8-2024

From Inbreeding to Admixture: How the Diverse Consequences of Gene Flow Shape Evolutionary Dynamics and Population Viability

Amy L. Springer
Utah State University

FROM INBREEDING TO ADMIXTURE: HOW THE DIVERSE CONSEQUENCES OF GENE FLOW SHAPE EVOLUTIONARY DYNAMICS AND POPULATION VIABILITY

by

Amy L. Springer

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

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

Zachariah Gompert, Ph.D. Major Professor	Carol von Dohlen, Ph.D. Committee Member
Luis Gordillo, Ph.D. Committee Member	Paul Wolf, Ph.D. Committee Member
Karen Mock, Ph.D. Committee Member	D. Richard Cutler, Ph.D. Vice Provost of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2024

ABSTRACT

From Inbreeding to Admixture: how the Diverse Consequences of Gene Flow Shape Evolutionary Dynamics and Population Viability

by

Amy L. Springer, Doctor of Philosophy

Utah State University, 2024

Major Professor: Zachariah Gompert, Ph.D.
Department: Biology

Gene flow is the evolutionary process whereby genetic material from one population is spread to another. Gene flow—or the absence thereof—can critically affect the evolutionary trajectory of a population. While a severe lack of gene flow can increase extinction risk via the deleterious effects of inbreeding, gene flow across divergent populations (i.e. admixture) can have diverse consequences spanning the spectrum from outbreeding depression to evolutionary rescue. Given the wide variety of potential consequences, the study of gene flow remains central to advancing our understanding of evolutionary dynamics and conservation. But gaps remain in our understanding of how patterns of gene flow alter the evolutionary dynamics of r-selected, highly-fecund organisms like insects. To help address these knowledge gaps, in this dissertation I investigated how ecological factors, population dynamics, and gene flow jointly affect evolutionary outcomes and conservation risk in insects. Specifically, I showed that (1) the combination of heat and host stress interact non-additively to increase the severity of inbreeding depression in seed beetles (*Callosobruchus maculatus*), (2) both isolation by distance and isolation by resistance have shaped patterns of genetic structure diversity in the endemic Hayden's ringlet butterfly (*Coenonympha haydenii*), and (3) admixture among divergent populations of seed beetles both facilitates adaptation to

a novel, stressful food source and alters the predictability of evolutionary change during evolutionary rescue.

(183 pages)

PUBLIC ABSTRACT

From Inbreeding to Admixture: how the Diverse Consequences of Gene Flow Shape Evolutionary Dynamics and Population Viability

Amy L. Springer

When *Homo sapiens* traveled out of Africa and interbred with the Neanderthals of Europe, human genes spread into the Neanderthal population. Likewise, genes from Neanderthals spread into the human genome—genes humans still carry to this day. The process whereby genetic material from one population is spread to another is known as gene flow. Gene flow—or the absence thereof—can have critical consequences for a population's health and survival. On one hand, if no gene flow occurs, mating among close relatives can lead to high levels of inbreeding. Inbreeding can have devastating consequences for the health of populations, in some cases even leading to extinction. On the other extreme, gene flow between distantly-related populations or species can result in hybridization, also known as admixture. The evolutionary consequences of admixture vary widely. While some admixture events have negative consequences and result in an evolutionary dead end (i.e. crossing a horse and a donkey to produce a sterile mule), other admixture events improve population health and survival (i.e. when mixed-breed dogs show greater genetic health than their purebred parents, an outcome colloquially known as "hybrid vigor"). Understanding the varied effects of gene flow is critical to understanding evolution: patterns of gene flow can affect a population's extinction risk, determine whether or not a population will successfully adapt to a changing environment, and can even alter how well we can predict evolutionary change. In this dissertation, I explored how inbreeding, environmental stress, barriers to gene flow, and admixture affect evolutionary outcomes and conservation risk in insects. Specifically, I showed that (1) the combination of heat stress and a poor food source interact non-additively to increase the severity of inbreeding depression in seed beetles (*Callosobruchus maculatus*), (2) barriers to gene flow (i.e. rivers, mountains) have

shaped patterns of genetic structure and genetic health in the endemic Hayden's ringlet butterfly (*Coenonympha haydenii*), and (3) admixture among populations of seed beetles alters both their ability to survive and adapt to a novel, poor food source and our ability to predict evolutionary change at a genomic level.

to my parents, Jeff and Diana Springer

who gave me my first genetics book,
tolerated all the dead insects I brought home,
and who always believed I could.

-and-

to my grandfather, Eugene Springer

who always found time to draw with me,
and whose fascination with fancy pigeons rivaled
even that of Charles Darwin's

ACKNOWLEDGMENTS

First, I would like to thank my advisor, Zach Gompert, and all my committee members. Zach, you have the patience of a saint. Thank you for helping me to be a clearer thinker, a better writer, and a more rigorous scientist. I'm sure everyone already knows what a cheerful, brilliant, and generous person you are, so I will not further elaborate on those qualities here. Instead, I will remember the terrifying dark and ominous music you played, how you got me banned from a Wyoming gas station for buying too many soda cups, and how you cackled mercilessly at me when I came to your office drenched in water after the eyewash station broke and flooded the lab and everyone nearby. Thanks for that. To the rest of my committee members—Paul Wolf, Luis Gordillo, Carol von Dohlen, Karen Mock, and Frank Messina—thank you for your guidance and support over the years. Luis, your cheerfulness and enthusiastic welcome always made my day better. I also want to give special thanks to the late Frank Messina, who always made time to stop by and chat or offer his wisdom and expertise, and whose conversation and good humor will be greatly missed. Frank, I will fondly remember the quizzical skepticism you expressed in response to my every experimental design. I know you weren't sure if any of my beetle experiments would work... but they did.

I would also like to thank my fellow lab members who went to great measures to drag me out the door and make sure I was included in whatever chaos they decided to pursue that day. If not for you, I might never have ventured out of my apartment. Thank you for accepting me into your world and being my friends. Sam, you are such a brilliant scientist and loyal friend. I will never forget being your camping "spouse" during that first cold summer of fieldwork in the Grand Tetons, the many hours we spend slumped on a couch watching episodes of Friends instead of doing our work, and all the delicious food you made for us. Mal, I don't know how I would have survived without you (and Erica!). You are one of the most kind and resilient human beings I have ever met. Thank you for being my

roommate and one of my dearest friends. Tara, your effervescence, generosity, and cheerful company made everyone's lives brighter and work easier.

To all the students and technicians who helped me through my experiments—thank you. There is no way I could have completed my experiments without you. Daniel, Cam, and Angélica, thank you for your dedication, patience, and many hours of careful assistance. Megan, thank you for being my field partner, bunk mate, and friend, and for many hours of audio books and sorely-needed company during so many long days. I promise I will finish your painting someday when life is less busy!

Finally, thank you to all my friends, family and mentors back home who were there for me through this journey. In particular, I want to thank my dad, who has proofread and commented on every scientific paper I've ever written, and my mom, who took care of all my creatures during my absence and has been so excited to be able to call me Dr. Springer one day. To my first mentor, Brian—I would never have pursued research without you, nor would I have finished this PhD without you. Your mentorship has meant the world to me. Dr. Zani—you are, and have always been, a spark of light and a breath of fresh air in a confusing world. Thank you for ever challenging me to do more; to be more. Thank you as well to my best friends Amanda and Paige, who supported me in every possible way and spent an immeasurable number of hours on Skype with me, and to my spouse, Vadym, who followed me across the country in pursuit of my career. Vadik, your loyalty, patience, forgiveness, and dedication have been unwavering. I don't know how you put up with me all these years. Finally, to all my family in Australia who cheered me on from the start and stayed up into the small hours of the morning to watch my defense, your support has meant so much to me. These have been busy years, but I always thought of you even when I couldn't find time to write. With this PhD business finally over, I hope I might visit the great southern land again soon!

Amy L. Springer

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Gene flow is a fundamental evolutionary process, and the presence of gene flow—or lack thereof—can have a profound impact on the evolutionary trajectory of a population. A severe lack of gene flow can result in inbreeding depression, whereby mating with close relatives causes a decrease in fitness, which over time can increase a population's risk of extinction [Frankham, 1995, O'Grady et al., 2006]. Conversely, gene flow between different species or highly divergent populations can lead to outbreeding depression, whereby hybrid incompatibilities or the breakdown of adaptive gene complexes decreases the fitness of admixed individuals [Bhargav et al., 2022, Calvo-Baltanás et al., 2021, Kim et al., 2018, Turissini and Matute, 2017, Verhoeven et al., 2011]. Given the vast variety of evolutionary outcomes that can arise from different patterns of gene flow, the study of this process remains central to furthering our understanding of evolutionary dynamics and conservation.

Insects are one of the most abundant and diverse groups of organisms on earth, and they provide a vast array of vital ecosystem services [Cardoso et al., 2011, Hallmann et al., 2017]. However, insects are declining at troubling rates across the globe [Forister et al., 2011, Hallmann et al., 2017]. In light of this fact, there is a critical need to advance understanding of how environmental change will affect insects, and how gene flow might alter evolutionary dynamics in these species. Unfortunately, despite their crucial role in ecosystems, insects are largely ignored in conservation policy, and conservation efforts remain underfunded [Cardoso et al., 2011]. Furthermore, while conservation genetic strategies are well-developed for terrestrial vertebrate species, gaps remain in our understanding of how processes like inbreeding will alter the evolutionary dynamics of rapidly-reproducing, high-fecundity organisms like insects. Indeed, evidence suggests that evolution can be extremely rapid in highly fecund species with short generation times, a fact that could significantly alter conservation outcomes for these species [Christie et al., 2012].

To help address these gaps in our knowledge, in this dissertation I investigate how ecological factors and population dynamics, especially small population size and hybridization, jointly influence population viability in insects. In particular, I consider the variable impacts of gene flow on population viability in two insect species: seed beetles (*Callosobruchus maculatus*), and the Hayden's ringlet butterfly (*Coenonympha haydenii*).

In my second chapter, I investigate how environmental stress impacts the degree of inbreeding depression (i.e. a decline in fitness caused by inbreeding, or mating among close relatives due to a severe lack of gene flow) in seed beetles, *C. maculatus*. Environmental stress can have a profound effect on the magnitude of inbreeding depression a population experiences [Armbruster and Reed, 2005, Fox and Reed, 2011, Joubert and Bijlsma, 2010, Liao and Reed, 2009]. Quantifying this effect is crucial to better understand the viability of threatened populations, which are often simultaneously subject to both inbreeding and environmental stress. But the degree to which inbreeding-stress interactions vary across populations within a species or between closely related species remains relatively understudied [Fox et al., 2007]. In this chapter, I assess the magnitude of inbreeding-stress interactions in three populations of seed beetles, *Callosobruchus maculatus*. In particular, I asked 1) to what degree do environmental stressors—and interactions among environmental stressors—affect the magnitude of inbreeding depression?, 2) to what degree do individual stressors (inbreeding and environmental stress) vs. interactions among those stressors impact overall fitness? and 3) To what degree do these effects vary by lineage?

In my third chapter, I address the impact of barriers to gene flow on patterns of genetic structure in the endemic Hayden's ringlet butterfly, *Coenonympha haydenii*. Genetic structure, or the organization of genetic diversity across geographic space, is a key evolutionary pattern. The study of genetic structure can reveal migratory routes [e.g., Gompert et al., 2021, Hemstrom et al., 2022], ecological specialization [e.g., Chaturvedi et al., 2018, Ferrari et al., 2012, Michell et al., 2023, Nosil et al., 2008], or even the genesis of speciation [Avise et al., 2000, Harvey et al., 2017, Mayr, 1942], all of which are critical for understanding the evolutionary viability of a population. The development of genetic structure is driven by a

combination of genetic drift, gene flow, and natural selection [Wright, 1931], but how severe range restriction (i.e. endemism) impacts the processes that dominate the development of genetic structure remains unclear. In this study, I explored patterns of genetic diversity and structure in the Hayden's ringlet butterfly, a species found only in the greater Yellowstone area. Specifically, I asked to what degree genetic structure in the Hayden's ringlet can be explained by barriers to gene flow related to (a) geographic distance and genetic drift alone (isolation by distance), (b) geographic or ecological barriers to gene flow (isolation by environment), and/or (c) local adaptation to larval host plants among sites (isolation by adaptation).

In my fourth chapter, I address to what degree admixture (i.e. gene flow between genetically divergent lineages) facilitates adaptation to a novel, stressful environment in cowpea seed beetles, *Callosobruchus maculatus*. I further address how admixture combined with environmental stress jointly impact the predictability of evolutionary change. Admixture is common in nature [Mallet, 2005], and can be a vital source of adaptive potential [Lewontin and Birch, 1966, Pereira et al., 2014, Rieseberg et al., 1999]. Admixture can generate extreme phenotypes (transgressive segregation), transfer globally beneficial alleles (i.e. adaptive introgression) and reverse inbreeding depression (masking of the genetic load), all of which could increase the adaptive potential of hybrids in novel or marginal environments [Buerkle et al., 2000, Crow, 1948, De Carvalho et al., 2010, Durkee et al., 2023, Gompert et al., 2006, Oziolor et al., 2019]. Conversely, hybrid incompatibilities could decrease the adaptive potential of hybrids [Bhargav et al., 2022, Calvo-Baltanás et al., 2021, Kim et al., 2018, Turissini and Matute, 2017, Verhoeven et al., 2011]. In this experiment, I assessed the impact of admixture on patterns of evolutionary rescue and parallelism in cowpea seed beetles (*Callosobruchus maculatus*) during adaptation to lentil, a novel, stressful host plant. Specifically, I asked (1) if admixture facilitates adaptation to lentil, (2) if parallelism was higher in admixed or non-admixed lineages, and (3) to what degree parallelism in admixed lineages was associated with selection on globally adaptive alleles, epistatic effects, and/or hybrid incompatibilities.

Finally, in my fifth chapter I summarize and the results of my three research projects (found in chapters 2, 3, and 4), and address the overall impact of my findings.

REFERENCES

P Armbruster and DH Reed. Inbreeding depression in benign and stressful environments. *Heredity*, 95(3):235, 2005.

John C Avise et al. *Phylogeography: the History and Formation of Species*. Harvard university press, 2000.

V Vikram Bhargav, Joanna R Freeland, and Marcel E Dorken. Evidence of hybrid breakdown among invasive hybrid cattails (typha× glauca). *Heredity*, 129(3):195–201, 2022.

C Alex Buerkle, Robert J Morris, Marjorie A Asmussen, and Loren H Rieseberg. The likelihood of homoploid hybrid speciation. *Heredity*, 84(4):441–451, 2000.

Vanesa Calvo-Baltanás, Jinge Wang, and Eunyoung Chae. Hybrid incompatibility of the plant immune system: an opposite force to heterosis equilibrating hybrid performances. *Frontiers in Plant Science*, 11:576796, 2021.

Pedro Cardoso, Terry L Erwin, Paulo AV Borges, and Tim R New. The seven impediments in invertebrate conservation and how to overcome them. *Biological conservation*, 144(11): 2647–2655, 2011.

Samridhi Chaturvedi, Lauren K Lucas, Chris C Nice, James A Fordyce, Matthew L Forister, and Zachariah Gompert. The predictability of genomic changes underlying a recent host shift in Melissa blue butterflies. *Molecular Ecology*, 27(12):2651–2666, 2018.

Mark R Christie, Melanie L Marine, Rod A French, and Michael S Blouin. Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences*, 109(1):238–242, 2012.

James F Crow. Alternative hypotheses of hybrid vigor. *Genetics*, 33(5):477, 1948.

Dulcineia De Carvalho, Pär K Ingvarsson, Jeffrey Joseph, Leonie Suter, Claudio Sedivy, DAVID MACAYA-SANZ, Joan Cottrell, Berthold Heinze, Ivan Schanzer, and Christian

Lexer. Admixture facilitates adaptation from standing variation in the european aspen (populus tremula l.), a widespread forest tree. *Molecular Ecology*, 19(8):1638–1650, 2010.

Lily F Durkee, Laure Olazcuaga, Rachael Szymanski, Brett A Melbourne, and Ruth A Hufbauer. Genetic mixing facilitates adaptation to a novel environmental constraint. *Ecological Entomology*, 2023.

Julia Ferrari, Joan A West, Sara Via, and H Charles J Godfray. Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. *Evolution*, 66(2):375–390, 2012.

Matthew L Forister, Joshua P Jahner, Kayce L Casner, Joseph S Wilson, and Arthur M Shapiro. The race is not to the swift: Long-term data reveal pervasive declines in california's low-elevation butterfly fauna. *Ecology*, 92(12):2222–2235, 2011.

Charles W Fox and David H Reed. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution*, 65(1):246–258, 2011.

CW Fox, KL Scheibly, BP Smith, and WG Wallin. Inbreeding depression in two seed-feeding beetles, *Callosobruchus maculatus* and *Stator limbatus* (Coleoptera: Chrysomelidae). *Bulletin of Entomological Research*, 97(1):49–54, 2007.

Richard Frankham. Inbreeding and extinction: a threshold effect. *Conservation Biology*, 9 (4):792–799, 1995.

Zachariah Gompert, James A Fordyce, Matthew L Forister, Arthur M Shapiro, and Chris C Nice. Homoploid hybrid speciation in an extreme habitat. *Science*, 314(5807):1923–1925, 2006.

Zachariah Gompert, Amy Springer, Megan Brady, Samridhi Chaturvedi, and Lauren K Lucas. Genomic time-series data show that gene flow maintains high genetic diversity despite substantial genetic drift in a butterfly species. *Molecular Ecology*, 30(20):4991–5008, 2021.

Caspar A Hallmann, Martin Sorg, Eelke Jongejans, Henk Siepel, Nick Hofland, Heinz Schwan, Werner Stenmans, Andreas Müller, Hubert Sumser, Thomas Hörren, et al. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS one*, 12(10):e0185809, 2017.

Michael G Harvey, Glenn F Seeholzer, Brian Tilston Smith, Daniel L Rabosky, Andrés M Cuervo, and Robb T Brumfield. Positive association between population genetic differentiation and speciation rates in new world birds. *Proceedings of the National Academy of Sciences*, 114(24):6328–6333, 2017.

William B Hemstrom, Micah G Freedman, Myron P Zalucki, Santiago R Ramírez, and Michael R Miller. Population genetics of a recent range expansion and subsequent loss of migration in monarch butterflies. *Molecular Ecology*, 31(17):4544–4557, 2022.

D Joubert and R Bijlsma. Interplay between habitat fragmentation and climate change: inbreeding affects the response to thermal stress in *Drosophila melanogaster*. *Climate Research*, 43(1/2):57–70, 2010.

Bernard Y Kim, Christian D Huber, and Kirk E Lohmueller. Deleterious variation shapes the genomic landscape of introgression. *PLoS Genetics*, 14(10):e1007741, 2018.

Richard C Lewontin and LC Birch. Hybridization as a source of variation for adaptation to new environments. *Evolution*, pages 315–336, 1966.

W Liao and DH Reed. Inbreeding–environment interactions increase extinction risk. *Animal Conservation*, 12(1):54–61, 2009.

James Mallet. Hybridization as an invasion of the genome. *Trends in ecology & evolution*, 20(5):229–237, 2005.

Ernst Mayr. *Systematics and the origin of species, from the viewpoint of a zoologist*. Harvard University Press, 1942.

Craig T Michell, Natascha Wagner, Marko Mutanen, Kyung Min Lee, and Tommi Nyman. Genomic evidence for contrasting patterns of host-associated genetic differentiation across shared host-plant species in leaf-and bud-galling sawflies. *Molecular Ecology*, 2023.

Patrik Nosil, Scott P Egan, and Daniel J Funk. Heterogeneous genomic differentiation between walking-stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. *Evolution*, 62(2):316–336, 2008.

Elias M Oziolor, Noah M Reid, Sivan Yair, Kristin M Lee, Sarah Guberman VerPloeg, Peter C Bruns, Joseph R Shaw, Andrew Whitehead, and Cole W Matson. Adaptive introgression enables evolutionary rescue from extreme environmental pollution. *Science*, 364(6439):455–457, 2019.

Julian J O'Grady, Barry W Brook, David H Reed, Jonathan D Ballou, David W Tonkyn, and Richard Frankham. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological conservation*, 133(1):42–51, 2006.

Ricardo J Pereira, Felipe S Barreto, and Ronald S Burton. Ecological novelty by hybridization: experimental evidence for increased thermal tolerance by transgressive segregation in tigriopus californicus. *Evolution*, 68(1):204–215, 2014.

Loren H Rieseberg, Margaret A Archer, and Robert K Wayne. Transgressive segregation, adaptation and speciation. *Heredity*, 83(4):363–372, 1999.

David A Turissini and Daniel R Matute. Fine scale mapping of genomic introgressions within the drosophila yakuba clade. *PLoS genetics*, 13(9):e1006971, 2017.

Koen JF Verhoeven, Mirka Macel, Lorne M Wolfe, and Arjen Biere. Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society B: Biological Sciences*, 278(1702):2–8, 2011.

Sewall Wright. Evolution in Mendelian populations. *Genetics*, 16(2):97, 1931.

CHAPTER 2

MEASURING THE EFFECT OF ENVIRONMENTAL STRESS ON INBREEDING DEPRESSION ALONE OBSCURES THE RELATIVE IMPORTANCE OF INBREEDING - STRESS INTERACTIONS ON OVERALL FITNESS IN *CALLOSOBRUCHUS MACULATUS*

Abstract

Environmental stress can have a profound effect on inbreeding depression. Quantifying this effect is of particular importance in threatened populations, which are often simultaneously subject to both inbreeding and environmental stress. But while the prevalence of inbreeding-stress interactions is well known, the importance and broader applicability of such interactions in conservation are not clearly understood. We used seed beetles, *Callosobruchus maculatus*, as a model system to quantify how environmental stressors (here host quality and temperature stress) interact with inbreeding as measured by changes in the magnitude of inbreeding depression, δ, as well as the relative importance of inbreeding-stress interactions to overall fitness. We found that while both environmental stressors caused substantial inbreeding-stress interactions as measured by change in δ, the relative importance of these interactions to overall survival was modest. This suggests that assessing inbreeding-stress interactions within the framework of δ alone may give an inaccurate representation of the relevance of interactions to population persistence. Furthermore, we found that the effect of environmental stress on fitness, but not inbreeding depression, varied strongly among populations. These results suggest that the outcomes of inbreeding-stress interactions are not easily generalized, an important consideration in conservation settings.

