *Drosophila melanogaster*: Consequences for thermotolerance. *American Zoologist* 38(3): 503-517.

Fisher, R. A. 1958. The Genetical Theory of Natural Selection. Clarendon, England.

Fogleman, J. C. 1979. Oviposition site preference for substrate temperature in *Drosophila melanogaster. Behavior Genetics* 9: 407-412.

Geer, B. W., Heinstra, P. W. H. and McKechnie, S. W. 1993. The biological basis of ethanol tolerance in *Drosophila. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 105(2): 203-229.

Geer, B. W., Langevin, M. L. and McKechnie, S. W. 1985. Dietary ethanol and lipid synthesis in *Drosophila melanogaster. Biochemical Genetics* 23(7-8): 607-622.

Gibson, J. B., May, T. W. and Wilks, A. V. 1981. Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* in relation to environmental variation: Ethanol levels in breeding sites and allozyme frequencies. *Oecologia* 51(2): 191-198.

Gibson, J. B. and Wilks, A. V. 1988. The alcohol dehydrogenase polymorphism of *Drosophila melanogaster* in relation to environmental ethanol, ethanol tolerance and alcohol dehydrogenase activity. *Heredity* 60: 403-414.

Hageman, J., Eisses, K. T., Jacobs, P. J. M. and Scharloo, W. 1990. Ethanol in *Drosophila* cultures as a selective factor. *Evolution* 44(2): 447-454.

Hagstrum, D. W. and Milliken, G. A. 1991. Modeling differences in insect developmental times between constant and fluctuating temperatures. *Annals of the Entomological Society of America* 84(4): 369-379.

Hand, D. J. and Taylor, C. C. 1987. Multivariate Analysis of Variance and Repeated Measures. London, Chapman & Hall.

Hanski, I. 1999. Metapopulation Ecology. Oxford, Oxford University Press.

Harding, E. K., Crone, E. E., Elderd, B. D., Hoekstra, J. M., McKerrow, A. J., Perrine, J. D., Regetz, J., Rissler, L. J., Stanley, A. G., Walters, E. L. and Group, N. H. W. 2001. The scientific foundations of habitat conservation plans: A quantitative assessment. *Conservation Biology* 15: 488-500.

Heinstra, P. W. H., Scharloo, W. and Thorig, G. E. W. 1987. Physiological significance of the alcohol dehydrogenase polymorphism in larvae of *Drosophila. Genetics* 117(1): 75-84.

Hellmann, J. J., Weiss, S. B., McLaughlin, J. F., Boggs, C. L., Ehrlich, P. R., Launer, A. E. and D., M. in review. Death of a butterfly population: An overview of a long-term data set. *Oecologia.*

Hickey, D. A. and McLean, M. D. 1980. Selection for ethanol tolerance and *Adh* allozymes in natural populations of *Drosophila melanogaster. Genetical Research* 36(1): 11-15.

Hilborn, R. and Mangel, M. 1997. The Ecological Detective: Confronting Models with Data. Princeton, NJ, Princeton University Press.

Hoekstra, J. M., Clark, J. A., Fagan, W. F. and Boersma, P. D. in press. A comprehensive review of Endangered Species Act recovery plans. *Ecological Applications.*

Hoffmann, A. A. and Parsons, P. A. 1993a. Direct and correlated responses to selection for desiccation resistance: a comparison of *Drosophila melanogaster* and *D. simulans. Journal of Evolutionary Biology* 6(5): 643-657.

Hoffmann, A. A. and Parsons, P. A. 1993b. Selection for adult desiccation resistance in *Drosophila melanogaster*: Fitness components, larval resistance and stress correlations. *Biological Journal of the Linnean Society* 48(1): 43-54.

Huey, R. B. 1991. Physiological consequences of habitat selection. *American Naturalist* 137: S91-S115.

Huey, R. B. and Berrigan, D. 2001. Temperature, demography, and ectotherm fitness. *American Naturalist* 158(2): 204-210.