Keywords: inbreeding depression, environmental stress, *Callosobruchus maculatus*, hierarchical Bayesian models, population management

Introduction

It is now widely recognized that environmental stress can have a profound effect on the magnitude of inbreeding depression that a population experiences [Armbruster and Reed, 2005, Fox and Reed, 2011, Joubert and Bijlsma, 2010, Liao and Reed, 2009]. But the relationship between environmental stress and inbreeding depression, while generally positive, has also been found to vary widely across systems [Armbruster and Reed, 2005]. Many systems show a synergistic interaction between inbreeding depression and environmental stress, while others show no relationship, and still others suggest that environmental stress can actually decrease the magnitude of inbreeding depression [e.g., Armbruster and Reed, 2005, Dahlgaard and Hoffmann, 2000, Fox and Reed, 2011, Miller, 1994]. For example, environmental stress appeared to decrease the magnitude of inbreeding depression in bladder campion plants and certain root parasites [Sandner and Matthies, 2016a,b]. This high degree of variation in the response to inbreeding-stress combinations suggests that inbreeding-environment interactions may not be easily generalizable across populations or species. Variable outcomes might be explained by differences in population history (e.g., purging history), or other causes [Hedrick and Garcia-Dorado, 2016, Sandner and Matthies, 2016a]. Knowing whether information regarding the effect of inbreeding-environment interactions in one threatened population can be dependably applied to other populations is of critical importance in the application of conservation policy. But the degree to which inbreeding depression varies across populations within a species or between closely related species remains relatively understudied [Fox et al., 2007].

Whereas inbreeding-stress interactions have been well-studied within the framework of changes in inbreeding depression (i.e. studies assessing changes in the magnitude of inbreeding depression under benign vs. stressful conditions, see, e.g. Armbruster and Reed 2005, Fox and Reed 2011), studies comparing the relative importance of inbreeding-stress interactions vs. additive effects on a population's overall fitness are less common. For threatened and endangered populations, which are likely to experience both inbreeding depression and environmental stress simultaneously, understanding the relative impact of inbreeding-stress

interactions vs. additive effects is of particular importance. If inbreeding-stress interactions have a far greater impact on fitness than the additive effects of individual stressors, failing to account for the interaction term could cause conservationists to underestimate a population's risk of extinction. Indeed, simulations conducted by Liao and Reed [2009] suggest that synergistic interactions between inbreeding and environmental stress could decrease the time for a population to go extinct by as much as 28.5%. In contrast, if the interaction term is negligible as compared to the additive effects of inbreeding or environmental stress, spending time and resources trying to minimize inbreeding-stress interactions could lead to a costly misallocation of conservation efforts. As such, understanding how inbreeding, environmental stressors, and their interactions compare in their relative effects on fitness—as well as how much this varies among populations or species—is crucial for conservation efforts [Armbruster and Reed, 2005, Kristensen et al., 2008, 2003, Pray et al., 1994, Reed et al., 2002, 2012].

In this study, we used the cowpea seed beetle, *Callosobruchus maculatus*, as a model organism to assess the effect of two environmental stressors on both the magnitude of inbreeding depression and overall fitness across lineages. *Callosobruchus maculatus* is a widspread pest of stored legumes. Originally a pest of cowpea *(Vigna unguiculata)* in sub-Saharan Africa, *C. maculatus* is now found in warm climates across the globe where it feeds on various species of grain legumes, especially from the tribe Phaseoleae [e.g., mung bean, adzuki bean, and cowpea; Kébé et al., 2017, Tuda et al., 2006]. Adults lay eggs the on surface of host seeds, and upon hatching, larvae burrow into a single seed where they remain for the entirety of their development. Under standard laboratory conditions emerging adults do not feed, meaning beetles obtain all resources from a single seed. Thus, the "natural" habitat of *C. maculatus* populations infesting legume crop stores can be easily and precisely replicated under laboratory conditions [Messina, 1991, Tuda et al., 2014]. Because larvae spend their entire life inside a single seed, host quality and temperature are the two primary environmental variables juvenile beetles experience. These life history characteristics make *C. maculatus* ideal for realistically manipulating ecological conditions.

Fox and Reed [2011] examined the degree to which inbreeding depression increases along two axes of stress (temperature and host) and across two populations of cowpea beetle (South India, SI, and Burkina Faso, BF). They found that inbreeding depression (as measured by haploid lethal equivalents, HLE) increased in a roughly linear fashion with the magnitude of environmental stress, but the overall effect of environmental stress on the magnitude of inbreeding depression varied across populations of *C. maculatus*. Specifically, they found that inbreeding depression in the BF lineage was less sensitive to changes in temperature stress than it was the SI lineage. We expand here on the work of Fox and Reed [2011] by using an additional cowpea beetle lineage to further our understanding of the generalizability of inbreeding-stress interactions. We also use a more stressful host species, green pea (average survival from hatched egg to adulthood on green pea, *Pisum sativum*, is around 30-50 percentage points lower than survival on either cowpea, *Vigna unguiculata*, or mung bean, *Vigna radiata*; see Messina et al., 2018), to more clearly assess two-way vs. three-way interactions among stressors. Finally, we contrast the effect of inbreeding-stress interactions as measured by inbreeding depression with the effect of inbreeding-stress interactions on overall fitness. This dual perspective allows us to more clearly assess the conservation implications of interactions between environmental stress and inbreeding. In particular, we addressed the following questions: 1) to what degree do environmental stressors—and interactions among environmental stressors—affect the magnitude of inbreeding depression?, 2) to what degree do individual stressors (inbreeding and environmental stress) vs. interactions among those stressors impact overall fitness? and 3) To what degree do these effects vary by lineage?

Materials and Methods

Experimental Design

We used three lineages of *Callosobruchus maculatus* for this experiment. The South India (SI) lineage was collected from mung bean *(Vigna radiata)* in Tirunelveli, India in 1979 [Messina, 1991, Mitchell, 1991], and has been maintained in captivity for in excess

of 450 generations assuming an average generation time of 30 days. Two cowpea-adapted lineages were obtained from Dr. Charles Fox at the University of Kentucky [Messina et al., 2018]. Each was originally collected from infested cowpeas and have been maintained in the laboratory on this host continuously since their intial collection. The Burkina Faso (BF) lineage was collected from cowpea *(V. unguiculata)* pods in a field in Ouagadougou, Burkina Faso in 1989 [Messina, 1993], and is estimated to have been maintained in captivity for in excess of 325 generations. The North American lineage was collected from California (CA) and was originally maintained by Dr. Peter Credland at the University of London [Tuda et al., 2014]. The CA lineage is estimated to have been maintained in laboratory culture for at least 100 generations. After all three lineages were obtained, cultures were maintained at Utah State University at 25°C in 2 L jars containing 750 g beans for approximately 75 generations (BF and CA) or >100 generations (SI). New generations were founded approximately every 25 days by transferring ∼2000 (estimated by volume) newly-emerged beetles to new 2 L culture jars. Recent genomic analyses have shown that the mung-adapted lineage (SI) used in this experiment has a variance effective population size of (N_e) = 1149.6 individuals (95% CI = 1077.4–1229.8), indicating that significant bottlenecks or purging are unlikely to have occurred in the recent demographic history of this lineage [Gompert and Messina, 2016]. The effective population sizes of CA and BF are expected to be similar or higher than that of SI given their extensive shared culturing history.

We used a full-factorial experimental design to assess the effect of two external stressors (host and temperature) and one internal stressor (inbreeding) on two fitness components (adult female weight and survival) across three populations of *Callosobruchus maculatus* (SI, BF and CA) (Fig. 2.1). This design gives us eight distinct treatment groups: one control treatment, three single-stress treatments, three double-stress treatments, and one triple-stress treatment. These eight combinations of stressors allow us to consider the importance of both two-way and three-way non-additive effects of stressors on overall fitness.

To equalize the genetic contribution of all families to each treatment group and thereby control for family effects, we used the block design developed by Roff [1998] and used by

Fox and Reed [2011]. In this block design, full-sibling offspring from two unrelated families are paired in four distinct crosses: two full-sibling inbred crosses (one for each family), and two reciprocal outcrosses (i.e., a male from family A paired with a female from family B and a female from family A paired with a male from family B, see Fig. 2.1). This design ensures equal contribution of alleles across treatment groups. Using reciprocal outcrosses helps account for family-specific maternal/paternal effects. In addition, in this experiment we introduced a split-brood design by dividing the eggs laid by each pair of beetles evenly across the eight treatment groups, further controlling for family-specific effects.

To create blocks, we first took 54 random pairs of virgin beetles from each population and allowed them to lay eggs. Single-egg seeds from these founding pairs were then isolated in 48-well tissue-culture plates in order to obtain 54 full-sibling families of up to 24 virgin beetles each. Successful full-sibling families were randomly paired to form up to 26 blocks per population (where each block comprised two unique, unrelated full-sibling families).

We conducted four types of crosses within each block: matings within each of the two full-sibling families (inbreeding treatment, offspring inbreeding coefficient of $F = 0.25$), and reciprocal outcrosses between the two full-sibling families (outbreeding treatment, $F = 0$) (Fig. 2.1). We created up to five replicates of each cross type within each block (for example, by conducting five full-sibling crosses from ten members of a single full-sibling family) to increase sample sizes and to account for within-family variation in inbreeding depression.

We chose to use green pea, *Pisum sativum*, as the novel host for this experiment because peas impose a moderate level of stress as compared to the relatively high-quality hosts (cowpea and mung bean) used in Fox and Reed [2011]. Messina et al. [2018] found that in all cases, survival in green pea was well below the >90% survival seen in cowpea or mung, with survival on green pea ranging from ∼40% to 72% dependent upon lineage. Thus, green pea can be viewed as not just a novel host, but a truly stressful host. All host seeds used in this experiment were organically grown and ordered in bulk to ensure uniform host quality. We acquired both cowpea and mung beans from Azure Standard (Dufur, OR), and green peas from Sun Organic Farm (San Marcos, CA).

All pairs of beetles from the above blocks were randomly assigned either their natural host (control; mung bean for SI, cowpea for BF and CA) or green pea (stressful host) as their first oviposition substrate. After 24 hours, if more than 8 eggs had been laid by a given pair, the pair was transferred to a fresh petri dish with the alternate host. If fewer than 8 eggs had been laid, pair was left in the same petri dish for one additional day. Pairs were then transferred daily between the stressful host and the native host. This design was used to account for the possiblity that 1) younger beetles lay more viable eggs (see, e.g., Fox et al. 2003, Fox and Reed 2010), and 2) females may preferentially lay larger, or otherwise more fit, eggs on their native host. This split-brood design allowed us to compare the effect of all experimental treatments within each full-sibling family.

After 5–7 days, all seeds from each petri dish bearing a single egg were divided equally into two plastic bags, which were left partially unsealed to provide adequate air exchange, and placed into the heat stress treatment (37°C) or the control temperature treatment (27°C). Thus, the temperature exposure treatment in our study was from egg hatch to adult emergence. All beetles were reared in Percival incubators (model Nos. AR-22L and I-36VL for heat and control temperature treatments respectively) under a 12 hr light:12 hr dark light cycle.

We measured performance in terms of survival (in all lines) and female adult mass (in BF and SI). Adult female mass was chosen as one of our fitness components because female size is often good proxy for fecundity in insects, including in *Callosobruchus* [Credland et al., 1986, Messina, 1993]. After 15 days, bags were checked daily for emergence of adult beetles. Adult beetles were removed from the bags once every 24 hours and stored at -20°C in 48-well tissue-culture plates for subsequent mass measurements. Forty-five days after the date the parental pairs (F1) were formed, the bags were removed from incubator and frozen to prevent the development of the next generation (F3). Development time for seed beetles at 25°C is generally less than 35 days on suitable hosts [Fox, 1993, Fox et al., 2011, Messina, 1991, Messina and Durham, 2013]. Thus, we measured survival for each treatment as emergence to 45 days. For any given bag, survival to adulthood was measured as the

number of beans with exit holes divided by the total number of beans. We collected mass data from frozen female BF and CA beetles using a Mettler Toledo mass balance (model XPE105) with a precision of ±0.01 mg.

Statistical Analysis

We fit Bayesian generalized linear models to quantify the individual and combined effects of inbreeding and the two environmental stressors (i.e., low quality host and high temperature) on *C. maculatus* survival and female mass (an approximation of fecundity). The output of a Bayesian model is a posterior probability distribution for model parameters of interest (in this case, the effect of environmental stressors, and the derived parameters δ, and HLE) based on experimental data, our mathematical model, and prior assumptions. To increase the efficiency of the computational model fitting process, we fit our model to each lineage separately. This is mathematically equivalent to fitting a single model for all three lineages and including population as a factor with a non-hierarchical prior (Kruschke 2014). The resulting posteriors from these separate analyses can be directly compared and summarized across lineages, allowing us to make statistical inferences about differences among populations. Generating multiple summaries of a posterior distribution in a Bayesian analysis does not result in an increased risk of type I errors, and is not subject to the problem of multiple testing as seen in conventional frequentist analysis (Kruschke 2014).

Linear Model

We assumed that the number of beetles that survived to the adult stage for each block (j) and treatment (k) was described by a binomial sampling distribution, that is $y_{jk} \sim \text{binomial}(p_{jk}, n_{jk})$, where p_{jk} is the survival probability and n_{jk} is the total sample size for the block and treatment. We further assumed that the logit probability of survival for block j and treatment k (denoted p_{jk}) was a linear function of a block and treatment specific error term (ϵ_j) and eight treatment covariates: an intercept, the three individual stress treatments (inbreeding, host, and temperature), three two-way interactions (inbreeding $\times$ host, inbreeding $\times$ temperature, and host $\times$ temperature), and the single three-way

interaction (inbreeding × host × temperature), such that

$$\log\left(\frac{p_{jk}}{1-p_{jk}}\right) = \beta^C + \beta^I \mathbb{1}_{jk}^I + \beta^H \mathbb{1}_{jk}^H + \beta^T \mathbb{1}_{jk}^T + \beta^{I\times H} \mathbb{1}_{jk}^{I\times H} +$$

$$\beta^{I\times T} \mathbb{1}_{jk}^{I\times T} + \beta^{H\times T} \mathbb{1}_{jk}^{H\times T} + \beta^{I\times H\times T} \mathbb{1}_{jk}^{I\times H\times T} + \epsilon_{jk}$$

Here I, H and T superscripts denote inbreeding, host and temperature stress treatments and the $\mathbb{1}$ are binary indicator variables set to 1 when the all of the relevant stress treatments apply. We included the error terms (ϵ_{jk}) to allow for over-dispersion among blocks (i.e. treatment-specific block effects) relative to simple binomial sampling expectations. Specifically, ϵ_{jk} allows for a random effect for each block x treatment combination to account for the fact that individual pairings within a family block are not independent. We placed minimally-informative priors on the eight regression coefficients, such that $\beta \sim \text{Normal}(\mu = 0, \tau = 1e^{-6})$. Here τ is the precision of the prior ($\tau = \frac{1}{\sigma^2}$). We modeled the ϵ_{jk} terms hierarchically by assuming normal priors with means of zero and treatment-specific precision parameters. Minimally-informative priors were placed on the eight precision parameters, $\tau_\epsilon \sim \text{gamma}(0.1, 0.01)$.

We fit a similar model for female mass data, but instead assumed a normal sampling distribution and the identity link function. We included the same eight covariates, that is, the intercept (β^C), the three individual stress treatments (inbreeding, host, and temperature), three two-way interactions (inbreeding × host, inbreeding × temperature, and host × temperature), and the single three-way interaction (inbreeding × host × temperature). We likewise placed the same minimally-informative normal priors on the regression coefficients (the β parameters), and assumed a minimally-informative gamma prior for the precision parameter of the normal sampling distribution, that is $\tau \sim \text{gamma}(0.1, 0.01)$. We estimated a separate precision parameter for each block and treatment, and included the random effect term ϵ_{jk} for each block × treatment combination.

We fit the models via Markov chain Monte Carlo (MCMC) using the `rjags` (version 4-8)

interface with `JAGS` (version 4.2.0) [Plummer, 2003, 2013]. For each model (i.e., lineage and performance metric), we ran three chains, each with a burn-in period of 2000 iterations, a thinning interval of 100 (survival) or 50 (mass), and 200,000 (survival) or 50,000 (mass) post-burn-in iterations. We evaluated convergence to the posterior distribution by examining sample history plots, calculating parameter effective sample sizes, and by calculating the Gelman-Rubin scale reduction factor [Gelman and Rubin, 1992].

We focused our inferences on Bayesian point estimates (posterior medians) and 95% credible intervals (CIs, specifically, equal-tail probability intervals) of the regression coefficients, and several derived parameters. Specifically, from the posterior samples, we calculated posterior probability distributions for expected survival probabilities and mass for each treatment (across blocks) and differences in expectations between treatments.

Calculation of Inbreeding Depression

Within each model, we also calculated the coefficient of inbreeding depression from the posterior distributions for each of the four inbred-outbred treatment pairs: oubred vs. inbred, outbred-host stress vs. inbred-host stress, outbred-temperature stress vs. inbred-temperature stress, and outbred-host and temperature stress vs. inbred-host and temperature stress. The coefficient of inbreeding depression, δ, is defined as

$$\delta = \frac{W_o - W_i}{W_o} \tag{2.1}$$

where W_o and W_i denote relative fitnesses of outbred (o) and inbred (i) lines, and δ thus represents the percent change in fitness attributable to inbreeding. δ is bounded between zero and 1, where 1 represents a 100% decline in fitness (i.e. survival or mass) due to inbreeding and 0 represents the case where no inbreeding depression occurred. In the context of our Bayesian model, δ was calculated as a derived parameter by subtracting the posterior samples (MCMC output) for fitness in the inbred group from the posterior samples for fitness in the outbred group, then dividing the result by posterior samples for fitness in the outbred group. The output of this calculation is a posterior distribution for δ for

each environmental treatment group. We likewise calculated the number of haploid lethal equivalents (HLE) over the posterior for each outbred vs. inbred treatment comparison as

$$\text{HLE} = \log\left(\frac{W_i}{W_o}\right)/0.25. \tag{2.2}$$

Here, 0.25 denotes the inbreeding coefficient, F, or the probability that an individual received two identical copies of an allele from same ancestor. Because our inbreeding treatment included solely the offspring of full-sibling matings, all beetles in our inbred treatment groups will have an inbreeding coefficient of F = 0.25. As with δ, HLE was calculated as a derived parameter using the posterior distributions from our Bayesian linear models. Haploid lethal equivalents (HLE) can be interpreted as the number of lethal loci required to produce the observed drop in fitness associated with inbreeding in a haploid population. Thus, if HLE = 4, it would indicate that this population carries the equivalent of four lethal alleles, though in reality the population may carry a different number of deleterious alleles of lesser effect. Finally, we calculated the effect of environmental stress on the magnitude of inbreeding depression as the posterior differences between δ^{stress} and $\delta^{control}$ (i.e. $\delta^T - \delta^C$ and $\delta^H - \delta^C$), and the two-way interaction between heat and temperature stress on inbreeding depression as $\delta^{H+T} - \delta^H - \delta^T + \delta^C$.

Results

We mated 200, 254, and 358 pairs of virgin beetles each from the CA, BF and SI lineages respectively to produce a total of 17, 20, and 23 complete blocks. A block was considered complete if each of the four distinct cross types (inbred pairs from two families and their reciprocal outcrosses) are represented by at least one replicate pair within that block. From these pairs, we collected a total of 31,239 single-egg seeds (CA = 7,464 beans, BF = 10,077 beans, SI = 13,698 beans). After excluding data showing evidence of F1 beetles having mated and produced a second generation, we were left with survival data for 30,746 single-egg seeds (CA = 7,316 beans, BF = 9,932 beans, SI = 13,498 beans), which we used in our analysis. On average, each treatment group contained survival data from

1,281 beans.

Survival

Host stress, temperature stress, and the combination of both all substantially increased the magnitude of inbreeding depression (as captured by δ) for survival in *Callosobruchus maculatus* (posterior probabilities [p.p.] $\delta^{H} > \delta^{\text{control}} \geq 0.99, 0.99, 0.99$; p.p. $\delta^{T} > \delta^{\text{control}} = 0.99, 0.99, 0.98$; p.p. $\delta^{H+T} > \delta^{\text{control}} = 0.91, 0.99, 0.99$ for SI, CA, and BF lineages respectively) (see Table 2.2). In the benign environment, inbreeding depression decreased survival by 12.7–14.0%, while under host stress it decreased survival by 45.4–48.7% across lineages (see Fig. 2.2a). Temperature stress had a more variable effect on inbreeding depression, decreasing survival by 42.3%, 66.6%, and 74.4% for BF, SI, and CA lineages respectively. Finally, inbreeding depression decreased survival by 74.8–100% percent across lineages when both environmental stressors (temperature and host) were present (Fig. 2.2a). Analogous results in terms of haploid lethal equivalents (HLE) are shown in in Fig. 2.2a.

Although inbreeding depression increased substantially under the combination of temperature and host stress relative to the control (δ^{H+T} was 5.6×, 7.1×, and 5.7× greater than δ^{control} for SI, CA and BF respectively), the magnitude of this increase was equal or less than additive relative to the effects of the individual stressors. Specifically, we saw a modest trend towards a negative interaction between host and temperature stress on inbreeding depression (p.p. $\delta^{H\times T} < 0 = 0.81, 0.73$, and 0.55 for SI, CA, and BF respectively, see Table 2.2). In other words, the combination of host and temperature stress on inbreeding depression had an effect equal to or less severe than the sum of their separate effects.