Huey, R. B. and Stevenson, R. D. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist* 19: 357-366.

Jones, J. S., Coyne, J. A. and Partridge, L. 1987. Estimation of thermal niche of *Drosophila melanogaster* using a temperature-sensitive mutation. *The American Naturalist* 130: 83-90.

Junge-Berberovic, R. 1996. Effect of thermal environment on life histories of free living *Drosophila melanogaster* and *D. subobscura*. *Oecologia* 108(2): 262-272.

Kareiva, P., Andelman, S., Doak, D., Elderd, B., Groom, M., Hoekstra, J., Hood, L., James, F., Lamoreux, J., LeBuhn, G., McCulloch, C., Regetz, J., Savage, L., Ruckelshaus, M., Skelly, D., Wilbur, H., Zamudio, K. and Group, N. H. W. 1998. Using science in habitat conservation plans. Washington, D. C., American Institute of Biological Sciences.

Kindvall, O. 1995. The impact of extreme weather on habitat preference and survival in a metapopulation of the bush-cricket *Metrioptera bicolor* in Sweden. *Biological Conservation* 73(1): 51-58.

Kindvall, O. 1996. Habitat heterogeneity and survival in a bush cricket metapopulation. *Ecology* 77(1): 207-214.

Kingsolver, J. G. 2000. Feeding, growth, and the thermal environment of cabbage white caterpillars, Pieris rapae L. *Physiological and Biochemical Zoology* 73(5): 621-628.

Kohane, M. J. and Parsons, P. A. 1986. Environment dependent fitness differences in *Drosophila melanogaster:* Temperature, domestication and the alcohol dehydrogenase locus. *Heredity* 57: 289-304.

Lande, R. and Orzack, S. H. 1988. Extinction dynamics of age-structured populations in a fluctuating environment. *Proceedings of the National Academy of Sciences of the United States of America* 85(19): 7418-7421.

Leigh, E. G. 1981. The average lifetime of a population in a varying environment. *Journal of Theoretical Biology* 90(2): 213-239.

Levins, R. 1968. Evolution in Changing Environments. Some Theoretical Explorations. Princeton, NJ, Princeton University Press.

Liu, S. S., Zhang, G. M. and Zhu, J. 1995. Influence of Temperature-Variations on Rate of Development in Insects - Analysis of Case-Studies from Entomological Literature. *Annals of the Entomological Society of America* 88(2): 107-119.

Loeb, J. and Northrop, J. H. 1917. On the influence of food and temperature upon the duration of life. *Journal of Biological Chemistry* 32: 103-121.

Louda, S. M. and Rodman, J. E. 1996. Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). *Journal of Ecology* 84: 229-237.

Ludwig, D. and Cable, R. M. 1933. The effect of alternating temperature on the pupal development of *Drosophila melanogaster* Meigen. *Physiological Zoology* 6: 493-508.

MacArthur, R. H. and Wilson, E. O. 1967. The Theory of Island Biogeography. Princeton, NJ, Princeton University Press.

Mangel, M. and Tier, C. 1993. Dynamics of metapopulations with demographic stochasticity and environmental catastrophes. *Theoretical Population Biology* 44(1): 1-31.

Mangel, M. and Tier, C. 1994. Four facts every conservation biologist should know about persistence. *Ecology* 75(3): 607-614.

McCarthy, J. J., Canziani, O. F., Leary, N. A., Dokken, D. J. and White, K. S., Eds. 2001. Climate Change 2001: Impacts, Adaptation & Vulnerability. Cambridge, UK, Cambridge University Press.

McCullugh, P. and Nelder, J. A. 1989. Generalized Linear Models. Boca Raton, FL, Chapman & Hall/CRC.

McDonald, J. 1998. Critical habitat designation under the Endangered Species Act: a road to recovery? *Environmental Law* 28: 671-700.

McKechnie, S. W. and Geer, B. W. 1993. Micro-evolution in a wine cellar population: an historical perspective. *Genetica* 90(2-3): 201-215.

McKechnie, S. W. and McKenzie, J. A. 1983. Polymorphism of alcohol dehydrogenase (*Adh*) in a winery cellar population of *Drosophila melanogaster:* Gene-frequency association with temperature and genotypic differences in progeny production. *Evolution* 37(4): 850-853.

McKenzie, J. A. 1975. The influence of low temperature on survival and reproduction in populations of *Drosophila melanogaster. Australian Journal of Zoology* 23: 237-247.

McKenzie, J. A. and McKechnie, S. W. 1978. Ethanol tolerance and the *Adh* polymorphism in a natural popuation of *Drosophila melanogaster. Nature* 272: 75-76.

McKenzie, J. A. and McKechnie, S. W. 1979. A comparative study of resource utilization in natural populations of *Drosophila melanogaster* and *D. simulans. Oecologia* 40: 299-309.

McLaughlin, J. F., Hellmann, J. J., Boggs, C. L. and Ehrlich, P. R. in review. The route to extinction: Population dynamics of a threatened butterfly. *Oecologia.*

Mercot, H., Defaye, D., Capy, P., Pla, E. and David, J. R. 1994. Alcohol tolerance, *Adh* activity, and ecological niche of *Drosophila* species. *Evolution* 48(3): 746-757.

Munks, R. J. L. and Turner, B. M. 1994. Suppression of heat-shock protein synthesis by short-chain fatty acids and alcohols. *Biochimica Et Biophysica Acta-Molecular Cell Research* 1223(1): 23-28.

[NCDC] National Climate Data Center. 2001. Daily surface data for Monroe 4 SW and Wenatchee Experimental Station weather stations.

Natural Resources Defense Council v. United States Department of the Interior. 1991. 113 F.3d 1121. (9th Cir. 1997).

Northern Spotted Owl v. Lujan. 1997. 758 F. Supp 621, No. C88-573Z. (U. S. District Court 1991).

Oakeshott, J. G., Gibson, J. B., Anderson, P. R., Knibb, W. R., Anderson, D. G. and Chambers, G. K. 1982. Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in *Drosophila melanogaster* on different continents. *Evolution* 36(1): 86-96.

Palabost, L. 1972. The influence of density on the larval viability of *Drosophila melanogaster. Drosophila Information Service* 49: 118.

Park, T. 1954. Experimental studies of interspecies competition. II. Temperature, humidity, and competition in two species of *Tribolium. Physiological Zoology* 27(3): 177-238.

Park, T. 1962. Beetles, competition, and populations. *Science* 138(3548): 1369-1375.

Parsell, D. A. and Lindquist, S. 1994. Heat shock proteins and stress tolerance. The Biology of Heat Shock Proteins and Molecular Chaperones. R. I. Morimoto, a. Tissieres and C. Georgopoulos. New York, Cold Springs Harbor Laboratory Press: 457-494.

Parsons, P. A. 1977. Larval reaction to alcohol as an indicator of resource utilization differences between *Drosophila melanogaster* and *D. simulans. Oecologia* 30: 141-146.

Parsons, P. A. and Spence, G. E. 1981. Longevity, resource utilization and larval Preferences in *Drosophila:* Interspecific and intraspecific variation. *Australian Journal of Zoology* 29(5): 671-678.

Parsons, P. A. and Stanley, S. M. 1981. Comparative effects of environmental ethanol on *Drosophila melanogaster* and *D. simulans* adults including geographic differences in *D. melanogaster*. Genetic studies of Drosphila populations. J. B. Gibson and J. G. Oakeshott. Canberra, Australian National University Press: 47-57.

Parsons, P. A., Stanley, S. M. and Spence, G. E. 1979. Environmental ethanol at low concentrations: longevity and development in the sibling species *Drosophila melanogaster* and *Drosophila simulans*. *Australian Journal of Zoology* 27(5): 747-754.