All stress treatments—inbreeding, host, temperature, and every combination thereof—decreased *C. maculatus* survival to adulthood (p.p. for reduced survival relative to the control > 0.99 for all stress treatments) (Figs. 2.3a and 2.4a). Across lineages, survival to adulthood under host stress was 36.7–57.5 percentage points lower than under benign conditions, while under temperature stress survival decreased by 27.5–66.3 percentage points (see Fig. 2.3a). Inbreeding depression had less of an impact on survival (8.9–11.0 percentage point decrease) than either host or temperature stress, and showed less variation across lin-

eages. Across all lineages, adding inbreeding stress to environmental stress lowered survival by an additional 8.1–18.1 percentage points compared to the environmental stress treatment alone (compare I+H to H and I+T to T in Fig. 2.3a). Finally, both the combination of temperature and host stress and the combination of all three stressors (inbreeding, temperature, and host) imposed such severe stress that survival dropped to less than 2% across lineages.

In contrast to our results for inbreeding depression (δ), the reduction in fitness under combinations of two stressors showed a trend for being greater than additive (p.p. for $\beta_{I\times H} < 0 =$ 0.84-0.99, $\beta_{I\times T} < 0 =$ 0.90-0.99, $\beta_{H\times T} < 0 > 0.99$) (Fig. 2.4a). However, the only combination of stressors that showed a consistent non-additive impact on survival to adulthood was host×temperature (see Fig. 2.4a). While the 95% CIs for the host×inbreeding and temperature×inbreeding treatments overlapped zero for most lineages, there were credible interactions between host and inbreeding in BF and between temperature and inbreeding in CA (p.p. $\beta_{I\times H} < 0 = 0.99$ for BF and p.p. $\beta_{I\times T} < 0 = 0.99$ for CA). Three-way interactions showed considerable uncertainty and were not credibly different from zero.

Finally, there were no credible differences in the magnitude of inbreeding depression across any of our populations (see Table 2.3). In contrast, the effect of host stress varied credibly across all three populations (Table 2.3). In particular, survival both temperature and host stress had a credibly lower impact on survival in BF than in either SI or CA (Table 2.3). This trend was particularly pronounced for temperature stress, where CA and SI survival was 30 percentage points lower than in BF (95% CI = 0.18-0.43%). Moreover, survival in BF was affected more by host than temperature ($\beta_H - \beta_T =$ -0.39, 95%CI = -0.90–0.13, p.p. $< 0 = 0.93$), while survival in CA and SI was affected more by temperature than host (CA, $\beta_H - \beta_T = 0.65$, 95% CI = 0.23–1.13, p.p. $> 0 > 0.99$; SI, $\beta_H - \beta_T = 1.01$, 95% CI = 0.56–1.53, p.p. $> 0 > 0.99$).

Similarly, in the double-stress (I+H, I+T, and H+T) treatments, the CA and SI lineages showed credibly lower survival than BF (a 21 percentage point decrease; 95% CI =

0.15-1.27%, see Table 2.3). The effect of host stress on survival was also credibly different across all population pairs (p.p. that the absolute value of the difference in $\beta^H > 0 \geq 0.99$ for all three population pairs). The effect of temperature stress was credibly less severe than in either CA or BF (p.p. that ${\beta^T}_{\text{BF}} - {\beta^H}_{\text{CA}}$ and ${\beta^T}_{\text{BF}} - {\beta^H}_{\text{SI}} > 0 \geq 0.99$). However, no credible differences between populations were observed for interaction terms (i.e. $\beta_{I\times H}$, $\beta_{I\times T}$, and $\beta_{H\times T}$), indicating that the differences across populations in our double-stress groups are largely due to differences in the additive effects of each stressor across populations rather than differences in the magnitude of interactions across populations. No credible differences were observed between populations were observed in the triple-stress (inbreeding + host + temperature) treatment or for the three-way interaction term ($\beta_{I\times H\times T}$). Similar results were observed for the effects of external stressors on inbreeding depression (δ) (Fig. 2.2a).

Female Mass

Under benign conditions, we saw inbreeding depression with respect to female mass only in the CA lineage, where inbreeding decreased mass by 7.4% (95% CI = 2.0–12.5%, see Fig. 2.3b). In the single-stress treatments, we saw a trend for inbreeding depression under host stress in BF (p.p. $\delta^{\text{host}} > 0 = 0.901$), and under temperature stress in CA (p.p. $\delta^{\text{temp}} > 0 = 0.929$). However, evidence that either of these treatments increased inbreeding depression more than in the control treatment was moderate to marginal (p.p. $\delta^{\text{host}} > \delta^{\text{control}} = 0.770$ for BF; p.p. $\delta^{\text{temp}} > \delta^{\text{control}} = 0.615$ for CA, see Table 2.2). Analogous results in terms of haploid lethal equivalents (HLE) are shown in in Fig. 2.2b.

We were unable to obtain female mass data for the CA host-temperature combination stress treatment because survival was too low. However, the two-way combination of temperature and host stress showed a strong trend for increasing the magnitude of inbreeding depression in BF (Fig. 2.2b). Inbred BF beetles from the temperature plus host treatments were 49.2% (95% CI = -16.5–94.8%) smaller by mass than non-inbred BF beetles subjected to those same environmental stressors (as compared to 2.02%, 6.53%, and 3.24% for δ^{control}, δ^{host}, and δ^{temp} respectively). Furthermore, the trend for the magnitude of this increase

in inbreeding depression was more than additive: inbreeding depression increased by 41.7 percentage points more than expected by simply summing the effects of temperature and host stress (95% CI = -13.7–87.8 percentage points; p.p. $\delta^{H+T} > \delta^{H} + \delta^{T} - \delta^{C} = 0.96$). In contrast to our results for survival (where we saw a modest negative trend), here the trend for an interaction between host and temperature stress on inbreeding depression was strongly positive (Fig. 2.2b and Table 2.2).

There was a highly credible difference in female mass across lineages: under benign conditions, average mass for the BF lineage was 11% greater than for CA (CA, mean mass = 2.67 mg, 95% CI 2.57-2.78 mg; BF, mean mass = 3.00 mg, 95% CI 2.85-3.14 mg for BF, see Fig. 2.3b and Table 2.3). While inbreeding decreased female mass in both lineages, only CA showed a credible effect (BF, p.p. $\beta^{I} < 0 = 0.72$; CA, p.p. $\beta^{I} < 0 \geq 0.99$) (Figs. 2.3b and 2.4b). As with survival, there were no credible differences in the effect of inbreeding depression (β^{I}) on female mass across populations. However, the effect of host stress on female mass was considerably more severe in BF than in CA (p.p. β^{H} for BF $< \beta^{H}$ for CA ≥ 0.99). In the BF lineage, host stress decreased female mass at adulthood by 0.578 mg (95% CI = 0.357 to 0.801 mg decrease) as compared to the control group. While host stress showed no measurable effect on mass in CA, temperature stress decreased CA mass from 2.67 mg to 2.26 mg (a 0.41 mg drop; p.p. $\text{mass}^{\text{temp}} > \text{mass}^{\text{control}} > 0.99$) (Fig. 2.3b). In contrast, the effect of temperature stress on CA was considerably more severe in CA than in BF (p.p. β^{T} for CA $< \beta^{T}$ for BF $= 0.99$, see Fig. 2.4b).

We found no credible evidence of two-way interactions between stressors on mass in either BF or CA (Fig. 2.4b), and we saw no credible differences in the interaction terms ($\beta^{I\times H}$ and $\beta^{I\times T}$) across populations. As for survival, this indicates that the credible differences in female mass we saw in the double-stress treatment groups (i.e. I+H and I+T, see Table 2.3) can be attributed mainly differences in baseline female mass and the differential effects of additive stressors across populations rather than differences in the magnitude of interactions across populations. That said, we did find a strong positive trend for an interaction among all three stressors combined (inbreeding, host, and temperature stress)

on mass in BF (p.p. $\beta^{I\times H\times T} < 0 = 0.96$), such that beetles exposed to all three stressors were smaller than expected from the effects of the individual stressors. Due to exceedingly low survival in the host plus temperature treatment and in the inbreeding plus host plus temperature treatments, we were unable to collect mass data for CA (Table 2.1).

Discussion

The prevalence of interactions between inbreeding and environmental stress has become clear in recent years, garnering interest in what consequences this might hold for conservation [Armbruster and Reed, 2005, Fox and Reed, 2011, Kristensen et al., 2008, 2003, Liao and Reed, 2009, Pray et al., 1994, Reed et al., 2012]. But the significance and consistency of this phenomenon remain unclear. In this study we sought to shed light on these questions by approaching analyses from two different perspectives: first, we looked at the degree to which environmental stress (and interactions among stressors), resulted in inbreeding-stress interactions as measured by the magnitude of inbreeding depression (δ). Second, we looked at the degree to which interactions among each combination of our three stressors (inbreeding, host, and temperature) impacted overall fitness, as measured by survival and female mass. By comparing the effect of inbreeding-stress interactions as measured by the effect of environmental stressors on inbreeding depression vs. the effect of inbreeding-stress interactions on fitness, we sought to determine whether a substantial inbreeding-stress interaction as measured by δ implies that the effect of that interaction on overall fitness will be relevant for conservation. We used *C. maculatus* lineages from three different continents (CA from North America, BF from Africa, and SI from southwest Asia) to determine the degree of consistency in stress responses across lineages.

The magnitude of inbreeding-stress interactions as measured by inbreeding depression vs. overall fitness are not interchangeable

Both environmental stressors we used (host and temperature stress) had a strong effect on the magnitude of inbreeding depression, δ, for survival in our lineages. This is consistent with the general literature consensus that environmental stress generally increases the

severity of inbreeding depression [Armbruster and Reed, 2005, Fox and Reed, 2011]. Similarly, we found that each stressor individually (inbreeding, temperature, and host) had a strong impact on *C. maculatus* survival. Our environmental stressors (temperature and poor host quality) caused a greater reduction in survival in *C. maculatus* than did inbreeding. That said, under our experimental design beetles were subjected to only one generation of inbreeding (inbreeding coefficient F = 0.25). In the wild (or in captive breeding), populations may instead be subjected to prolonged bouts of inbreeding. Thus, the current study may underestimate the impact of inbreeding depression in wild or captive-bred populations. Conversely, in cases where inbreeding has been so prolonged that purging has occurred [see Bijlsma et al., 1999], our study may instead overestimate the impact of inbreeding depression.

Despite finding clear evidence that both environmental stressors produced an inbreeding-stress interaction as measured by their effect on δ, the survival model showed an inbreeding-stress interaction in only two treatment groups: inbreeding $\times$ host in BF and inbreeding $\times$ temperature in CA. This discrepancy illustrates that inbreeding-stress interactions showing credible effects within the context of relative fitness (i.e. outbred vs. inbred fitess) may show marginal effects within the context of overall fitness. Instead, the decline in survival in our treatment groups can be attributed largely to the individual, additive effects of each stressor rather than inbreeding-stress interactions. Thus, even in cases where the effect of an inbreeding-stress interaction as measured by inbreeding depression (i.e. δ or HLE) is substantial, we cannot assume that the importance of those interactions vs. additive effects of individual stressors on overall fitness will be similarly important. For example, if under benign conditions, inbreeding decreased survival from 100% to 90%, while under stressful conditions, inbreeding decreased survival from 2% to 1%, δ would increase from 0.1 to 0.5, indicating that a substantial inbreeding-stress interaction exists. But concluding from this change in δ that inbreeding-stress interactions have a substantial effect on population fitness would be false, as in this hypothetical scenario the additive effects of environmental stress alone caused a 98% drop in survival, rendering the effect of the inbreeding-stress in-

teraction negligible by comparison. Thus, using δ as the sole measure of the importance of inbreeding-stress interactions may give a false impression regarding the relative importance of inbreeding-stress interactions to overall fitness. Our findings highlight the importance of carefully parsing the effect of inbreeding-stress interactions as measured by inbreeding depression vs. the effect of inbreeding-stress interactions as measured by overall fitness—relevance in the first case may not imply relevance in the second.

Strength of interactions depends on the severity of the stressors

Because our study expands upon the work of Fox and Reed [2011], some of our results can be compared. Overall, Fox and Reed [2011] saw higher baseline survival and more severe inbreeding depression than we did ($\delta^{control} = 0.31$ for BF and 0.19 for SI, as compared to 0.13 and 0.14 in our study). We also saw a drastically different response to temperature stress in the SI lineage. Whereas Fox and Reed [2011] found that temperature stress decreased survival in SI by only 13 percentage points (88% survival in the control vs. 75% under temperature stress), we found that temperature stress decreased survival in SI by 63 percentage points (75.5% survival in the control vs. 12.1% under temperature stress). These differences may reflect the impact of confounding variables such as humidity, host seed quality, differences in rearing setup, and oxygen availability. Alternatively, there may have been significant changes in the genetic composition of the SI population between the earlier study and ours. Under most temperature regimes, seed beetles have a generation time of approximately 30 days or less [Fox et al., 2011]. Consequently the SI and BF lineages would have had over 75 generations in which to evolve differences between the Fox and Reed [2011] experiment and our own. Fricke and Arnqvist [2004] demonstrated that replicate populations of the same seed-beetle strain can rapidly diverge in mating behavior and reproduction under different laboratory conditions. Both local adaptation and genetic drift could cause substantial change in a population's response to both environmental stress and inbreeding. Accounting for the potential of evolutionary rescue may an important consideration to avoid over-generalizing the effect of inbreeding-stress interactions both across populations and over time [Carlson et al., 2014, Gonzalez et al., 2013].

The consensus of our study, Fox et al. [2011], and Fox and Reed [2011] suggest that the severity of an individual stressor may be critical to determining its potential for interactions with other stressors or with inbreeding. Fox et al. [2011], used mild stressors and found marginal evidence for interactions among stressors. The authors suggested that the range of stressors used were so mild that either a) the interactions between stressors were too small to detect, or b) stressors must reach a certain severity threshold before interactions occur between them. Fox and Reed [2011] used moderate stressors (e.g., survival in the most stressful treatment group was 33%) and found clear evidence for relevant interactions among them. In this study, we used combinations of stressors that in some cases severely limited survival, with three treatment groups showing survival of less than 2% (I+T, H+T, and I+H+T). Thus, our study expands upon Fox et al. [2011] and Fox and Reed [2011] to represent the far end of the spectrum: the effect of interactions when stressors are severe. In our most severe stress combinations, we found little evidence of interactions. This result provides strong support for the trend that Schou et al. [2015] found in their study of inbreeding-stress interactions in *Drosophila*: under severe stress, the rate of increase in inbreeding-stress interactions falls short of linear. In other words, the higher the stress level, the smaller the increase in inbreeding-stress interactions becomes.

Taken together, these trends illustrate that although the strength of interactions may increase with the severity of stress imposed when stressors are mild to moderate, there will be a point at which the interaction between two biological stressors will necessarily be less than additive due to limiting bounds on the values a trait can assume. For example, inbreeding depression, δ, has a maximum upper bound of 1, which would indicate that inbreeding decreased fitness by 100%. Consider inbreeding depression for survival in the CA lineage in our study: under a purely additive model, we would predict that δ^{H+T} should decrease survival by 108.8% (baseline δ plus additional effect of temperature and host $= 14\% + 60.1\% + 34.7\%$, see 2.2), which of course is impossible. Thus, when assessing the relevance of potential interactions in a conservation context, it may be helpful to first consider the magnitude of each stressor on its own.

Interactions vary with the fitness component measured

Our study also showed that effects of inbreeding and stress depend on fitness measure. We saw clear evidence of inbreeding depression and the impact of individual stressors on δ for survival, but these effects were less clear for adult female mass. The effect of interactions between environmental stressors also varied by fitness component. Using female mass as our measure of fitness, we found a strong trend for a positive interaction between host and temperature stress on inbreeding depression. This is consistent with Fox and Reed [2011] and with studies showing increased inbreeding depression under stressful conditions [e.g., Armbruster and Reed, 2005, Liao and Reed, 2009]. But we saw precisely the opposite when survival was the fitness measure: the interaction between temperature and host was negative (see Fig. 2.2). Hence, for survival, the effect of temperature plus host stress was less severe than the sum of their separate effects. Similarly, we did not see evidence for a three-way inbreeding×host×temperature interaction for survival, but there was a strong trend for a three-way interaction for mass.

The consistent differences between our survival vs. mass results underscore the fact that not all components of fitness will respond the same way to inbreeding or environmental stress This point was noted previously by Armbruster and Reed [2005] and Charlesworth and Charlesworth [1987] in their reviews of inbreeding literature. In our case, the differences in the stress response of mass vs. survival may have arisen in part because beetles with low mass were less likely to survive to adult emergence. Thus, the mean weight of beetles that emerged successfully may be higher than would have been observed had all beetles survived to emergence. In other cases, such for the three-way stress interaction on survival, the lack of effect might have occurred because the two-way stress treatment (temperature + host) alone decreased survival to less than 2%, a value too low to allow us to detect a more-than-additive three-way interaction. We note that our results closely parallel those of Schou et al. [2015], who also found that the magnitude of inbreeding depression was lower when measured using mass rather than survival in *Drosophila*, suggesting that this trend may be generalizable in certain cases. These results also reinforce the notion that

fitness components are not always directly comparable, and it would be advisable to avoid generalizing the results of a single fitness component. In addition, our results suggest that it is important to think carefully about which fitness measure (or combination of fitness components) is most relevant for the particular organism under study.

Effect of environmental stress differs across lineages

We found little evidence for variation in the magnitude of inbreeding depression among lineages. In wild populations that have undergone drastic bottlenecks or other significant demographic changes (as might be expected in endangered populations), population-specific responses may be more common. As the variance effective population size (N_e) for the the least-fecund lineage in our experiment was equal to $N_e = 1149.6$, it is unlikely that drastic bottlenecks have occurred in our beetle lineages' recent history [Gompert and Messina, 2016]. That said, the beetle lineages we used have been maintained in captivity for in excess of 100 generations (and over 450 generations in the case of SI), meaning that substantial levels of genetic drift and adaptation to captivity are likely to have occurred in our lineages.

We did observe, however, that the response to environmental stressors, but not their interactions with inbreeding depression, varied strongly across populations. Specifically, we found that the Burkina Faso (BF) lineage was less susceptible to environmental stress overall, and in particular showed far greater tolerance to temperature stress. In contrast, the South India (SI) and California (CA) populations were more susceptible to temperature stress than to host stress. In addition to differences in the mean effect of environmental stress, the variance in response to environmental stress seen in BF was also far higher than in the South India (SI) and California (CA) populations (see Fig. 2.3a). While we saw no credible differences in inbreeding-stress interactions across populations, we did see a modest trend for the host $\times$ temperature interaction being greater in BF than CA (see Fig.2.4a). Differences in response to temperature stress across populations (both mean and variance) may reflect local adaptation in each lineage to different stressors in their native environments, but given the differences in results between Fox and Reed [2011] and our study, it seems likely that divergent assay conditions and prior laboratory evo-

lution (adaptation to captivity and/or drift) may have played a role. Nevertheless, these differences suggest that the effect of environmental stress may not generalize well across populations. Inbreeding-stress interactions, however, were more consistent, suggesting that the magnitude of inbreeding-stress interactions may be somewhat more generalizable across populations within a single species than are the effects of individual stressors.

Conservation implications and future directions

In conclusion, we found that the magnitude and relevance of individual stressors and their interactions varied with 1) analysis perspective (i.e. measuring inbreeding-stress interactions in the context of inbreeding depression vs. fitness) 2) fitness component, 3) stressor severity, and 4) population. In all, our results suggest that inbreeding-stress interactions are both variable and complex. Critically assessing the aforementioned factors may help better clarify under which circumstances inbreeding-stress interactions are relevant for applied conservation. In particular, our results suggest that δ or HLE may be more sensitive measures for assessing the presence of inbreeding-stress interactions as they are based on relative rather than absolute fitness (specifically, inbred relative to outbred fitness). Thus, in situations where the goal is to determine the presence of an inbreeding-stress interaction, no matter how small, δ or HLE may prove more effective. However, in the context of conservation, it is arguably more important to understand not whether an inbreeding-stress interaction exists, but whether interactions effects are large enough to warrant conservation concern. Measures such as δ and HLE are not necessarily the most effective way to determine the relative importance of interactions vs. the additive effects of stressors. Thus, we suggest that placing inbreeding-stress interactions within the context of overall fitness (i.e. comparing additive vs. interactive effects directly) will yield more informative results regarding the conservation relevance of inbreeding-stress interactions than will looking at inbreeding-stress interactions in terms of δ or HLE alone.

In addition to exercising caution when interpreting the relevance of inbreeding-stress interactions within the framework of δ alone, our study showed that the magnitude of inbreeding-stress interactions varied with both fitness component and degree of stress im-

posed. This is consistent with previous research ([Armbruster and Reed, 2005, Charlesworth and Charlesworth, 1987, Schou et al., 2015]), and underscores the inbreeding-stress interactions can vary widely depending on the severity of stress a population experiences and which fitness measure is used. Future studies exploring the relationship between the magnitude of stressors and the resulting interaction between them could help explain some of this variation, and shed further light on how broadly inbreeding-stress interactions can be generalized. For conservation purposes, fitness measures of direct relevance to population persistence or management (in particular survival and fecundity) may be of greater value than fitness measures with less obvious connections to population persistence.

Finally, investigating the role of local adaptation and population demographic history (in particular the effects of small population size or bottlenecks) may help us better explain and predict variation in inbreeding-stress interactions across populations. While we know that inbreeding-stress interactions are a widespread phenomenon, how such interactions may change under adaptive evolution, evolutionary rescue, or purging is unclear. While our study did not show substantial differences in inbreeding depression or inbreeding-stress interactions across populations, we did see large differences in how populations responded to environmental stress. Furthermore, our results contrasted sharply with those of Fox et al. [2011], raising the question of whether stress responses may not only generalize poorly across populations, but across time as well. In light of these results, we suggest that for management purposes, generalizations about the effects of inbreeding-stress interactions across space (i.e. geography) and time (i.e. across many generations in a single population, especially when evolutionary rescue or purging is thought to be occurring) be approached with caution.

In summary, research over the past two decades has revealed substantial variation in magnitude and direction of inbreeding-stress interactions populations experience. Elucidating the various causes of such variation may help us not only to better predict a population's conservation risk, but to develop a deeper understanding of eco-evolutionary dynamics as a whole. Until then, carefully considering the many nuances that can affect the magnitude

and relevance of inbreeding-stress interactions may help us make sound judgements with regard to conservation.