Patlis, J. M. 2001. Paying tribute to Joseph Heller with the Endangered Species Act: when critical habitat isn't. *Stanford Environmental Law Journal* 20: 133-217.

Philippi, T. and Seger, J. 1989. Hedging Ones Evolutionary Bets, Revisited. *Trends in Ecology & Evolution* 4(2): 41-44.

Possingham, H. P., Andelman, S. J., Noon, B. R., Trombulak, T. and Pulliam, H. R. 2001. Making smart conservation decisions. Conservation Biology: Research Priorities for the Next Decade. M. E. Soule and G. H. Orians. Washington, D. C., Island Press.

Powsner, L. 1935. The effects of temperature on the durations of the developmental stages of *Drosophila melanogaster*. *Physiological Zoology* 8(4): 474-520.

Sang, J. H. 1949. The ecological determinants of population growth in a *Drosophila* culture. III. Larval and pupal survival. *Physiological Zoology* 22(3): 183-202.

Scheiring, J. F., Davis, D. G., Ranasinghe, A. and Teare, C. A. 1984. Effects of larval crowding on life-history parameters in *Drosophila melanogaster* Meigen (Diptera, Drosophilidae). *Annals of the Entomological Society of America* 77(4): 329-332.

Shaffer, M. L. 1981. Minimum Population Sizes for Species Conservation. *Bioscience* 31(2): 131-134.

Siddiqui, W. H. and Barlow, C. A. 1972. Population growth of *Drosophila melanogaster* (Diptera: Drosophilidae) at constant and alternating temperatures. *Annals of the Entomological Society of America* 65(5): 993-1001.

Smith, S. E. 1999. How 'critical' is a critical habitat?: The United States Fish and Wildlife Service's duty under the Endangered Species Act. *Dickinson Journal of Environmental Law & Policy* 8: 343-381.

Sokal, R. R. and Rohlf, F. J. 1981. Biometry. New York, W. H. Freeman and Company.

Soule, M. 1987. Viable Populations for Conservation. New York, Cambridge University Press.

Stearns, S. C. and Koella, J. 1986. The evolution of phenotypic plasticity in life-history traits: predictions for norms of reaction for age- and size-at-maturity. *Evolution* 40: 893-913.

Tuljapurkar, S. D. and Orzack, S. H. 1980. Population-Dynamics in Variable Environments .1. Long-Run Growth-Rates and Extinction. *Theoretical Population Biology* 18(3): 314-342.

[USFWS] U. S. Fish and Wildlife Service. 1995. Draft recovery panning guidance. Washington, D. C., U. S. Fish and Wildlife Service.

[USFWS] U. S. Fish and Wildlife Service. 1999a. Final designation of critical habitat for the Rio Grande silvery minnow. *Federal Register* 64: 36274-36290.

[USFWS] U. S. Fish and Wildlife Service. 1999b. Notice of intent to clarify the role of habitat in endangered species conservation. *Federal Register* 64: 31871-31874.

[USFWS] U. S. Fish and Wildlife Service. 1999c. Report to Congress, Endangered and Threatened Species Recovery Program, 1996. Washington, D. C., U. S. Fish and Wildlife Service.

[USFWS] U. S. Fish and Wildlife Service. 2000. Flood of court orders preclude new listing of threatened and endangered species in FY2001. News release, November 22, 2000. 2001.

[USFWS] U. S. Fish and Wildlife Service. 2001a. Threatened and Endangered Species System (TESS): Listed species summary (April 30, 2001).

[USFWS] U. S. Fish and Wildlife Service. 2001b. Threatened and Endangered Species System (TESS): Species with critical habitat (April 13, 2001).

VanDelden, W. 1982. The alcohol dehydrogenase polymorphism in *Drosophila melanogaster*: Selection at an enzyme locus. *Evolutionary Biology* 15: 187-222.

VanDelden, W., Boerema, A. C. and Kamping, A. 1978. The alcohol dehydrogenase polymorphism in populations of *Drosophila melanogaster*. 1. Selection in different environments. *Genetics* 90: 161-191.