Acknowledgments

We thank Megan Brady for help with the experiment and Charles Fox for providing beetle cultures. This work was funded by the National Science Foundation (NSF GRFP awarded to Amy Springer, fellow 2017239847), the Utah Agricultural Experiment Station (paper number 9276), and Utah State University.

Data Archival Location

Data files for mass and survival and computer scripts used to analyse the data are available on DRYAD (https://doi.org/10.5061/dryad.z8w9ghx8s).

REFERENCES

Armbruster, P. and D. Reed, 2005. Inbreeding depression in benign and stressful environments. Heredity 95:235.

Bijlsma, R., J. Bundgaard, and W. F. Van Putten, 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. Journal of Evolutionary Biology 12:1125–1137.

Carlson, S. M., C. J. Cunningham, and P. A. Westley, 2014. Evolutionary rescue in a changing world. Trends in Ecology & Evolution 29:521–530.

Charlesworth, D. and B. Charlesworth, 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237–268.

Credland, P. F., K. M. Dick, and A. W. Wright, 1986. Relationships between larval density, adult size and egg production in the cowpea seed beetle, *Callosobruchus maculatus*. Ecological Entomology 11:41–50.

Dahlgaard, J. and A. A. Hoffmann, 2000. Stress resistance and environmental dependency of inbreeding depression in *Drosophila melanogaster*. Conservation Biology 14:1187–1192.

Fox, C., M. Bush, and W. Wallin, 2003. Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. Functional Ecology 17:811–820.

Fox, C., K. Scheibly, B. Smith, and W. Wallin, 2007. Inbreeding depression in two seed-feeding beetles, *Callosobruchus maculatus* and *Stator limbatus* (Coleoptera: Chrysomelidae). Bulletin of Entomological Research 97:49–54.

Fox, C. W., 1993. A quantitative genetic analysis of oviposition preference and larval performance on two hosts in the bruchid beetle, *Callosobruchus maculatus*. Evolution 47:166–175.

Fox, C. W. and D. H. Reed, 2010. Inbreeding depression increases with maternal age in a seed-feeding beetle. Evolutionary Ecology Research 12:961–972.

———, 2011. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. Evolution 65:246–258.

Fox, C. W., R. C. Stillwell, W. G. Wallin, C. L. Curtis, and D. H. Reed, 2011. Inbreeding-environment interactions for fitness: complex relationships between inbreeding depression and temperature stress in a seed-feeding beetle. Evolutionary Ecology 25:25–43.

Fricke, C. and G. Arnqvist, 2004. Divergence in replicated phylogenies: the evolution of partial post-mating prezygotic isolation in bean weevils. Journal of Evolutionary Biology 17:1345–1354.

Gelman, A. and D. B. Rubin, 1992. Inference from iterative simulation using multiple sequences. Statistical Science 7:457–472.

Gompert, Z. and F. J. Messina, 2016. Genomic evidence that resource-based trade-offs limit host-range expansion in a seed beetle. Evolution 70:1249–1264.

Gonzalez, A., O. Ronce, R. Ferriere, and M. E. Hochberg, 2013. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution.

Hedrick, P. W. and A. Garcia-Dorado, 2016. Understanding inbreeding depression, purging, and genetic rescue. Trends in Ecology & Evolution 31:940–952.

Joubert, D. and R. Bijlsma, 2010. Interplay between habitat fragmentation and climate change: inbreeding affects the response to thermal stress in *Drosophila melanogaster*. Climate Research 43:57–70.

Kébé, K., N. Alvarez, M. Tuda, G. Arnqvist, C. W. Fox, M. Sembène, and A. Espíndola, 2017. Global phylogeography of the insect pest *Callosobruchus maculatus* (Coleoptera: Bruchinae) relates to the history of its main host, *Vigna unguiculata*. Journal of Biogeography 44:2515–2526.

Kristensen, T. N., J. S. F. Barker, K. S. Pedersen, and V. Loeschcke, 2008. Extreme temperatures increase the deleterious consequences of inbreeding under laboratory and semi-natural conditions. Proceedings of the Royal Society of London B: Biological Sciences 275:2055–2061.

Kristensen, T. N., J. Dahlgaard, and V. Loeschcke, 2003. Effects of inbreeding and environmental stress on fitness–using *Drosophila buzzatii* as a model organism. Conservation Genetics 4:453–465.

Liao, W. and D. Reed, 2009. Inbreeding–environment interactions increase extinction risk. Animal Conservation 12:54–61.

Messina, F. J., 1991. Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. Oecologia 85:447–455.

———, 1993. Heritability and 'evolvability' of fitness components in *Callosobruchus maculatus*. Heredity 71:623–629.

Messina, F. J. and S. L. Durham, 2013. Adaptation to a novel host by a seed beetle (Coleoptera: Chrysomelidae: Bruchinae): effect of source population. Environmental Entomology 42:733–742.

Messina, F. J., A. M. Lish, and Z. Gompert, 2018. Variable responses to novel hosts by populations of the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). Environmental Entomology 47:1194–1202.

Miller, P. S., 1994. Is inbreeding depression more severe in a stressful environment? Zoo Biology 13:195–208.

Mitchell, R., 1991. The traits of a biotype of *Callosobruchus maculatus* (f.)(Coleoptera: Bruchidae) from South India. Journal of Stored Products Research 27:221–224.

Plummer, M., 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. *in* Proceedings of the 3rd International Workshop on Distributed Statistical Computing, vol. 124, P. 10. Vienna, Austria.

———, 2013. rjags: Bayesian graphical models using MCMC. R package version 3.

Pray, L. A., J. M. Schwartz, C. J. Goodnight, and L. Stevens, 1994. Environmental dependency of inbreeding depression: implications for conservation biology. Conservation Biology 8:562–568.

Reed, D. H., D. A. Briscoe, and R. Frankham, 2002. Inbreeding and extinction: the effect of environmental stress and lineage. Conservation Genetics 3:301–307.

Reed, D. H., C. W. Fox, L. S. Enders, and T. N. Kristensen, 2012. Inbreeding–stress interactions: evolutionary and conservation consequences. Annals of the New York Academy of Sciences 1256:33–48.

Roff, D. A., 1998. Effects of inbreeding on morphological and life history traits of the sand cricket, *Gryllus firmus*. Heredity 81:28.

Sandner, T. M. and D. Matthies, 2016a. The effects of stress intensity and stress type on inbreeding depression in *Silene vulgaris*. Evolution 70:1225–1238.

———, 2016b. Interactions of inbreeding and stress by poor host quality in a root hemiparasite. Annals of Botany 119:143–150.

Schou, M. F., V. Loeschcke, and T. N. Kristensen, 2015. Inbreeding depression across a nutritional stress continuum. Heredity 115:56–62.

Tuda, M., K. Kagoshima, Y. Toquenaga, and G. Arnqvist, 2014. Global genetic differentiation in a cosmopolitan pest of stored beans: effects of geography, host-plant usage and anthropogenic factors. PLoS ONE 9:e106268.

Tuda, M., J. Rönn, S. Buranapanichpan, N. Wasano, and G. Arnqvist, 2006. Evolutionary diversification of the bean beetle genus *Callosobruchus* (Coleoptera: Bruchidae): traits associated with stored-product pest status. Molecular Ecology 15:3541–3551.

Tables and Figures

Table 2.1: Mass sample sizes by lineage and treatment

	Control	Inbred (I)	Host (H)	Temp (T)	I+H	I+T	H+T	I+H+T
BF	371	349	200	241	94	139	9	3
CA	291	237	71	49	49	19	0	0

Table 2.2: Point estimates and 95% credible intervals for the effect of host stress, temperature stress, and host-temperature stress interactions (H×T) on inbreeding depression, δ, using both survival and mass and fitness measures. Values greater than zero represent treatments or interactions that increased the severity of inbreeding depression, while values less than zero represent those that decreased the severity of inbreeding depression. Host-temperature interactions were cacluated as $\delta^{H\times T} = \delta^{H+T} - \delta^{H} - \delta^{T} + \delta^{C}$.

		Host ($\delta^H - \delta^C$)	Temp ($\delta^T - \delta^C$)	H×T
Survival	SI	0.32 (0.19, 0.43)	0.53 (0.23, 0.70)	-0.18 (-1.77, 0.21)
	BF	0.35 (0.20, 0.48)	0.30 (0.01, 0.49)	-0.03 (-0.58, 0.38)
	CA	0.35 (0.16, 0.51)	0.60 (0.35, 0.76)	-0.08 (-0.31, 0.21)
Mass	BF	0.04 (-0.07, 0.16)	0.01 (-0.09, 0.11)	0.42 (-0.14, 0.88)
	CA	-0.06 (-0.19, 0.06)	0.02 (-0.12, 0.15)	–

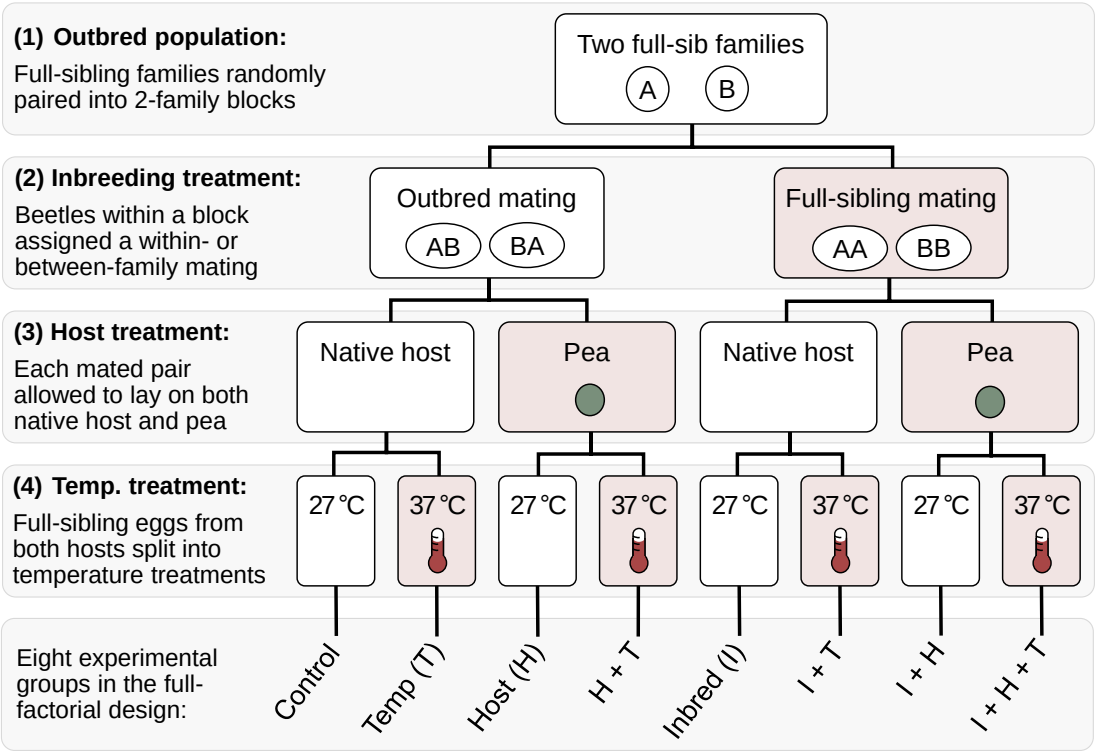


Fig. 2.1: Flow chart showing how beetles were assigned to treatment groups within a block. We used a split-brood design in order to divide the offspring of each beetle pair across all temperature-host treatment combinations. Red-shaded boxes indicate stressful treatments, while white boxes indicate benign conditions. This experimental design was implemented for all blocks across all lineages (BF, CA, and SI).

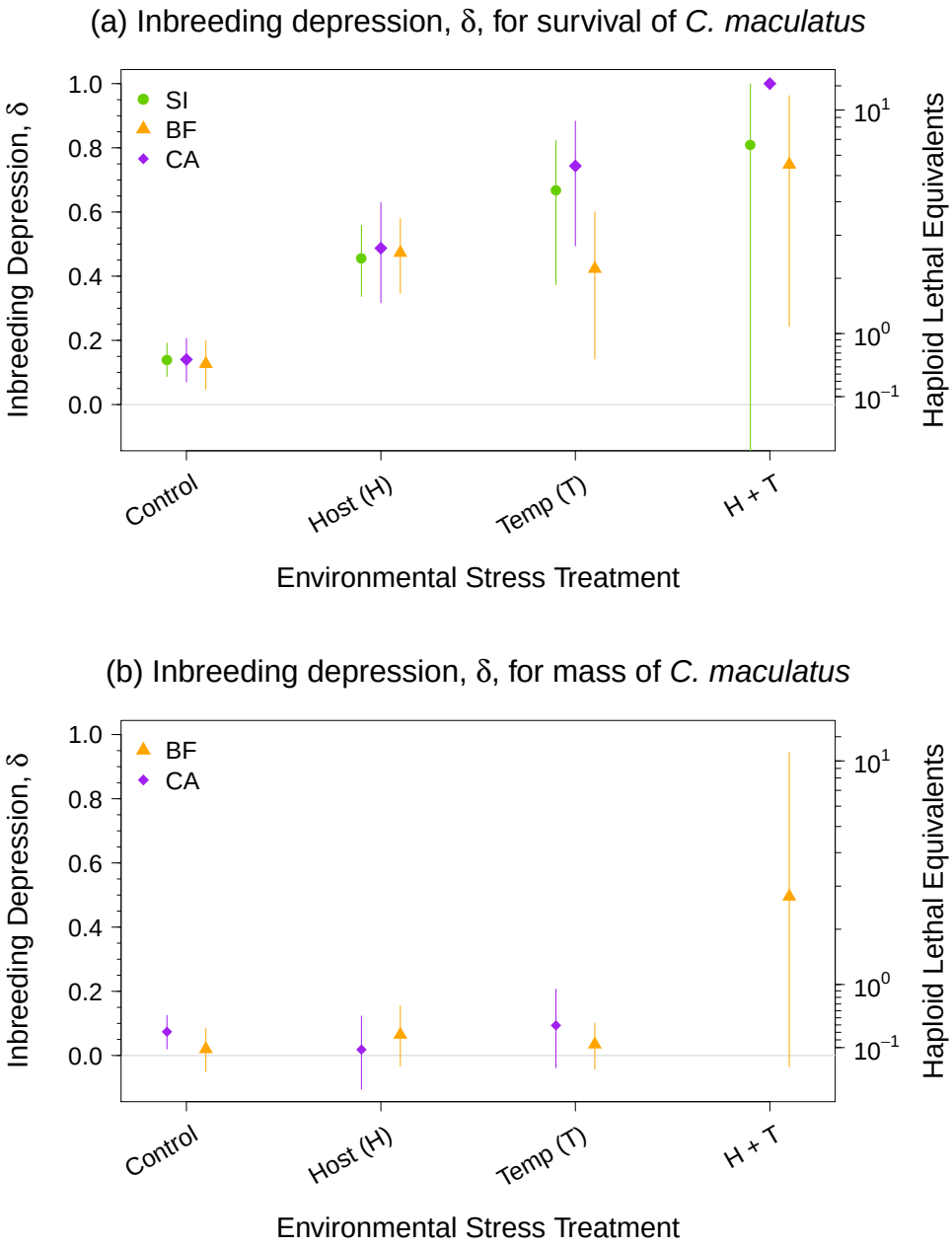


Fig. 2.2: Model posterior summaries for inbreeding depression (δ) and haploid lethal equivalents (HLE) using (a) survival and (b) mass as the measure of fitness. Points represent the median value of the posterior for each treatment-lineage combination, while vertical bars denote the 95% credible interval. Values of δ range from 0 to 1, with zero indicating no inbreeding depression, and 1 indicating that inbreeding reduced fitness by 100%. HLE is represented on a log scale, with the power of 10 increasing by 0.1 with each hash mark.

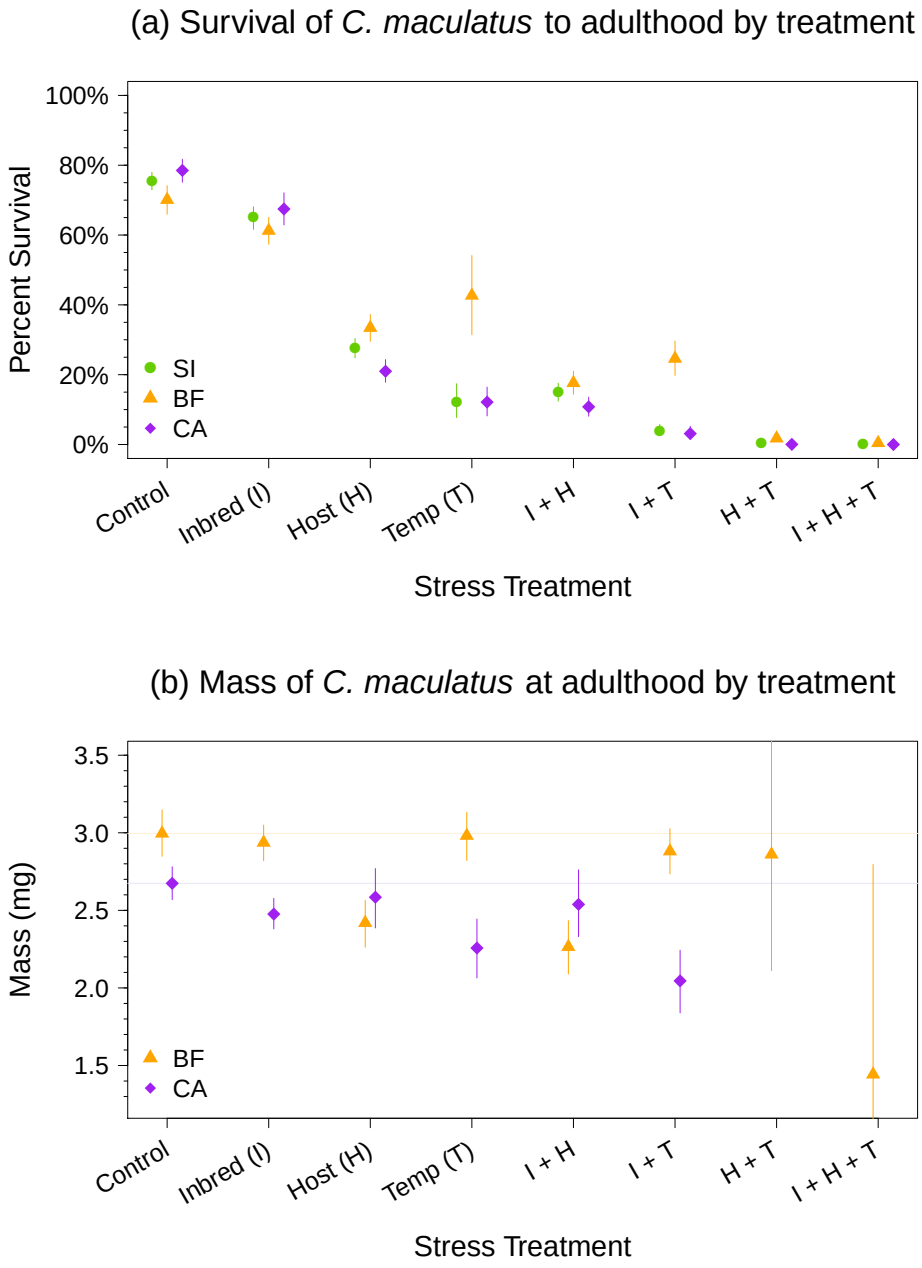


Fig. 2.3: Model posterior summaries for (a) percent survival and (b) female mass by treatment group and lineage. Points represent the median value of the posterior for a given treatment-lineage combination, and vertical bars denote the 95% credible interval. Horizontal lines in (b) represent the median mass for the control treatment by lineage.

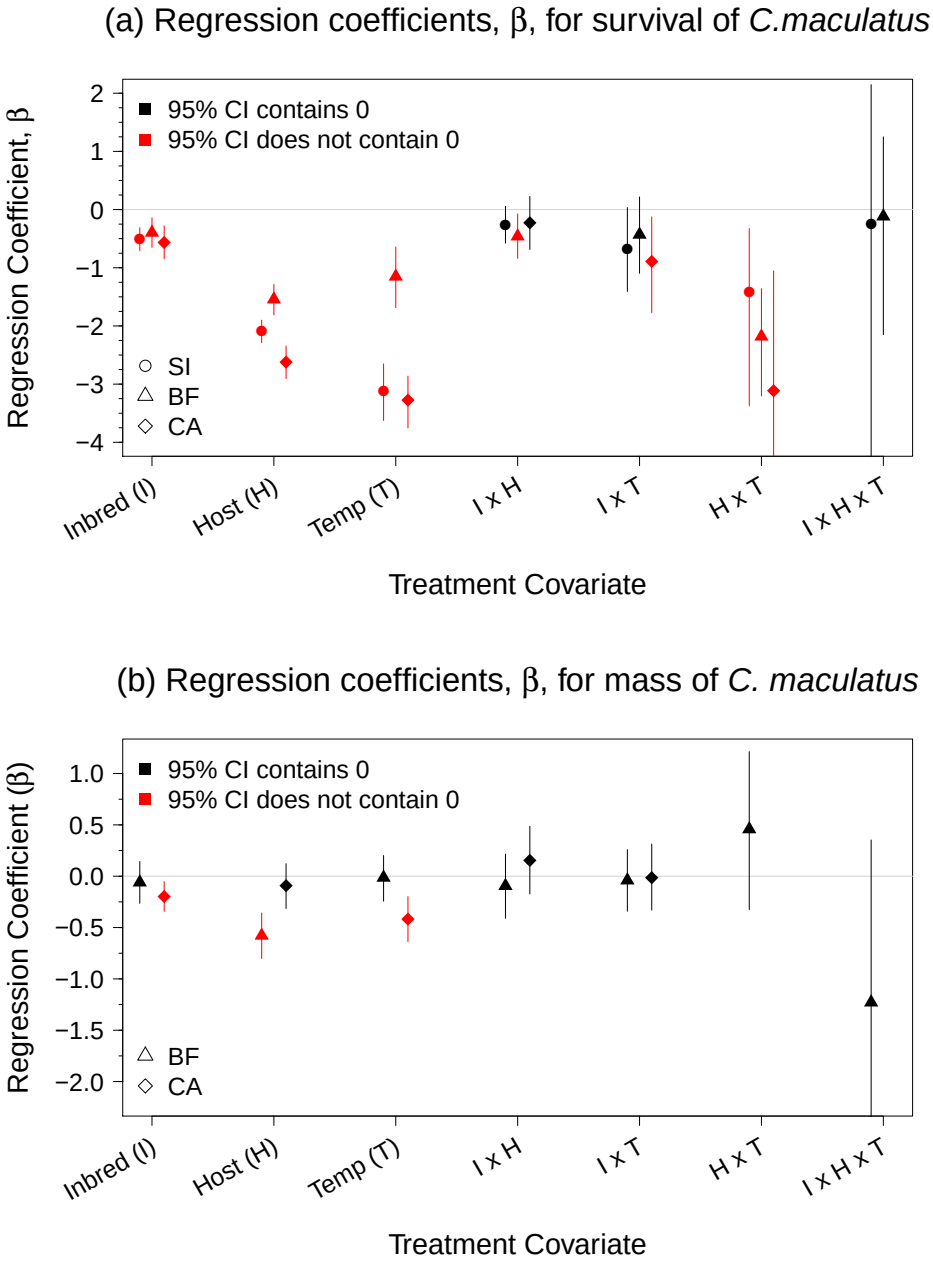


Fig. 2.4: Model posterior summaries for regression coefficients (β) for each of the seven treatment covariates from the linear models for (a) survival and (b) mass. Regression coefficients are presented on a logit scale where points indicate the median value of β from the posterior and vertical bars indicate 95% credible intervals. The horizontal gray bar indicates the line $y = 0$. Regression coefficients falling below zero indicate variables which decreased survival or mass as compared to the control, while those above zero increased survival or mass.

Table 2.3: Point estimates and 95% credible intervals for differences between populations by treatment group for both survival and mass data. Differences between population pairs were calculated as derived parameters by taking the difference between the posterior samples for the first population and the second, then summarizing the output. Negative estimates indicate that the second population had a higher survival or mass value than the first, while positive estimates indicate that the second population had a lower survival or mass value than the first. Credible differences between populations (in either direction) are shown in bold.

		Control	Inbred	Host
Survival	BF-CA	-0.08 (-0.14, -0.03)	-0.06 (-0.12, 0.00)	0.13 (0.07, 0.18)
	BF-SI	-0.05 (-0.10, -0.01)	-0.04 (-0.09, 0.01)	0.06 (0.01, 0.11)
	CA-SI	0.03 (-0.01, 0.07)	0.02 (-0.03, 0.08)	-0.07 (-0.10, -0.02)
Mass	BF-CA	0.32 (0.14, 0.51)	0.46 (0.31, 0.61)	-0.16 (-0.40, 0.07)

		Temperature	I+H	I+T
Survival	BF-CA	0.30 (0.19, 0.43)	0.07 (0.03, 0.11)	0.21 (0.16, 0.27)
	BF-SI	0.30 (0.18, 0.43)	0.03 (-0.02, 0.07)	0.21 (0.15, 0.26)
	CA-SI	0.00 (-0.07, 0.06)	-0.04 (-0.08, -0.01)	-0.01 (-0.03, 0.02)
Mass	BF-CA	0.73 (0.48, 0.97)	-0.28 (-0.55, -0.01)	0.84 (0.59, 1.09)

		H+T	I+H+T
Survival	BF-CA	0.02 (0.01, 0.03)	0.00 (0.00, 0.01)
	BF-SI	0.01 (0.00, 0.03)	0.00 (0.00, 0.01)
	CA-SI	0.00 (-0.01, 0.00)	0.00 (0.00, 0.00)
Mass	BF-CA	–	–

CHAPTER 3

CONSIDERABLE GENETIC DIVERSITY AND STRUCTURE DESPITE NARROW ENDEMISM AND LIMITED ECOLOGICAL SPECIALIZATION IN THE HAYDEN'S RINGLET, *COENONYMPHA HAYDENII*

Abstract

Understanding he processes that underlie the development of population genetic structure is central to the study of evolution. Patterns of genetic structure, in turn, can reveal signatures of isolation by distance, barriers to gene flow, or even the genesis of speciation. However, it is unclear how severe range restriction might impact the processes that dominate the development of genetic structure. In narrow endemic species, is population structure likely to be adaptive in nature, or rather the result of genetic drift? In this study, we investigated patterns of genetic diversity and structure in the narrow endemic Hayden's ringlet butterfly. Specifically, we asked to what degree genetic structure in the Hayden's ringlet can be explained by isolation by distance, isolation by resistance (in the form of geographic or ecological barriers to migration between populations), and isolation by environment (in the form of differences in host plant availability and preference). We employed a genotyping-by-sequencing (GBS) approach coupled with host preference assays, Bayesian modeling, and population genomic analyses to answer these questions. Our results suggest that despite their restricted range, levels of genetic diversity in the Hayden's ringlet are comparable to those seen in more widespread butterfly species. Hayden's ringlets showed a strong preference for feeding on grasses relative to sedges, but neither larval preference nor potential host availability at sampling sites correlated with genetic structure. We conclude that geography, in the form of isolation by resistance and simple isolation by distance, was the major driver of contemporary patterns of differentiation in this narrow endemic species.

Keywords: *Coenonympha haydenii*, population structure, hierarchical Bayesian models, narrow endemism

Introduction

Determining the evolutionary processes underlying the development of population genetic structure can provide important insights into the causes and potential consequences of evolution. Patterns of genetic structure, or the organization of genetic diversity across geographic space, can help reveal contemporary gene flow and migratory routes [e.g., Gompert et al., 2021, Hemstrom et al., 2022], ecological specialization [e.g., Chaturvedi et al., 2018, Ferrari et al., 2012, Michell et al., 2023, Nosil et al., 2008], patterns of admixture [e.g., Prüfer et al., 2014], or even the initial stages of speciation [Avise et al., 2000, Harvey et al., 2017, Mayr, 1942]. The development of genetic structure is driven by three major evolutionary processes: genetic drift, gene flow, and natural selection [Wright, 1931]. But the degree to which each of these processes dominate—and what patterns of structure might arise as a result—depends heavily on geographic, ecological, and demographic conditions.

Narrow endemism (restriction of a species' range to a limited geographic area relative to dispersal capacity) is a condition that would, at first glance, appear to limit the potential for genetic structure to develop. Historically, it was predicted that narrow endemic species should show low levels of genetic diversity [Frankham, 1997, Soltis and Soltis, 1991]. At small population sizes, genetic drift will more readily drive alleles to fixation, leading to loss of diversity over time [Gillespie, 2001, Montgomery et al., 2000, Rivera-Ortíz et al., 2015, Wright, 1931]. Low levels of genetic diversity coupled with a narrowly limited geographic range (relative to dispersal capacity and habitat heterogeneticy) would seem to leave little genetic or geographic potential for differentiation to arise among populations. But a growing body of evidence suggests that endemic species—particularly plants—can show both high levels of genetic diversity [Forrest et al., 2017, Medrano and Herrera, 2008] as well as substantial genetic structure [Hobbs et al., 2013, Jiménez-Mejías et al., 2015, Turchetto et al., 2016]. But is genetic structure in endemic species likely to be adaptive in nature [see Robitzch et al., 2023], or simply the result of limited gene flow and drift?

To help tease apart the processes driving the development of genetic structure, we can categorize the patterns of structure into three major cases. These three cases are not mutually exclusive; the question is the degree to which each case contributes to overall patterns of structure. In the simplest case, population genetic structure can arise from a combination of geographic distance and genetic drift alone [Wright, 1943]. This pattern is known as isolation by distance (IBD), and occurs when intrinsic limitations to dispersal lead to non-random mating and the accumulation of genetic differences across space via genetic drift, even in a perfectly uniform environment [Slatkin, 1993, Wright, 1943]. If narrow endemic species experience a greater degree of genetic drift due population size limitations, then it might be predicted that IBD should more often be a key driver of patterns of structure in such species.

In cases where the environment connecting populations is not uniform, geographic or ecological barriers (e.g., mountains, rivers, low host availability) can reduce rates of gene flow among populations. Reduced rates of gene flow, in turn, can drive differentiation among isolated populations via genetic drift [Rivera-Ortíz et al., 2015]. Geographically or ecologically favorable conditions, on the other hand, can create corridors of increased gene flow, homogenizing populations [Sharma et al., 2013, Slatkin, 1987]. These conditions can result in patterns of genetic differentiation correlated with functional connectivity (i.e., heterogeneity in resistance of the landscape to gene flow) rather than physical distances (i.e. isolation by resistance, or IBR [McRae, 2006, Moreno-Contreras et al., 2023, Thomas et al., 2015]. Because narrow endemics occur within a limited geographic range, in some cases there simply may not be enough environmental variation within a narrow endemic's range to result in substantial patterns of IBR. However, in many cases narrow endemic species are associated with ecologically unique environments and may be ecological specialists [for example, species endemic to white sands or serpentine soils; see Anacker, 2014, Anacker et al., 2011, Lavergne et al., 2004, Metzler, 2014, Nery et al., 2023]. If narrow endemism is coupled with niche specialization, then narrow endemic species might be more likely to experience habitat fragmentation—especially if they also exhibit limited dispersal capacity—allowing

isolation by resistance to develop even on a fine geographic scale.

Finally, if individual populations occupy ecologically divergent environments (as opposed to geographic or environmental barriers existing between two or more equivalent environments), natural selection can drive population divergence via local adaptation, resulting in a pattern known as isolation by environment (which subsumes isolation by adaptation) [Driscoe et al., 2019, Funk et al., 2011, Luna et al., 2023, Nosil et al., 2008, 2009, Orsini et al., 2013, Wang and Bradburd, 2014]. Isolation by environment is specifically characterized genetic differentiation increasing with environmental differences between populations that are independent of geographic distances [Sexton et al., 2014, Wang and Bradburd, 2014]. This can occur as a result of direct selection at loci affecting fitness, as well as indirect selection at neutral loci [Nosil et al., 2008, 2009]. Thus, this pattern is the result of divergent selection coupled with reduced effective rates of gene flow (either via increased immigrant mortality or reduced hybrid fitness) increasing the potential for genetic hitchhiking, as well as limiting the extent to which gene flow erases the effects of natural selection [Nosil et al., 2008,?, 2009, Wang and Bradburd, 2014]. Natural selection is more likely to overcome the effects of genetic drift when effective population sizes are large, and higher levels of standing genetic variation provide more raw material upon which natural selection can act. While narrow endemism implies that a species occurs over a limited geographic range, it does not imply that population sizes and genetic diversity levels are necessarily low. To the contrary, depending on various factors such as body size and local carrying capacity, small geographic ranges (from a human perspective) can support large, viable populations of some species. Moreover, the degree to which genetic diversity—particularly adaptive genetic diversity—decreases with range size reduction is predicted to be initially slow, with major reductions in diversity occurring only after the majority of a species' range has been eliminated [Exposito-Alonso et al., 2022]. As narrow endemic species have increasingly been shown to harbor unexpectedly high levels of genetic diversity [Forrest et al., 2017, Medrano and Herrera, 2008], there is a possibility that patterns of genetic structure in such species could be adaptive in nature.

As narrow endemism is associated with increased extinction risk [Frankham, 1998, Pitman and Jørgensen, 2002], determining the degree to which genetic structure in narrow endemic species reflects patterns of local adaptation vs. genetic drift could have vital consequences for conservation. In species where genetic structure has arisen largely as a result of anthropogenic habitat fragmentation and drift [Johansson et al., 2007, Ripperger et al., 2013], populations might be better managed as a single unit. On the other hand, populations showing patterns of local adaptation might harbor vital adaptive variation, as well as show an increased risk for outbreeding depression due to high immigrant mortality or low hybrid fitness if not managed separately [Frankham et al., 2011]. More broadly, understanding the nature of genetic structure in narrow endemic species could help shed light on the degree to which range size might influence the processes that dominate the development of genetic structure.

In this study, we characterize patterns of genetic diversity and structure in the narrow endemic Hayden's ringlet butterfly. The Hayden's ringlet, *Coenonympha haydenii*, is a brown Satyrid butterfly found only in mountain meadows and forest clearings of southwestern Montana, southeastern Idaho, and western Wyoming (i.e., the Greater Yellowstone Ecosystem) [Debinski and Pritchard, 2002, Howe and Bauer, 1975, Pyle, 1981, Scott, 1992]. Known for both high local abundances [Caruthers and Debinski, 2006] and weak flying ability [Glassberg, 2001, Kaufman and Brock, 2003], it is possible that enough genetic variation and dispersal limitations could exist in this species to result in population genetic structure even at small spatial scales. Larvae of *C. haydenii* are thought to feed on one or more species of grasses (family Poaceae) or sedges (family Cyperaceae) [Debinski and Pritchard, 2002, Feltwell, 1993, Glassberg, 2001, Pyle, 1981]. Female Hayden's ringlets are associated with moist, hydric meadows or bogs [Pyle, 1981, Scott, 1992], and population sizes decline during periods of drought [Debinski et al., 2013]. This is consistent with the possibility that *C. haydenii* could be specialized on one or more endemic Yellowstone wetland species like sedges. Conversely, the congeneric and sympatric common ringlet (*Coenonympha tullia*) is known to be a broad generalist, even feeding successfully on introduced species such as

Kentucky bluegrass [Debinski and Pritchard, 2002]. If the Hayden's ringlet is also able to utilize multiple host plants, it is possible natural selection could be driving local adaptation to host plant use across its range, particularly in disrupted environments where novel, invasive grass species dominate. These factors make the Hayden's ringlet an ideal system for investigating the processes driving patterns of genetic structure in narrow endemic species. Specifically, in this study we asked the following questions: (1) how much genetic diversity and structure exists within the Hayden's ringlet, and (2) to what degree is the development of genetic structure in this narrow endemic species associated with (a) geographic distance and genetic drift alone (isolation by distance), (b) geographic or ecological barriers to dispersal between populations (isolation by resistance), and/or (c) ecological differences and local adaptation to larval host plants among sites (isolation by environment). This will provide much-needed data regarding host use and population connectivity in an iconic Yellowstone butterfly, as well as contribute another example of how genetic structure can develop in a narrow endemic species.

Materials and Methods

Butterfly Sample Collection

Over the course of two years, we collected adult *C. haydenii* specimens of both sexes from 14 sampling sites across the species' range (see Fig. 3.1a). We surveyed for *C. haydenii* presence at two additional locations in the Yellowstone Plateau region (AVP and GLR, see Table S1) and along approximately 10 miles of trail on the John D. Rockefeller Jr. Memorial Parkway, but we only observed a single Hayden's ringlet across this entire region. Due to low abundance between Yellowstone National Park and Grand Teton National Park, we were unable to collect butterflies from this area. At each of the 14 sites where Hadyen's ringlet populations were abundant, we collected an average of 27 butterflies per location (see Table S4 for specific sample sizes). Male butterflies were immediately frozen to preserve tissue for subsequent DNA extraction, while females were maintained temporarily in the lab for egg collection and oviposition preference assays and frozen afterwards. Butterfly specimens

sampled within Yellowstone and Grand Teton National Parks were collected under permits YELL-2018-SCI-8064, YELL-2019-SCI-8064, GRTE-2018-SCI-0041, and GRTE-2019-SCI-0055.

DNA Sequencing, Alignment, and Variant Calling

We used Qiagen DNeasy 96 Blood and Tissue Kits to extract DNA from the thoracic tissue of 287 butterfly specimens representing 14 sampling locations (see Fig. 3.1 and Table S4). When available, an equal number of male and female specimens were chosen for sequencing from each site. Reduced-representation restriction-fragment based DNA libraries were prepared for genotyping-by-sequencing (GBS) following methods similar to those in Gompert et al. [2014a]. Briefly, whole-genome DNA was digested using Mse1 and EcoR1 enzymes, ligated to custom barcode sequences, and amplified via PCR. Barcoded DNA fragments were then pooled across samples, purified, and size-selected using a BluePippin. DNA fragments between 300-450 bp were selected for sequencing. The resulting DNA fragment libraries were sequenced on the University of Texas Illumina HiSeq 4000 sequencing platform. The resulting DNA sequences were first filtered to remove PhiX sequences and poly-G tails. PhiX is a bacterial sequence introduced during HiSeq sequencing as an internal control. We used `SAMtools` version 1.10 and custom scripts to find and remove all reads that aligned to the PhiX reference genome, leaving 347,375,794 individual reads. Barcode sequences were then removed from these remaining reads using custom Perl scripts, allowing us to match each DNA sequence to the individual butterfly from which it came.

To date, no reference genome has been published for the Hayden's ringlet. In the absence of a full reference genome, we constructed a *de novo* set of reference contigs for *Coenonympha haydenii* using the program `CD-hit` version 4.8.1 [Li and Godzik, 2006]. See the Supplemental Information for further details regarding our construction of the reference contig set. Reads that were aligned to this reference contig set using `BWA` version 0.7.17-r1188 [Li and Durbin, 2009]. We used the `BWA aln` algorithm, with the total number of mismatches allowed per read (-n) set to 5, or approximately 6% of each read. We set seed length (-l) equal to 20 bp, and the maximum allowed mismatches in the seed sequence (-k)

equal to 2.

We identified sites with single nucleotide polymorphisms (SNPs) in our genomic data using `samtools` and `bcftools` version 1.9 [Li et al., 2009]. We used the original consensus caller (-c) to call variants, and set the threshold probability (-p) for accepting variants to 0.01 (i.e., we only called variants if the posterior probability the nucleotide was invariant was less than 0.01). Variants were then filtered for quality using custom Perl scripts. We retained variable sites for which there were at least 2x more reads than the number of individuals we sequenced (i.e., mean coverage per $\geq$2x), contained a minimum of 10 reads for the alternative allele (to filter out possible sequencing errors), and had a phred-scaled mapping quality >30. We removed variant sites with base-quality rank-sum test, mapping-quality rank-sum test, and read-position rank-sum test p-values less than 0.001, 0.0001, and 0.001 respectively. We also removed any variable sites missing data for 20% or more of the individuals we sequenced. We set a maximum read depth of 8000 (3 standard deviations greater than the mean coverage level across loci) to remove possible paralogs/gene families, and removed all SNPs located less than 2 bps apart along a contig. After quality filtering, we were left with a total of 9313 SNPs for downstream analysis.

Assessing Patterns of Genetic Diversity and Structure

To measure overall levels of genetic diversity in the Hayden's ringlet, we calculated both Watterson's θ (θ_W) and nucleotide diversity (π). We estimated both diversity statistics and their 95% block bootstrap intervals using the program `ANGSD` version 0.933-71-g604e1a4 [Korneliussen et al., 2014], which uses the full set of aligned contigs (not our quality-filtered SNP set) to account for uncertainty in the number of segregating sites present. We then calculated per-base-pair values of both θ_W and π based on the estimated number of bases sequenced from `ANGSD` using `R` version 4.2.2 [R Core Team, 2022].

To summarize patterns of genetic structure in the Hayden's ringlet, we first used the program `ENTROPY` version 2.0 to estimate admixture proportions [Gompert et al., 2014b, Shastry et al., 2021]. `ENTROPY` is a program similar to the admixture model in `STRUCTURE`, but has the added feature of accounting for uncertainty in genotypes as captured by geno-

type likelihoods. It uses a Bayesian framework to co-estimate genotypes and the proportion of a particular individual's genome that would be derived from each of K hypothetical source populations. The purpose of this in our case was not to estimate the optimal value of K, but rather to assess patterns of coarse vs. fine-scale substructure within the species. To this end, we ran `ENTROPY` for all K-values between two and seven using our 9313-SNP set as input. For each value of K, we ran 10 Markov chain Monte Carlo (MCMC) chains with a 10,000-step burn-in period, 20,000 sampling iterations, a thinning interval of 5, and a Dirichlet initialization value of 50. As an additional summary of genetic structure, we then conducted a PCA in `R` using the (unscaled) posterior genotype estimates from `ENTROPY`.

We used Nei's F_{ST} [Nei, 1973] to quantify the magnitude of the genetic differentiation among the sampled populations. To calculate this, we first used `estpEM` version 0.1 [Soria-Carrasco et al., 2014] to obtain a maximum likelihood estimate of allele frequencies for each SNP (N = 9313) for each population (N = 14) of Hayden's ringlets we sampled. The program `estpEM` uses an expectation-maximization (EM) algorithm to account for uncertainty in genotypes arising from finite coverage and sequencing error [Soria-Carrasco et al., 2014]. We set the tolerance level for EM convergence to 0.001, the maximum number of EM iterations to 20, and used our filtered genotype likelihood files split by population as input. With the allele frequency estimates for each population generated by `estpEM`, we then calculated pairwise Nei's F_{ST} values for each combination of populations, as well as overall F_{ST} across all populations. Briefly, we calculated the mean F_{ST} across all 9313 loci using the formula $F_{ST} = \frac{1/L \sum_{i=1}^{L}(H_T - H_S)}{1/L \sum_{i=1}^{L}(H_T)}$ where H_T is the expected heterozygosity for the total population (i.e. across all subpopulation), H_S is the average of the expected heterozygosities within each subpopulation, and L is the number of loci [Lucek et al., 2019]. These calculations were completed in `R`.

Tests for Isolation by Distance and Resistance

To determine the degree to which patterns of genetic structure in the Hayden's ringlet correlate to the geographic distances among sites (i.e. isolation by distance), we first conducted a Mantel test. We used the logit of pairwise F_{ST} and the natural log of euclidean

distances among sites to produce our genetic and geographic distance matrices for comparison. The Mantel test was conducted in R version 4.2.2 using the package `vegan` version 2.6-4 [Oksanen et al., 2022, R Core Team, 2022]. We used the Pearson correlation method, and ran the test for 999 permutations.