Via, S. and Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39: 505-522.

[WSU-PAWS] Washington State University Public Agricultural Weather System. 2001. Daily weather observations for WSU TFREC weather station.

Weiss, S. B., White, R. R., Murphy, D. D. and Ehrlich, P. R. 1987. Growth and dispersal of larvae of the checkerspot butterfly *Euphydryas editha*. *Oikos* 50(2): 161-166.

Worner, S. P. 1992. Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environmental Entomology* 21: 689-699.

VITA

Jonathan M. Hoekstra is the eldest of four sons born to Donald J. Hoekstra and Barbara J. Armstrong. While growing up, Jonathan spent many days roaming a local Nature Center and watching the woods, ponds and critters change with the seasons and decline as the surrounding neighborhoods encroached. He even ventured into environmental activism when some nearby woods were cleared for a new junior high school – he left notes telling the construction crews to stop. After graduating as a valedictorian from Holland High School in Holland, Michigan, Jonathan attended Stanford University in Palo Alto, California where he earned B. S. With Distinction and M.S. degrees in Biological Sciences. After toiling in the Business School library, slinging hash in the kitchens, and managing the Sunday Flicks, Jonathan landed an undergraduate research assistant job with the Center for Conservation Biology where his research interests in insect ecology and conservation biology developed. He also worked as a teaching assistant and won awards for Excellence in Teaching and Exceptional Contributions in Teaching. Following graduation, Jonathan spent a year as an endangered species biologist for the U.S. Fish and Wildlife Service in Ventura, California before moving to Seattle to study with Joel Kingsolver at the University of Washington. In addition to his dissertation research, Jonathan collaborated with several colleagues at the University of Washington and elsewhere on a diversity of projects ranging from the evolution of a novel sex determination in field mice, to conservation of endangered grasshoppers, a synthesis on the strength of natural selection in the wild, and analyses of

how science is used in implementing the Endangered Species Act. Other publications include:

Hoekstra, J. M., Clark, J. A., Fagan, W. F. and Boersma, P. D. In press. A comprehensive review of Endangered Species Act recovery plans. *Ecological Applications.*

Hoekstra, J. M., Fagan, W. F. and Bradley, J. E. In press. A critical role for critical habitat in the recovery planning process? Not yet. *Ecological Applications.*

Harvey, E., **Hoekstra, J. M.**, O'Connor, R. J. and Fagan, W. F. In press. Recovery plan revisions: progress or just process? *Ecological Applications.*

Boersma, P. D., Kareiva, P., Fagan, W. F., Clark, J. A. and **Hoekstra, J. M.** In press. How good are endangered species recovery plans? *BioScience.*

Hoekstra, H., **Hoekstra, J. M.**, Berrigan, D., Vignieri, S., Hill, C., Hoang, A., Gibert, P., Beerli, P. and Kingsolver, J. G. 2001. Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Sciences* 98:9157-9160.

Hoekstra, H. and **Hoekstra, J. M.** 2001. The evolution of novel genotypes: the role of mutation, selection and meiotic drive in maintaining XY females. *Evolution* 55:190-197.

Kingsolver, J. G., Hoekstra, H., **Hoekstra, J. M.**, Berrigan, D., Vignieri, S., Hill, C., Hoang, A., Gibert, P. and Beerli, P. 2001. The strength of phenotypic selection in natural populations. *The American Naturalist* 157:245-261.

Feder, M. E. Karr, T. L., Yang, W., **Hoekstra, J. M.** and James, A. C. 1999. Interaction of *Drosophila* and its endosymbiont *Wolbachia*: natural heat shock and the overcoming of sexual incompatibility. *American Zoologist* 39:363-373.

Hoekstra, J. M. 1998. Conserving Orthoptera in the wild: lessons from *Trimerotropis infantilis*. *Journal of Insect Conservation* 2:179-185.