To identify geographic or ecological barriers to dispersal (i.e. isolation by resistance) separating *C. haydenii* sites, we used the statistical method Estimating Effective Migration Surfaces (`EEMS`), developed by Petkova et al. [2016]. `EEMS` is based on the stepping-stone model of migration, and estimates effective migration rates by comparing the actual degree of genetic differentiation found among sites to the expectation under a null isolation-by-distance model. The model uses a resistance distance, which is a distance from circuit theory, to integrate over all possible dispersal paths between pairs of populations [Petkova et al., 2016]. In contrast to some other circuit-theory based approaches [e.g., McRae, 2006, McRae et al., 2008], resistance distances are not defined *a priori* based on habitat features, but instead inferred from the data as part of model fitting [Petkova et al., 2016]. This allows the identification of geographic regions among sites that might be serving as either environmental or geographic barriers or conduits to gene flow. We ran `EEMS` using a grid density of N-demes = 50, 100, and 150 demes. The number of demes corresponds to the number of nodes EEMS produces in the triangular grid to which individual samples can be assigned. For each grid density level, we ran three MCMC chains of 4,000,000 steps each, a burn-in of 2,000,000 steps, and thinning interval of 9999.

Tests for Ecological Divergence Among Localities and Populations

We collected potential host plant specimens from 9 of our 14 sampling sites and species presence data from 12 of our 14 sampling sites as a measure of community assemblage. These data were collected to assess whether ecological differences among sampling sites correlate with genetic structure in the Hayden's ringlet (i.e. isolation by environment/adaptation). We additionally collected these data to serve as a record of potential host plants likely to be encountered by Hayden's ringlet populations across their range, and to inform which plant species would make the strongest candidates for oviposition and larval preference assays.

The larvae of the Hayden's ringlet are suggested to feed generally on grasses [Debinski and Pritchard, 2002, Glassberg, 2001, Kaufman and Brock, 2003], at least some of which may overlap with the host genera used by the closely-related common ringlet butterfly, *C. tullia*. As such, we collected voucher specimens of each unique species of *Poa*, *Stipa*, and *Melica* grasses found in sampling site meadows where Hayden's ringlets were observed. All three of these grass genera are known to be suitable hosts for the congeneric and often sympatric common ringlet butterfly (*Coenonympha tullia*). It has also been suggested that Hayden's ringlets may be able to feed on sedges (family Cyperaceae) [Feltwell, 1993, Pyle, 1981], so we collected voucher specimens of all species of *Carex* sedges we found as well. After collection, plant specimens from different sites were classified by morphotype (or species where possible), and differences in community assemblage among sites were assessed using the Sørensen index. The Sørensen index measures the number of species shared between two sites as compared to the total number of species present across both sites, with greater weight given to shared than to non-shared species [Hao et al., 2019].

As a second test for potential host use differences among populations of Hayden's ringlets, we conducted both female preference and larval preference assays and assessed differences in preference among populations. Since the preferred host(s) of the Hayden's ringlet are unknown, these assays were also conducted to determine whether this species is generally more likely to use grasses (Poaceae) or sedges (Cyperaceae) as their larval host. For these assays, we chose to compare preference for Hood's sedge (*Carex hoodii*) vs. Kentucky bluegrass (*Poa pratensis*). Both species are abundant throughout *C. haydenii's* range, and represent the two plant families Hayden's ringlets are hypothesized to feed upon: sedges (Cyperaceae) and grasses (Poaceae). Furthermore, the results of our plant community assemblage surveys showed that these species were the most well-represented members of their genus across our sampling sites, with *Carex hoodii* being observed at 10 out of 12 meadows and *Poa pratensis* being observed at 7 out of 12 meadows we collected Hayden's ringlets from.

Whereas feral Kentucky bluegrass was the most common species of *Poa* we found at

our sampling sites for this study, it is also a non-native species. Kentucky bluegrass was introduced to the Great Plains and Rocky Mountain region as a forage crop for domestic livestock [McArthur et al., 1995]. It is now one of the most abundant and widespread feral exotic plants in the region [Kauffman et al., 2023, Kay, 2001, McArthur et al., 1995], often reaching very high densities in meadows [Kay, 2001] and representing up to 40-50% of the vegetation cover in certain riparian regions across Yellowstone and the Grand Tetons [Kauffman et al., 2023]. Feral Kentucky bluegrass is especially prevalent in meadows overgrazed by bison and elk [Kauffman et al., 2023, Kay, 2001], and now represents one of the dominant grass species in the Lamar Valley of Yellowstone National Park [Hunter et al., 2018].

Due to the high abundance of this exotic species throughout the range of the Hayden's ringlet and its propensity to alter the ecology and community structure of meadows it invades [Sanderson et al., 2017], the presence of Kentucky bluegrass could impose a strong selective pressure on Hayden's ringlet populations, setting the stage for local adaptation. The response of butterfly species to the presence of novel, exotic plant species is both well-documented and varied, with non-native species in some cases creating an ecological trap [for example, if adult butterflies preferentially lay their eggs on an unsuitable, exotic host, e.g. Davis and Cipollini, 2014], and in other cases providing a lifeline for endangered species whose native host has gone extinct [Braga, 2023, Graves and Shapiro, 2003]. In either case, the invasion of exotic plant species can have a substantial ecological and evolutionary impact on butterfly populations, even in remote areas. Indeed, in another Yellowstone area butterfly (genus *Lycaeides*), certain local populations have adapted to feed on feral roadside alfalfa (*Medicago sativa*), with alfalfa-adapted populations showing reduced oviposition preference for their native hosts [Chaturvedi et al., 2018, Forister et al., 2020]. Similarly, the congener of the Hayden's ringlet, *Coenonympha tullia*, is both found in the greater Yellowstone area and known to successfully utilize Kentucky bluegrass as a larval host [Debinski and Pritchard, 2002]). Unlike the Hayden's ringlet, the common ringlet has rapidly expanded its range across the United States over the past 60 years [Wiernasz, 1983, 1989]. It is possible the

ability to feed on exotic species like Kentucky bluegrass could have played a role in this range expansion. Together, this makes Kentucky bluegrass both an ecologically relevant species to test as a potential host for the Hayden's ringlet, as well as a plant species with reasonable potential to be correlated with patterns of local adaptation in this species.

Finally, we chose harebell (*Campanula rotundifolia*) as a control group because it is a common herbaceous flower in the area [Craighead, 2005]. Harebell is often found growing in meadows in association with grassland communities [Stevens et al., 2012], and thus may realistically be encountered by *C. haydenii* larvae in the wild. Harebell stem leaves are also long and narrow like those of grasses and sedges [Craighead, 2005, McGhan, 2023], which allowed us to control for leaf shape and size during our larval preference assays.

We conducted oviposition preference assays following standard procedures described in Forister et al. [2009], and assessed differences in preference across populations using a hierarchical Bayesian model. Briefly, we collected adult female butterflies from eight of our sampling sites (see Table S4) and placed them individually in plastic cups containing three plant samples each: Hood's sedge (*Carex hoodi*), Kentucky bluegrass (*Poa pratensis*), and harebell (*Campanula rotundifolia*). All plant specimens used for these assays (from all three species) were collected from a meadow in greater Yellowstone area where Hayden's ringlets were abundant. Females were maintained in these cups for 72 hours, after which we counted the number of eggs adhered by each butterfly to each species of plant. Since female butterflies were given the choice of three plant species for oviposition, we modeled the number of eggs laid on each host plant multinomially. Specifically, we assumed the number of eggs laid on each host to follow the distribution multinomial$(P_{1:3}, n)$, where $P_{1:3}$ are the probabilities of oviposition on each host of the three host plants, and n is the total number of eggs laid. Each butterfly population was allowed its own oviposition probability values to account for potential differences in preference across populations. The oviposition probabilities ($P_{1:3}$) from each population were assigned a Dirichlet prior with $\alpha = \tau * S$. Here, the vector τ represents the global probability of oviposition on each host plant across all populations and S is a scaling factor that describes that variability in preference among

populations. Finally, τ was assigned a Dirichlet hyperprior with $\alpha = 1$, and S a uniform hyperprior with lower and upper bounds of 1 and 200, respectively. We fit our model using `rjags` version 4.3.1 [Plummer, 2003, 2013]. We ran three MCMC chains of 80,0000 sampling steps each, with a burn-in of 10,000 steps and thinning interval of 50. We checked convergence of the MCMC chains using the Gelman diagnostic [Gelman and Rubin, 1992].

After female oviposition preference assays were complete, all eggs laid in the oviposition cups were gently removed from their substrate and stored in vented petri dishes under ambient temperature and light conditions until they hatched (approximately 10 days). Within one day of hatching, we performed larval preference assays following standard protocols [Gamberale-Stille et al., 2014, Gómez Jiménez et al., 2014, Gu and Walter, 1999, Wang et al., 2017] to assess differences in larval feeding preferences across populations. We tested up to 40 neonate larvae each from 10 of our sampling sites (see Table S4). Larvae were placed in the center of petri dishes equidistant from three 1-cm long leaf segments representing each of our test species (Kentucky bluegrass, Hood's sedge, and harebell). We took pictures of the leaf tissue flattened between glass slides both before and after the 72 hour herbivory trial with a Canon EOS M6 camera. We used the program `ImageJ` version 1.52A [Schneider et al., 2012] to trace outlines around each leaf image and calculate leaf surface area both before and after herbivory. The surface area lost by each leaf was calculated as the surface area before herbivory minus the surface area after herbivory (measured in cm^2). In addition, each leaf was manually assigned a binary value indicating whether signs of herbivory (i.e. jagged leaf margins) were observed (see the Supplemental Information for more details about our ImageJ protocol).

We estimated larval preferences among populations using a hierarchical Bayesian model. In our model, we assumed leaf area lost during the herbivory assays could be attributed to two main causes: (1) larval feeding, and (2) shrinkage of the leaf tissue due to moisture loss over time. We assumed total leaf area loss to follow a normal distribution with a mean and standard deviation as follows:

$$\text{total leaf area lost} \sim \text{N}(\text{shrinkage} + \text{herbivory} * \mathbb{1},\ \sigma_{loss}).$$

Here, $\mathbb{1}$ is a binary indicator set equal to 1 if herbivory was observed, and equal to 0 if no herbivory was observed. Thus, in cases where herbivory was observed, mean leaf area lost was defined as the sum of shrinkage plus larval herbivory. If no herbivory was observed, mean leaf area lost was defined as shrinkage only. We defined herbivory as following a normal distribution where the mean (μ_{herb}) and standard deviation (σ_{herb}) were allowed to vary by each unique plant species $\times$ butterfly population combination. Shrinkage was defined as following a normal distribution where the mean (μ_{shrink}) and standard deviation (σ_{shrink}) were allowed to vary by host plant only since the population each caterpillar was obtained from should have no effect on the amount of moisture lost by each leaf over time. The standard deviation parameters σ_{loss} , σ_{shrink}, and σ_{herb} were all assigned gamma priors with parameters $k = 2$ and $\theta = 0.1$, while μ_{shrink} was assigned a normal prior with $\mu = 0$ and $\sigma = 2$. Meanwhile, μ_{herb} was defined as the sum of population and host effects multiplied by the probability of the caterpillar eating (P). The host effect was distributed normally with a mean of μ_{host} and standard deviation of 0.5, with μ_{host} assigned a normal prior of $\text{N}(0, 20)$. A normal prior was also placed on the population effect, but with a sum-to-zero constraint for model indentifiability and a gamma prior for the standard deviation ($k = 2$, $\theta = 0.1$). For each host plant species $\times$ butterfly population combination, the total number of trials where larvae consumed leaf tissue was assigned to a binomial distribution with n = number of trials and p = the probability of a larva eating. We wrote this model in the language STAN [Stan Development Team, 2022b] and implemented it using the R-interface `RStan` version 2.21.5 [Stan Development Team, 2022a]. We used a warm-up period of 15,000 steps and ran the model for 30,000 Hamiltonian Monte Carlo (HMC) steps.

Tests for Isolation by Environment

We quantified the degree to which patterns of genetic structure in the Hayden's ringlet are explained by geographic distance (i.e. isolation by distance) vs. ecological distance (i.e.

ecological differences between the sites themselves, isolation by environment) using three different metrics of ecological distance: (i) the potential host plants available, (ii) oviposition preference distances, and (iii) larval herbivory preference distances. Each ecological distance was analyzed separately. For this, we used a Bayesian linear mixed model introduced by Gompert et al. [2014a], which extends a similar maximum-likelihood model from Clarke et al. [2002]. This model accounts for the lack of independence among sampling site pairs (i.e. the genetic distance between populations A vs. B is not independent from the genetic distance between populations A vs. C because both comparisons include population A) [Gompert et al., 2014a]. We modeled the effect of geographic and ecological distance on logit F_{ST} as follows:

$$\text{logit}\left(F_{ST}{}_{ij}\right) = \beta_0 + \beta_{geo} X_{ij}^{geo} + \beta_{eco} X_{ij}^{eco} + \lambda_i + \lambda_j.$$

Where X_{ij}^{geo} is the geographic distance (calculated as Euclidean distances) between each pair of sites and X_{ij}^{eco} is either (i) the potential host plant community dissimilarity (as measured by the Sørensen index), (ii) the median difference in oviposition preference for Kentucky bluegrass (*Poa pratensis*), or (iii) the median difference in larval preference for Kentucky bluegrass (*Poa pratensis*) for each pairwise combination of populations. Population random effects are represented by λ_i and λ_j. All distances were centered and standardized prior to running the model to account for differences in unit scale. We fit this model in R using `rjags` version 4.3.1 [Plummer, 2003, 2013]. We ran 3 MCMC chains of 5000 sampling steps each, with a burn-in of 2000 steps and thinning interval of 5. We fit the full model shown above, along with sub-models including only geographic distance, only ecological distance, or neither distance (i.e. a null model). Deviance information criterion was used to compare the relative performance of the full model and sub-models for each ecological variable.

Results

Moderate genetic diversity and population structure exist in Hayden's Ringlet

Estimates of nucleotide diversity across populations of the Hayden's ringlet varied from $\pi = 0.00284$ at JSM (95% bootstrap interval 0.00281-0.00288) to $\pi = 0.00344$ at USL (95% bootstrap interval 0.00342-0.00347) (see Table 3.2). Estimates of θ_W were similar, ranging from a low of 0.00280 (95% bootstrap interval 0.00277-0.00282) at JSM to a high of 0.00360 (95% bootstrap interval 0.00359-0.00362) at BNP (see Table 3.2). Genetic structure across sites was moderate but notable, with an overall F_{ST} of 0.10. Pairwise F_{ST} comparisons (see Table 3.3) ranged from 0.0181 to 0.1191. The population pairs that showed the highest degree of genetic differentiation were USL vs. JSM ($F_{ST} = 0.1191$) and USL vs. PSP ($F_{ST} = 0.1071$). Meanwhile, the least-differentiated population pairs were TRL vs. BNP ($F_{ST} = 0.0181$) and HRP vs. MRF ($F_{ST} = 0.0186$). JSM and SKI, which are located very closely in geographic space (∼5 km apart, see Fig. 3.1a and Table 3.3) nevertheless showed a degree of differentiation comparable to population pairs much further apart in geographic space ($F_{ST} = 0.0609$). Principal component analysis (PCA) shows individuals clustering by sampling site (see Fig. 3.1b). In particular, we saw that PC 1 separates the northern Hayden's ringlet populations from southern populations, while PC 2 separates the southern populations of Hayden's ringlets along a NE to SW gradient. The PCA does not perfectly mirror the map of our sampling locations, but is nevertheless suggestive of isolation by distance.

Admixture analysis (Fig. 3.2) showed the presence of meaningful structure across populations of Hayden's ringlets across multiple levels of K. The most prominent pattern was a clinal split between the northern and southern populations of Hayden's ringlets at K=2. Higher values of K revealed additional substructure within the species. At $K = 3$, `ENTROPY` split the southern populations of Hayden's ringlets along a North-South axis. In particular, we saw the southernmost population of Hayden's ringlets, PSP, being separated from the remainder of the populations. Similarly, $K = 4$ split the northern populations across a roughly West-East axis, separating northern populations east of the Gallatin mountain range (BNP, HNV, TRL) from those west of this range (GNP, WTC). Higher levels of K continued to refine the northeast-to-southwest clinal pattern seen across the southern

populations of Hayden's ringlets. A small number of individual butterflies (specifically from BCR, MRF, GNP, and HNV) showed ancestry values that differed considerably from both the typical values of their own population, as well as those of other populations we surveyed. This suggests that these individuals could be migrants or of mixed ancestry. Overall, our admixture analysis suggests that the greatest degree of genetic differentiation in the Hayden's ringlet exists between northern and southern populations, with additional substructure occurring within those geographic regions.

Isolation by distance and resistance both contribute to population structure in *C. haydenii*

We saw a strong signal for isolation by distance (see Fig. 3.4), with the Mantel test showing a significant and strong correlation between geographic and genetic distance in the Hayden's ringlet (R = 0.7, P = 0.001). In addition to isolation by distance (IBD), `EEMS` analysis showed several geographic areas with credibly increased or reduced relative migration rates (see Fig. 1c and 1d). Results for each of the three chains for grid sizes of 50, 100, and 150 were similar (see Fig. S1). There were several geographic areas within *C. haydenii*'s range where genetic differentiation among populations was either lower (low resistance) or higher (high resistance) than expected under a null IBD model alone, a pattern consistent with isolation by resistance. In particular, we saw a region of credibly reduced relative migration rates separating the northern and southern populations in our study, consistent with results from PC1 of the PCA (see Fig. 1d and 1b). This geographic region of credibly reduced gene flow produced by the `EEMS` model corresponds to the location of the Yellowstone plateau, roughly following the southern edge of the geothermally active Yellowstone volcanic area (see Fig. 3.1a). There was also a region of credibly increased relative migration connecting the majority of the southern populations of Hayden's ringlets with the exception of PSP, the southernmost population. This region of increased connectivity among southern Hayden's ringlet populations follows the river valley region known as Jackson hole, a low-elevation region between the Teton and Gros Ventre mountain regions (see Fig. 3.1a). The southernmost population (PSP)—which showed credibly lower levels of

gene flow with the remaining ringlet populations than expected under a null IBD model—is separated from the Jackson hole valley region by the Wyoming mountain range.

C. haydenii shows strong preference for grass host, but limited evidence for isolation by environment

All populations of *C. haydenii* we assessed laid credibly more eggs on Kentucky bluegrass than expected if females had no oviposition host preference (posterior probability [p.p.] for percent oviposition on *P. pratensis* $> 33\% > 0.98$ for all, see Fig. S5). Oviposition rates on Kentucky bluegrass varied from a low of 51% (95% CI 33-65%) to a high of 74% (95% CI 61-87%) across populations. The median global preference for oviposition on Kentucky bluegrass across populations was 57% (95% CI 47-67%; p.p. percent oviposition on *P. pratensis* $> 33\% > 0.99$), while the global preference for oviposition on Hood's sedge, *Carex hoodii*, was only 24% (95% CI 17-35%; p.p. percent oviposition on *C. hoodii* $< 33\% = 0.96$). Median global preference for oviposition on harebell, our control species, was the lowest at only 17% (95% CI 10-26%; p.p. preference for *C. rotundifolia* $< 0.33 > 0.99$), 16 percentage points lower than expected if butterflies distributed their eggs equally across available substrates. The strength of oviposition preference varied credibly between several Hayden's ringlet population pairs, with both TRL and BTB showing credibly higher rates of oviposition on *Poa pratensis* than PIN, HRP, and WTC (p.p. > 0.99 for all six comparisons).

As with oviposition, Hadyen's ringlet larvae showed a strong preference for Kentucky bluegrass, *Poa pratensis*. The species-level preference for Kentucky bluegrass produced by the Bayesian model was 71% (95% CI 64%-79%), meaning we would expect 71% of the leaf tissue consumed by a randomly sampled group of Hayden's ringlet larvae to be from Kentucky bluegrass when given a choice of Kentucky bluegrass, Hood's sedge, and harebell. Unlike in the female oviposition assays, no harebell herbivory was observed from any of the larvae we assayed. All populations we assayed showed a trend toward consuming more grass (*Poa pratensis*) than sedge (*Carex hoodii*), with every population consuming credibly more grass than sedge (p.p. consumed more grass than sedge > 0.99) except SKI (p.p. SKI

consumed more grass than sedge = 0.85).

The proportion of each host plant species eaten by larvae varied considerably by population. BCR and HRP showed the greatest preference for *Poa pratensis*, consuming 100% grass (95% CI 87-100% and 75-100% respectively) and 0% *Carex hoodii* sedge (95% CI 0-13% and 0-25% respectively). SKI, meanwhile, showed the lowest degree of herbivory preference, consuming 56.4% *Poa pratensis* grass (95% CI 44-71%) vs. 44% *Carex hoodii* sedge (95% CI 29-56%). Due to differences in total leaf tissue consumption across populations (25 out of 45 population pairs showed credible differences), we assessed differences in host preference across populations as differences in the proportion of grass vs. sedge leaf tissue consumed (see the Supplemental Information for details). We saw credible differences in the proportion of grass vs. sedge leaf tissue consumed for 21 out of 45 pairwise population comparisons. The pairs with the greatest differences in preference were BCR vs. SKI and HRP vs. TRL, with BCR and HRP consuming 42.1 (95% CI 27-55) and 41.9 (95% CI 17-55) percentage points more *Poa pratensis* grass and 42.1 (95% CI 27-55) and 41.9 (95% CI 17-55) percentage points less *Carex hoodii* than SKI and TRL respectively.

Despite finding credible differences in larval feeding and oviposition preferences across populations, we found no evidence that these differences correlated with genetic distances among Hayden's ringlet populations. The credible intervals for both the effect of larval preference and oviposition preference on logit(F_{ST}) overlapped zero (p.p. $\beta_{herb} > 0 = 22\%$; p.p. $\beta_{ovipos} > 0 = 36\%$, see Fig. 3.4c-d). This suggests that there is no measurable correlation between either larval host preference or oviposition preference for Kentucky bluegrass and genetic distances among Hayden's ringlet populations. The deviance information criterion (DIC) values for sub-models testing only the effect of larval preference (mean DIC = -21.65) or oviposition preference (mean DIC = -16) on genetic distance were substantially greater than for models and sub-models that included geographic distance as a variable (mean DIC ranged from -66 to -60). This suggests that our geographic distance models (both sub-models and the full models) better predict genetic distances in the Hayden's ringlet than models including oviposition or larval preference alone. Similarly, we found

no measurable effect of potential host community distance (as measured by the Sørensen index) on degree of genetic differentiation in the Hayden's ringlet (see Fig. 3.4b). The credible interval for β_{comm} overlapped zero (p.p. $\beta_{comm} > 0 = 73\%$), indicating there was no credible effect of the availability of *Poa*, *Stipa*, *Melica*, and *Carex* species across sites on genetic differentiation in the Hayden's ringlet. The DIC value for the sub-model including only host community as a variable was 15, while the sub-model and full model including geographic distance ranged from -63 to -62, again suggesting that the sub-model including only host community information was less predictive than models containing geographic distance information. Taken together, our data suggest that isolation by adaptation to the host plant communities we measured (a form of isolation by environment) is unlikely to be a driver of patterns of contemporary genetic structure in the Hayden's ringlet.

Discussion

In this study, we assessed patterns of genetic diversity and structure in the narrow endemic Hayden's ringlet. We also assessed patterns of oviposition and larval host preference, and used Bayesian methods and `EEMS` modeling to assess the role of isolation by distance, barriers to disperal (i.e. isolation by resistance), and potential host availability and preference (i.e. isolation by environment) contribute to population structure in this species. Our results indicate that despite range restriction, the Hayden's ringlet shows genetic diversity levels comparable to other more widely-distributed species. The Hayden's ringlet also appears to consistently prefer grass (*Poa pratensis*) over sedge (*Carex hoodii*), but this host association is unlikely to be driving patterns of population structure. Instead, we found that both isolation by distance and barriers to dispersal were most closely associated with genetic distances in this species. We discuss the implications of these results in more detail below.

Narrow endemism not associated with notable genetic diversity reduction in the Hayden's ringlet

Despite its restricted distribution, the Hayden's ringlet showed levels of genetic diversity

comparable to more widely-distributed butterfly species. The average nucleotide diversity across Hayden's ringlet populations we sampled was $\pi = 0.003$, while nucleotide diversity in *Leptidea* sp., *Lycaeides melissa*, and *Parnassius mnemosyne* (all widely-distributed, non-migratory butterfly species) ranged from $\pi = 0.001$ to $\pi = 0.005$ [Gompert et al., 2014b, Talla et al., 2019, 2023]. In contrast, both migratory monarchs (*Danaeus plexippus*) and non-migratory *Heliconius* sp. showed comparatively high nucleotide diversity (π = 0.01-0.06 and 0.020-0.28, respectively [Hemstrom et al., 2022, Kryvokhyzha, 2014, Martin et al., 2016, Talla et al., 2020]. Migratory butterfly species have been shown to harbor higher levels of genetic diversity than non-migratory species in general, possibly due to greater population sizes and connectivity [García-Berro et al., 2023], so the substantial difference in nucleotide diversity between monarchs and Hayden's ringlets is not unexpected. However, *Heliconius* species are both non-migratory and have low dispersal ability [Kronforst and Fleming, 2001], so why this species group shows far higher genetic diversity levels than reported in other non-migratory species is unclear.

Many butterfly species have wide distributions, but are locally rare. The Hayden's ringlet, by contrast, is narrowly restricted in range, but locally prolific. Within their range, Hayden's ringlets are often so abundant they are the most common butterfly species surveyed [Caruthers and Debinski, 2006]. High local abundances in the Hayden's ringlet could be one factor contributing to the maintenance of genetic diversity in this species. Conversely, poor dispersal (as seen in *Lycaeides melissa* and *Parnassius mnemosyne*) [Gompert et al., 2010, Gorbach and Kabanen, 2010, Talla et al., 2019] or poor connectivity among populations could lead to high levels of genetic drift, reducing nucleotide diversity estimates in more widespread butterfly species. In particular, the widely-distributed *Lycaeides melissa* is known for low local population sizes, patchy distributions and metapopulation dynamics (Gompert et al. 2010, 2012, Scott 1992; but also see Guiney et al. 2010). While even low levels of gene flow can be enough to maintain nucleotide diversity across populations–even in the face of low effective population sizes for individual demes and substantial genetic drift [Gompert et al., 2021, Whitlock and Barton, 1997]–the more widespread a species

is, the more likely it is that insurmountable geographic barriers to gene flow (even if this barrier is distance alone) might exist within their distribution. This could cause widespread species to behave more similarly to multiple, smaller demes with no gene flow amongst them than a single, panmictic population. Thus, the genetic diversity levels maintained in widely-distributed butterfly species might be expected to be more similar to those of geographically restricted species than global census sizes alone would suggest [Gompert et al., 2010]. This could help explain why genetic diversity levels in non-migratory butterfly species do not appear to scale linearly with population size in nature (i.e. Lewontin's paradox) [Charlesworth and Jensen, 2022, Gompert et al., 2021, Lewontin et al., 1974].

In all, the similarity in diversity levels between the Hayden's ringlet vs. widely-distributed butterfly species suggest this is yet another case where narrow endemism is not associated with a notable reduction in genetic diversity. This adds to a growing body of research showing that even narrow endemic species can still harbor substantial genetic diversity [Forrest et al., 2017, Hobbs et al., 2013, Jiménez-Mejías et al., 2015, Medrano and Herrera, 2008, Robitzch et al., 2023]. That said, nucleotide diversity amongst eukaryotes ranges from approximately $\pi = 0.001$ to $\pi = 0.15$ [Charlesworth and Jensen, 2022], placing the Hayden's ringlet firmly on the low end for eukaryotes as a whole. Other butterfly species with similar nucleotide diversity levels to the Hayden's ringlet have been targeted for conservation efforts [Talla et al., 2023]. But neutral diversity should not be conflated with adaptive genetic diversity. Simulations suggest that loss of adaptive genetic diversity is likely to proceed more slowly than loss of neutral genetic diversity [Exposito-Alonso et al., 2022], so one must be cautious in presuming that species with low nucleotide diversity and a limited distribution necessarily lack adaptive genetic potential. Nucleotide diversity levels alone are not sufficient to interpret whether or not the Hayden's ringlet is a species of conservation concern. While its narrow distribution put the Hayden's ringlet at greater risk of extirpation due to natural disasters (e.g., catastrophic fires or volcanic activity across the entire Yellowstone area), high local abundances coupled with genetic diversity levels comparable to more widely-distributed butterfly species suggests that the Hayden's ringlet is not nec-

essarily at higher conservation risk due to genetic factors [i.e. inbreeding depression, etc.; see Frankham, 2005] than many other non-migratory, geographically widespread butterfly species.

Geography informs patterns of population genetic structure in the Hayden's ringlet

We saw clear evidence of population structure across the range of the Hayden's ringlet. The strongest signal of genetic differentiation was a geographic split between northern and southern populations of *C. haydenii*, with additional genetic substructure occurring within each of these groups.

The correlation between geographic and genetic distances in the Hayden's ringlet was R = 0.7, substantially higher than correlations seen in many other non-migratory butterfly species. Specifically, correlations between geographic and genetic distance for the Langue's metalmark (*Apodemia mormo langei*), heath fritillaries (*Melitaea athalia* and *Melitaea celadussa*), and checkerspots (*Euphydryas aurinia* and *Euphydryas editha*) ranged between R = 0.39 and R= 0.53 [Dupuis et al., 2018, Mikheyev et al., 2013, Tahami et al., 2021]. This suggests that isolation by distance is able to explain a greater degree of the population structure observed in the Hayden's ringlet than in other non-migratory butterfly species. The high correlation between genetic and geographic distances in the Hayden's ringlet suggests much of the population structure observed in this narrow endemic species can be attributed to genetic drift and limited dispersal.

Despite the clear patterns of genetic structure present in this species, F_{ST} values between populations of Hayden's ringlets were low to moderate. The scale of differentiation we observed is consistent with fine- to moderate-scale genetic population structure (F_{ST} between 0.01-0.2) seen in other non-migratory butterfly species [Hinojosa et al., 2023, Pertoldi et al., 2021, Talla et al., 2019, 2023], and on average greater than in migratory species like monarchs (F_{ST} = 0.0001) [Talla et al., 2020]. While the F_{ST} values we observed may be considered low in other groups of organisms, in many cases F_{ST} values between nominal species of butterflies are not considerably greater than what we found within populations of

the Hayden's ringlet [i.e. Tahami et al., 2021, Talla et al., 2019], and in some cases variation within butterfly species is higher than that observed between species. For example, in the El Segundo blue (*Euphilotes battoides allyni*), F_{ST} among populations of the same species ranged from 0.1 to 0.5 [Dupuis et al., 2020], while in heath fritillaries, F_{ST} between two nominal species (*Melitaea celadussa* and *Melitaea athalia*) was only 0.1-0.2 [Tahami et al., 2021]. Thus, our results are clearly in-line with results from other butterfly species, and consistent with expectations for a non-migratory species with limited dispersal ability.

We saw several geographic regions with credibly increased or reduced relative migration rates in the Hayden's ringlet. The largest of these was a wide region of credibly reduced relative gene flow between northern and southern *C. haydenii* populations corresponding to the southern border of the Yellowstone plateau and John D. Rockefeller, Jr. Memorial Parkway. Despite having visited two additional sites (Avalanche Peak AVP, Grassy Lake Reservoir GLR; see Table S1 for coordinates) and surveyed approximately 10 miles of trail in this region, we found no viable populations of Hayden's ringlets connecting our northern and southern sampling sites. Much of the habitat in this region consisted of dense lodgepole pine monocultures and previous burn sites [Parmenter et al., 2003, Rothermel, 1994, Turner and Simard, 2017]. Hayden's ringlets prefer open grassy meadows and sunny forest edges [Debinski and Pritchard, 2002, Kaufman and Brock, 2003], so this densely-forested region could present an ecological barrier to migration. Regardless, the fact that our field observations are consistent with the results from our EEMS model suggests that this geographic region presents a true barrier to gene flow for the Hayden's ringlet, and that isolation by resistance contributes to patterns of genetic structure in this species. Interestingly, the geographic split we found between northern and southern *C. haydenii* populations corresponds to a similar boundary observed between northern *Lycaeides idas* populations and southern, admixed *Lycaeides* [Gompert et al., 2010, 2012]. This suggests that a combination of geographic (elevation; mountain ranges) and ecological (forest type) conditions present in the John D. Rockefeller, Jr. Memorial Parkway may present a barrier to gene flow more generally, and could apply to other non-migratory butterfly species in the greater Yellowstone

ecosystem as well.

Despite being a non-migratory species known for poor flight [Glassberg, 2001, Kaufman and Brock, 2003], we nevertheless saw evidence of long-distance dispersal in *C. haydenii*. Several individuals in our admixture analysis matched neither the population from which they were sampled, nor any other population we sampled. In particular, one individual each from MRF, GNP, and HNV in our admixture plots did not match the admixture proportions of any other butterflies we sampled. These individuals appear to be either of mixed origin or migrants from an area we did not sample. One individual from BCR, on the other hand, appears to be a migrant from PSP (or near PSP). The distance between PSP and BCR is over 65 km, indicating that long-distance dispersal does occur in *C. haydenii* at least occasionally. Hayden's ringlets are notoriously poor fliers [Glassberg, 2001, Kaufman and Brock, 2003], so we expect typical dispersal distances in the Hayden's ringlet to be similar to those reported for other poor dispersers like *Lycaeides melissa*, *Parnassius* sp., and *Heliconius* sp. [Gompert et al., 2010, Gorbach and Kabanen, 2010, Kronforst and Fleming, 2001], which rarely disperse further than 2 km during their lifetime. We suggest that the instances of long-distance dispersal we report here are likely a result of rare gene flow events such as butterflies being blown long distances during adverse weather conditions. But as even small amounts of gene flow are sufficient to erase patterns of genetic differentiation, these occasional long-distance dispersal events likely still play a role in determining the magnitude of population genetic structure present in this species.

Strong preference for grass host, but no evidence of isolation by environment in the Hayden's ringlet

We observed strong oviposition and larval herbivory preference for Kentucky bluegrass (*Poa pratensis*) over Hood's sedge (*Carex hoodii*) in *C. haydenii*. Preference for grass was both strong and remarkably consistent, with all populations showing a credible preference for *Poa* in both oviposition and herbivory assays with the exception of SKI. While it has been previously suggested that Hayden's ringlets might feed on sedges due to their association with bogs and hydric habitats [Pyle, 1981, Scott, 1992], our evidence overwhelmingly

points to grasses as being the preferred host of the Hayden's ringlet. However, the fact that larvae did often feed on both the sedge and grass host, while completely refusing the control host, suggests that Hayden's ringlets may accept more than one host, and are more likely generalist feeders like their congener the common ringlet, *C. tullia*, than narrow host-specialists [Debinski and Pritchard, 2002, Scott, 1992]. This is consistent with preliminary host acceptance data we collected which showed that Hayden's ringlet larvae will consume tissue from many genera of grasses and sedges including *Stipa*, *Carex*, *Poa*, *Phleum*, and *Elymus* when given no other choice. Anecdotal evidence also suggests that Hayden's ringlet larvae can be reared to adulthood on *Carex* species [Stout, 2017], which would indeed suggest that the Hayden's ringlet is a broad generalist given their strong preference for *Poa*. That said, our study only compared only a single species of sedge with a single species of grass. It is possible these species alone are not sufficient to provide a full picture of *C. haydenii's* preference for grasses vs. sedges. Additional work is needed to further elucidate the degree of host specificity and preference in *C. haydenii*.

While the degree of preference for *Poa* varied credibly across populations, we saw no evidence of host-associated genetic differentiation across populations in the Hayden's ringlet. Neither potential host community differences nor differences in larval herbivory preference were predictive of genetic distances among Hayden's ringlet populations in our study. If the Hayden's ringlet is in fact a generalist feeder, and host use does not substantially impact larval fitness, then the composition and abundance of potential host species may have a limited effect on genetic differentiation. This could explain the absence of host-associated population structure we observed in this species. But how then do we interpret the phenotypic variation in host preference among populations we observed? It is possible the variation we saw reflects true variation for preference that exists among Hayden's ringlet populations in the wild. However, laboratory experiments must always be interpreted with caution with regard to their applicability in the field. In this case, we note that the Hayden's ringlet populations that showed the highest degree of herbivory preference also happened to be the populations that consumed the least total amount of plant material. Because

our preference measure was scaled by total tissue consumed, the lower the total level of consumption, the more sensitive (and stochastic) our preference measure will be to small differences in herbivory. In other words, when total consumption is low, each bite of tissue a larva consumes will have a proportionally larger impact on preference than that same bite of tissue in a case where total consumption is high. Thus, in cases where total herbivory was low, herbivory preferences have the potential to appear exaggerated compared to cases where larvae ate a greater amount of total leaf tissue.

If the Hayden's ringlet is not limited to feeding on a narrow endemic Yellowstone-area plant species, what might be driving current patterns of range restriction in the Hayden's ringlet? Since we only assayed two species of potential hosts, one of which is an invasive species, we cannot definitively say that host specialization is not a driver of genetic differentiation and narrow endemism in the Hayden's ringlet. But preliminary work we conducted on larval performance showed that Hayden's ringlet larvae can survive on Kentucky bluegrass through at least the 4th instar, at which point our larvae entered—and did not survive—diapause (likely due to mold and resultant fungal infection). Kentucky bluegrass, *Poa pratensis*, is one of the most widespread turf grass species in the United States [Huff et al., 2003]. It is ubiquitous along roadsides and in lawns, occurs in all 50 states, and is highly invasive across the northern Great Plains and Yellowstone region, forming high-density feral populations throughout Yellowstone and the Grand Teton National Parks [DeKeyser et al., 2015, Hunter et al., 2018, Kauffman et al., 2023, Kay, 2001, McArthur et al., 1995]. Counter to what might be expected if the presence of this novel, exotic species were exerting a strong selective pressure on Hayden's ringlet populations due to its unsuitability as a host, we saw no evidence that any of the populations we surveyed have developed a strong preference against feeding or ovipositing on this species. If Kentucky bluegrass is in fact a viable host for the Hayden's ringlet, it would strongly suggest that host specialization is not the key factor preventing contemporary range expansion in the Hayden's ringlet. Instead, other environmental factors not considered in this study, such as site elevation, temperature, rainfall, or forest cover, could play a greater role. In

particular, the fact that populations of Hayden's ringlets decline during periods of drought [Debinski et al., 2013] suggest that the Hayden's ringlet might be restricted to wetter habitats. Perhaps a factor other than host plant use could be driving *C. haydenii*'s association with wetland areas. Hayden's ringlets overwinter as larvae, so it is possible moisture levels could have an effect on larval survival through winter diapause. On the other hand, it has also been suggested that the Hayden's ringlet could be a narrow endemic today simply because it is a remnant species left behind from a larger, pre-glaciation distribution, and it's range simply has not yet returned to its former size [Pyle, 1981]. Unlike the Hayden's ringlet, the range of the common ringlet (*Coenonympa tullia*) is both able to use Kentucky bluegrass as a larval host and has expanded rapidly over the past 60 years [Debinski and Pritchard, 2002, Wiernasz, 1983, 1989]. This expansion is thought to have been driven in part by a shift from univolitinism to multivoltinism [Wiernasz, 1983, 1989]. Whether the Hayden's ringlet is univoltine or multivoltine does not appear to have been documented. If the Hayden's ringlet is obligately univoltine, this could help explain why the Hayden's ringlet has remained endemic, while its congener has become widespread in distribution. More exploration of the life history and ecological requirements of the Hayden's ringlet are necessary to more fully understand the causes of genetic structure and narrow endemism in this species.

Conclusions

Despite their restricted range, we found that the Hayden's ringlet harbors genetic diversity levels comparable to geographically widespread, non-migratory butterfly species with similar dispersal ability. We found strong evidence that the Hayden's ringlet prefers grasses (*Poa*) over sedges (*Carex*) as a larval host, but work to determine the degree of host specificity in this species remains to be done. Geography, specifically isolation by distance and isolation by resistance (i.e. barriers to dispersal such as mountain ranges and/or regions of poor habitat) appear to be the driving factors producing patterns of population structure in the Hayden's ringlet. We found no evidence that either host preferences or host availability were correlated with genetic divergence, and it does not appear that isolation

by environment is driving population divergence in this narrow endemic species. Instead, population structure in this species has likely developed largely via genetic drift, suggesting that the Hayden's ringlet would not necessarily benefit from being managed as more than one unit. That said, it is always possible that local adaptation to ecological factors we did not measure could be contributing to genetic structure in this species. Questions remain as to how evolutionary processes unfold in the face of narrow endemism, but in some cases at least, it appears that patterns of genetic diversity and structure in restricted vs. widespread species may not differ as greatly as one might initially suspect.

Acknowledgments

Thank you to Megan Brady for invaluable assistance with both field collections and preference experiments, to Angélica Traslaviña for many months of assistance with DNA extractions, and to both Camden Treat and Daniel Johnson for their extensive assistance cataloguing larval herbivory results. Support and resources from the Center for High Performance Computing at the University of Utah are gratefully acknowledged. This work was funded by the National Science Foundation (NSF GRFP awarded to AS, fellow 2017239847; NSF DEB 1844941 to ZG) and Utah State University.

REFERENCES

Anacker, B. L., 2014. The nature of serpentine endemism. American Journal of Botany 101:219–224.

Anacker, B. L., J. B. Whittall, E. E. Goldberg, and S. P. Harrison, 2011. Origins and consequences of serpentine endemism in the california flora. Evolution 65:365–376.

Avise, J. C. et al., 2000. Phylogeography: the History and Formation of Species. Harvard university press.

Braga, M. P., 2023. Are exotic host plants a life raft or a trap for butterflies? Current Opinion in Insect Science P. 101074.

Caruthers, J. C. and D. M. Debinski, 2006. Montane meadow butterfly species distributions in the greater yellowstone ecosystem. University of Wyoming National Park Service Research Center Annual Report 30:85–96.

Charlesworth, B. and J. D. Jensen, 2022. How can we resolve lewontin's paradox? Genome biology and evolution 14:evac096.

Chaturvedi, S., L. K. Lucas, C. C. Nice, J. A. Fordyce, M. L. Forister, and Z. Gompert, 2018. The predictability of genomic changes underlying a recent host shift in melissa blue butterflies. Molecular Ecology 27:2651–2666.

Clarke, R. T., P. Rothery, and A. F. Raybould, 2002. Confidence limits for regression relationships between distance matrices: estimating gene flow with distance. Journal of Agricultural, Biological, and Environmental Statistics 7:361–372.

Craighead, C., 2005. Common Wildflowers of Grand Teton National Park. Grand Teton Natural History Association.

Davis, S. L. and D. Cipollini, 2014. Do mothers always know best? oviposition mistakes and resulting larval failure of pieris virginiensis on alliaria petiolata, a novel, toxic host. Biological Invasions 16:1941–1950.

Debinski, D. M., J. C. Caruthers, D. Cook, J. Crowley, and H. Wickham, 2013. Gradient-based habitat affinities predict species vulnerability to drought. Ecology 94:1036–1045.

Debinski, D. M. and J. Pritchard, 2002. A field guide to butterflies of the Greater Yellowstone Ecosystem. Roberts Rinehart.

DeKeyser, E. S., L. A. Dennhardt, and J. Hendrickson, 2015. Kentucky bluegrass (poa pratensis) invasion in the northern great plains: a story of rapid dominance in an endangered ecosystem. Invasive Plant Science and Management 8:255–261.

Driscoe, A. L., C. C. Nice, R. W. Busbee, G. R. Hood, S. P. Egan, and J. R. Ott, 2019. Host plant associations and geography interact to shape diversification in a specialist insect herbivore. Molecular Ecology 28:4197–4211.

Dupuis, J. R., S. M. Geib, K. H. Osborne, and D. Rubinoff, 2020. Genomics confirms surprising ecological divergence and isolation in an endangered butterfly. Biodiversity and Conservation 29:1897–1921.

Dupuis, J. R., J. C. Oliver, B. M. Brunet, T. Longcore, J. J. Johnson, and F. A. Sperling, 2018. Genomic data indicate ubiquitous evolutionary distinctiveness among populations of california metalmark butterflies. Conservation Genetics 19:1097–1108.

Exposito-Alonso, M., T. R. Booker, L. Czech, L. Gillespie, S. Hateley, C. C. Kyriazis, P. L. Lang, L. Leventhal, D. Nogues-Bravo, V. Pagowski, et al., 2022. Genetic diversity loss in the anthropocene. Science 377:1431–1435.

Feltwell, J., 1993. The Encyclopedia of Butterflies. Prentice Hall.

Ferrari, J., J. A. West, S. Via, and H. C. J. Godfray, 2012. Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. Evolution 66:375–390.

Forister, M. L., C. C. Nice, J. A. Fordyce, and Z. Gompert, 2009. Host range evolution is not driven by the optimization of larval performance: the case of lycaeides melissa (lepidoptera: Lycaenidae) and the colonization of alfalfa. Oecologia 160:551–561.

Forister, M. L., S. A. Yoon, C. S. Philbin, C. D. Dodson, B. Hart, J. G. Harrison, O. Shelef, J. A. Fordyce, Z. H. Marion, C. C. Nice, et al., 2020. Caterpillars on a phytochemical landscape: The case of alfalfa and the melissa blue butterfly. Ecology and Evolution 10:4362–4374.

Forrest, A., M. Escudero, M. Heuertz, Y. Wilson, E. Cano, and P. Vargas, 2017. Testing the hypothesis of low genetic diversity and population structure in narrow endemic species: the endangered antirrhinum charidemi (plantaginaceae). Botanical Journal of the Linnean Society 183:260–270.

Frankham, R., 1997. Do island populations have less genetic variation than mainland populations? Heredity 78:311–327.

———, 1998. Inbreeding and extinction: island populations. Conservation biology 12:665–675.

———, 2005. Genetics and extinction. Biological conservation 126:131–140.

Frankham, R., J. D. Ballou, M. D. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster, 2011. Predicting the probability of outbreeding depression. Conservation Biology 25:465–475.

Funk, D. J., S. P. Egan, and P. Nosil, 2011. Isolation by adaptation in neochlamisus leaf beetles: Host-related selection promotes neutral genomic divergence. Molecular Ecology 20:4671–4682.

Gamberale-Stille, G., L. Söderlind, N. Janz, and S. Nylin, 2014. Host plant choice in the comma butterfly–larval choosiness may ameliorate effects of indiscriminate oviposition. Insect science 21:499–506.

García-Berro, A., V. Talla, R. Vila, H. K. Wai, D. Shipilina, K. G. Chan, N. E. Pierce, N. Backström, and G. Talavera, 2023. Migratory behaviour is positively associated with genetic diversity in butterflies. Molecular Ecology 32:560–574.

Gelman, A. and D. B. Rubin, 1992. Inference from iterative simulation using multiple sequences. Statistical Science 7:457–472.

Gillespie, J. H., 2001. Is the population size of a species relevant to its evolution? Evolution 55:2161–2169.

Glassberg, J., 2001. Butterflies through binoculars: the west. Oxford University Press.

Gómez Jiménez, M. I., C. E. Sarmiento, M. F. Díaz, A. Chautá, A. Peraza, A. Ramírez, and K. Poveda, 2014. Oviposition, larval preference, and larval performance in two polyphagous species: does the larva know best? Entomologia Experimentalis et Applicata 153:24–33.

Gompert, Z., L. K. Lucas, C. A. Buerkle, M. L. Forister, J. A. Fordyce, and C. C. Nice, 2014a. Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Molecular ecology 23:4555–4573.

———, 2014b. Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Molecular ecology 23:4555–4573.

Gompert, Z., L. K. Lucas, J. A. Fordyce, M. L. Forister, and C. C. Nice, 2010. Secondary contact between lycaeides idas and l. melissa in the rocky mountains: extensive admixture and a patchy hybrid zone. Molecular ecology 19:3171–3192.

Gompert, Z., L. K. Lucas, C. C. Nice, J. A. Fordyce, M. L. Forister, and C. A. Buerkle, 2012. Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. Evolution 66:2167–2181.

Gompert, Z., A. Springer, M. Brady, S. Chaturvedi, and L. K. Lucas, 2021. Genomic time-series data show that gene flow maintains high genetic diversity despite substantial genetic drift in a butterfly species. Molecular Ecology 30:4991–5008.

Gorbach, V. and D. Kabanen, 2010. Spatial organization of the clouded apollo population (parnassius mnemosyne) in onega lake basin. Entomological Review 90:11–22.

Graves, S. D. and A. M. Shapiro, 2003. Exotics as host plants of the california butterfly fauna. Biological conservation 110:413–433.

Gu, H. and G. Walter, 1999. Is the common sowthistle (sonchus oleraceus) a primary host plant of the cotton bollworm, helicoverpa armigera (lep., noctuidae)? oviposition and larval performance. Journal of Applied Entomology 123:99–105.

Guiney, M. S., D. A. Andow, and T. T. Wilder, 2010. Metapopulation structure and dynamics of an endangered butterfly. Basic and Applied Ecology 11:354–362.

Hao, M., J. J. Corral-Rivas, M. S. González-Elizondo, K. N. Ganeshaiah, M. G. Nava-Miranda, C. Zhang, X. Zhao, and K. Von Gadow, 2019. Assessing biological dissimilarities between five forest communities. Forest Ecosystems 6:1–8.

Harvey, M. G., G. F. Seeholzer, B. T. Smith, D. L. Rabosky, A. M. Cuervo, and R. T. Brumfield, 2017. Positive association between population genetic differentiation and speciation rates in new world birds. Proceedings of the National Academy of Sciences 114:6328–6333.

Hemstrom, W. B., M. G. Freedman, M. P. Zalucki, S. R. Ramírez, and M. R. Miller, 2022. Population genetics of a recent range expansion and subsequent loss of migration in monarch butterflies. Molecular Ecology 31:4544–4557.

Hinojosa, J. C., C. Montiel-Pantoja, M. Sanjurjo-Franch, I. Martínez-Pérez, K. M. Lee, M. Mutanen, and R. Vila, 2023. Diversification linked to larval host plant in the butterfly eumedonia eumedon. Molecular Ecology .

Hobbs, J.-P. A., L. Van Herwerden, D. R. Jerry, G. P. Jones, and P. L. Munday, 2013. High genetic diversity in geographically remote populations of endemic and widespread coral reef angelfishes (genus: Centropyge). Diversity 5:39–50.

Howe, W. H. and D. L. Bauer, 1975. the Butterflies of north America. Garden City, NY: Doubleday.

Huff, D. R., M. Casler, and R. Duncan, 2003. Kentucky bluegrass. Turfgrass biology, genetics, and breeding. Wiley, Hoboken, NJ Pp. 27–38.

Hunter, H. E., P. O. Husby, J. Fidel, and J. C. Mosley, 2018. Ecological health of grasslands and sagebrush steppe on the northern yellowstone range. Rangelands 40:212–223.

Jiménez-Mejías, P., M. Fernández-Mazuecos, M. E. Amat, and P. Vargas, 2015. Narrow endemics in european mountains: high genetic diversity within the monospecific genus pseudomisopates (plantaginaceae) despite isolation since the late pleistocene. Journal of Biogeography 42:1455–1468.

Johansson, M., C. R. Primmer, and J. Merilä, 2007. Does habitat fragmentation reduce fitness and adaptability? a case study of the common frog (rana temporaria). Molecular Ecology 16:2693–2700.

Kauffman, J. B., D. L. Cummings, C. Kauffman, R. L. Beschta, J. Brooks, K. MacNeill, and W. J. Ripple, 2023. Bison influences on composition and diversity of riparian plant communities in yellowstone national park. Ecosphere 14:e4406.

Kaufman, K. and J. Brock, 2003. Field guide to butterflies of north america. Hillstar Editions LC .

Kay, C., 2001. Long-term aspen exclosures in the Yellowstone ecosystem. US Department of Agriculture, Forest Service, Rocky Mountain Research ....

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen, 2014. ANGSD: Analysis of next generation sequencing data. BMC Bioinformatics 15:356.

Kronforst, M. R. and T. H. Fleming, 2001. Lack of genetic differentiation among widely spaced subpopulations of a butterfly with home range behaviour. Heredity 86:243–250.

Kryvokhyzha, D., 2014. Whole genome resequencing of heliconius butterflies revolutionizes our view of the level of admixture between species.

Lavergne, S., J. D. Thompson, E. Garnier, and M. Debussche, 2004. The biology and ecology of narrow endemic and widespread plants: a comparative study of trait variation in 20 congeneric pairs. Oikos 107:505–518.

Lewontin, R. C. et al., 1974. The genetic basis of evolutionary change, vol. 560. Columbia University Press New York.

Li, H. and R. Durbin, 2009. Fast and accurate short read alignment with burrows–wheeler transform. bioinformatics 25:1754–1760.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin, 2009. The sequence alignment/map format and samtools. Bioinformatics 25:2078–2079.

Li, W. and A. Godzik, 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

Lucek, K., Z. Gompert, and P. Nosil, 2019. The role of structural genomic variants in population differentiation and ecotype formation in timema cristinae walking sticks. Molecular ecology 28:1224–1237.

Luna, L. W., L. N. Naka, G. Thom, L. L. Knowles, A. O. Sawakuchi, A. Aleixo, and C. C. Ribas, 2023. Late pleistocene landscape changes and habitat specialization as promoters of population genomic divergence in Amazonian floodplain birds. Molecular Ecology 32:214–228.

Martin, S. H., M. Möst, W. J. Palmer, C. Salazar, W. O. McMillan, F. M. Jiggins, and C. D. Jiggins, 2016. Natural selection and genetic diversity in the butterfly heliconius melpomene. Genetics 203:525–541.

Mayr, E., 1942. Systematics and the origin of species, from the viewpoint of a zoologist. Harvard University Press.

McArthur, E. D., A. C. Blauer, S. B. Monsen, and S. C. Sanderson, 1995. Plant inventory, succession, and reclamation alternatives on disturbed lands in grand teton national park. *in* Proceedings: Wildland Shrub and Arid Land Restoration Symposium, Pp. 343–358. Intermountain Research Station.

McGhan, P. J. R., 2023. United states department of agriculture forest service, plant of the week: Harebell (campanula rotundifolia l.). `https://www.fs.usda.gov/wildflowers/plant-of-the-week/campanula_rotundifolia.shtml`. Accessed on 2023-01-27.

McRae, B. H., 2006. Isolation by resistance. Evolution 60:1551–1561.

McRae, B. H., B. G. Dickson, T. H. Keitt, and V. B. Shah, 2008. Using circuit theory to model connectivity in ecology, evolution, and conservation. Ecology 89:2712–2724.

Medrano, M. and C. M. Herrera, 2008. Geographical structuring of genetic diversity across the whole distribution range of narcissus longispathus, a habitat-specialist, mediterranean narrow endemic. Annals of Botany 102:183–194.

Metzler, E. H., 2014. The remarkable endemism of moths at white sands national monument in new mexico, usa, with special emphasis on gelechioidea (lepidoptera). Journal of Asia-Pacific Biodiversity 7:e1–e5.

Michell, C. T., N. Wagner, M. Mutanen, K. M. Lee, and T. Nyman, 2023. Genomic evidence for contrasting patterns of host-associated genetic differentiation across shared host-plant species in leaf-and bud-galling sawflies. Molecular Ecology .

Mikheyev, A. S., C. S. McBride, U. G. Mueller, C. Parmesan, M. R. Smee, C. Stefanescu, B. Wee, and M. C. Singer, 2013. Host-associated genomic differentiation in congeneric butterflies: Now you see it, now you do not. Molecular ecology 22:4753–4766.

Montgomery, M. E., L. M. Woodworth, R. K. Nurthen, D. M. Gilligan, D. A. Briscoe, and R. Frankham, 2000. Relationships between population size and loss of genetic diversity:

comparisons of experimental results with theoretical predictions. Conservation Genetics 1:33–43.

Moreno-Contreras, I., A. Llanes-Quevedo, L. A. Sánchez-González, M. d. C. Arizmendi, and A. G. Navarro-Sigüenza, 2023. Isolation by resistance explains genetic diversity in the Arremon brushfinches of northern Mesoamerica. Molecular Ecology 32:3450–3470.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proceedings of the national academy of sciences 70:3321–3323.

Nery, E. K., M. K. Caddah, M. F. Santos, and A. Nogueira, 2023. The evolution of ecological specialization underlies plant endemism in the atlantic forest. Annals of Botany P. mcad029.

Nosil, P., S. P. Egan, and D. J. Funk, 2008. Heterogeneous genomic differentiation between walking-stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. Evolution 62:316–336.

Nosil, P., D. J. Funk, and D. Ortiz-Barrientos, 2009. Divergent selection and heterogeneous genomic divergence. Molecular ecology 18:375–402.

Oksanen, J., G. Simpson, F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'hara, P. Solymos, M. Stevens, E. Szoecs, et al., 2022. Vegan: Community ecology package, r package version 2.6-4.

Orsini, L., J. Vanoverbeke, I. Swillen, J. Mergeay, and L. De Meester, 2013. Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. Molecular ecology 22:5983–5999.

Parmenter, A. W., A. Hansen, R. E. Kennedy, W. Cohen, U. Langner, R. Lawrence, B. Maxwell, A. Gallant, and R. Aspinall, 2003. Land use and land cover change in the greater yellowstone ecosystem: 1975–1995. Ecological Applications 13:687–703.

Pertoldi, C., A. Ruiz-Gonzalez, S. Bahrndorff, N. Renee Lauridsen, T. Nisbeth Henriksen, A. Eskildsen, and T. T. Høye, 2021. Strong isolation by distance among local populations

of an endangered butterfly species (euphydryas aurinia). Ecology and Evolution 11:12790–12800.

Petkova, D., J. Novembre, and M. Stephens, 2016. Visualizing spatial population structure with estimated effective migration surfaces. Nature genetics 48:94–100.

Pitman, N. C. and P. M. Jørgensen, 2002. Estimating the size of the world's threatened flora. Science 298:989–989.

Plummer, M., 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. *in* Proceedings of the 3rd International Workshop on Distributed Statistical Computing, vol. 124, P. 10. Vienna, Austria.

———, 2013. rjags: Bayesian graphical models using MCMC. R package version 3.

Prüfer, K., F. Racimo, N. Patterson, F. Jay, S. Sankararaman, S. Sawyer, A. Heinze, G. Renaud, P. H. Sudmant, C. De Filippo, et al., 2014. The complete genome sequence of a Neanderthal from the Altai Mountains. Nature 505:43–49.

Pyle, R. M., 1981. The Audubon society field guide to North American butterflies. Knopf; distributed by Random House.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL `https://www.R-project.org`.

Ripperger, S. P., M. Tschapka, E. K. Kalko, B. Rodriguez-Herrera, and F. Mayer, 2013. Life in a mosaic landscape: anthropogenic habitat fragmentation affects genetic population structure in a frugivorous bat species. Conservation Genetics 14:925–934.

Rivera-Ortíz, F., R. Aguilar, M. Arizmendi, M. Quesada, and K. Oyama, 2015. Habitat fragmentation and genetic variability of tetrapod populations. Animal Conservation 18:249–258.

Robitzch, V., P. Saenz-Agudelo, T. J. Alpermann, B. Frédérich, and M. L. Berumen, 2023. Contrasting genetic diversity and structure between endemic and widespread damselfishes are related to differing adaptive strategies. Journal of Biogeography 50:380–392.

Rothermel, R. C., 1994. Fire growth maps for the 1988 Greater Yellowstone Area fires, vol. 304. US Department of Agriculture, Forest Service, Intermountain Research Station.

Sanderson, M. A., H. Johnson, M. A. Liebig, J. R. Hendrickson, and S. E. Duke, 2017. Kentucky bluegrass invasion alters soil carbon and vegetation structure on northern mixed-grass prairie of the united states. Invasive Plant Science and Management 10:9–16.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012. Nih image to imagej: 25 years of image analysis. Nature methods 9:671–675.

Scott, J. A., 1992. The butterflies of North America: a natural history and field guide. Stanford University Press.

Sexton, J. P., S. B. Hangartner, and A. A. Hoffmann, 2014. Genetic isolation by environment or distance: which pattern of gene flow is most common? Evolution 68:1–15.

Sharma, S., T. Dutta, J. E. Maldonado, T. C. Wood, H. S. Panwar, and J. Seidensticker, 2013. Forest corridors maintain historical gene flow in a tiger metapopulation in the highlands of central india. Proceedings of the Royal Society B: Biological Sciences 280:20131506.

Shastry, V., P. E. Adams, D. Lindtke, E. G. Mandeville, T. L. Parchman, Z. Gompert, and C. A. Buerkle, 2021. Model-based genotype and ancestry estimation for potential hybrids with mixed-ploidy. Molecular ecology resources 21:1434–1451.

Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

———, 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

Soltis, P. S. and D. E. Soltis, 1991. Genetic variation in endemic and widespread plant species. Aliso: A Journal of Systematic and Floristic Botany 13:215–223.

Soria-Carrasco, V., Z. Gompert, A. A. Comeault, T. E. Farkas, T. L. Parchman, J. S. Johnston, C. A. Buerkle, J. L. Feder, J. Bast, T. Schwander, et al., 2014. Stick insect genomes reveal natural selection's role in parallel speciation. Science 344:738–742.

Stan Development Team, 2022a. RStan: the R interface to Stan. URL `https://mc-stan.org/`. R package version 2.21.5.

———, 2022b. Stan modeling language users guide and reference manual. URL `https://mc-stan.org/`. Version 2.30.

Stevens, C. J., J. Wilson, and H. A. McAllister, 2012. Biological flora of the british isles: Campanula rotundifolia. Journal of Ecology 100:821–839.

Stout, T., 2017. Personal communication. `http://www.raisingbutterflies.org/raising_butterflies_301/`.

Tahami, M. S., V. Dincă, K. M. Lee, R. Vila, M. Joshi, M. Heikkilä, L. Dapporto, S. Schmid, P. Huemer, and M. Mutanen, 2021. Genomics reveal admixture and unexpected patterns of diversity in a parapatric pair of butterflies. Genes 12:2009.

Talla, V., A. Johansson, V. Dincă, R. Vila, M. Friberg, C. Wiklund, and N. Backström, 2019. Lack of gene flow: narrow and dispersed differentiation islands in a triplet of leptidea butterfly species. Molecular ecology 28:3756–3770.

Talla, V., V. Mrazek, J. Höglund, and N. Backström, 2023. Whole genome re-sequencing uncovers significant population structure and low genetic diversity in the endangered clouded apollo (parnasssius mnemosyne) in sweden. Conservation Genetics Pp. 1–10.

Talla, V., A. A. Pierce, K. L. Adams, T. J. de Man, S. Nallu, F. X. Villablanca, M. R. Kronforst, and J. C. de Roode, 2020. Genomic evidence for gene flow between monarchs with divergent migratory phenotypes and flight performance. Molecular ecology 29:2567–2582.

Thomas, L., W. J. Kennington, M. Stat, S. P. Wilkinson, J. T. Kool, and G. A. Kendrick, 2015. Isolation by resistance across a complex coral reef seascape. Proceedings of the Royal Society B: Biological Sciences 282:20151217.

Turchetto, C., A. L. A. Segatto, G. Mäder, D. M. Rodrigues, S. L. Bonatto, and L. B. Freitas, 2016. High levels of genetic diversity and population structure in an endemic and rare species: implications for conservation. AoB Plants 8.

Turner, M. G. and M. Simard, 2017. Using spatial statistics and landscape metrics to compare disturbance mosaics. Learning Landscape Ecology: A Practical Guide to Concepts and Techniques Pp. 175–190.

Wang, I. J. and G. S. Bradburd, 2014. Isolation by environment. Molecular ecology 23:5649–5662.

Wang, Y., Y. Ma, D.-S. Zhou, S.-X. Gao, X.-C. Zhao, Q.-B. Tang, C.-Z. Wang, and J. J. van Loon, 2017. Higher plasticity in feeding preference of a generalist than a specialist: experiments with two closely related helicoverpa species. Scientific Reports 7:1–12.

Whitlock, M. C. and N. H. Barton, 1997. The effective size of a subdivided population. Genetics 146:427–441.

Wiernasz, D. C., 1983. Range expansion and rapid evolution in Coenonympha tullia (Lepidoptera): Ecological and genetic change in a new environment. Princeton University.

———, 1989. Ecological and genetic correlates of range expansion in coenonympha tullia. Biological Journal of the Linnean Society 38:197–214.

Wright, S., 1931. Evolution in Mendelian populations. Genetics 16:97.

———, 1943. Isolation by distance. Genetics 28:114.

Data Accessibility and Benefit-Sharing

Data Accessibility Statement

Raw sequence reads have been deposited in NCBI's SRA (BioProject PRJNA1036281). Scripts, ecological data and downstream genetic data are available from Dryad (`https://doi.org/10.5061/dryad.zw3r228g3`)

Benefit-Sharing Statement

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

Author Contributions

AS and ZG designed the research. AS performed the research. AS and ZG analyzed the data. AS wrote the paper, with guidance and editing from ZG.

Tables and Figures

Table 3.1: Collection locations and sample sizes for the total number of adult butterflies collected from each site, the total number of specimens from which DNA was extracted and sequenced, the number of female butterflies for which oviposition preference assays were conducted, the number of female butterflies that produced offspring for the larval herbivory assays, and the total number of larvae for which herbivory assays were conducted.

	Latitude	Longitude	Butterflies collected	DNA sequenced	Oviposition pref.	Mothers of larvae	Larval pref.
BCR	43.3007	-110.5530	30	24	0	6	28
BNP	44.9337	-110.7212	30	24	0	0	0
BTB	43.6382	-110.6820	10	10	9	7	40
GNP	45.4323	-111.2245	35	24	2	2	5
HNV	44.6823	-110.4945	30	23	0	0	0
HRP	43.8957	-110.6427	26	25	9	5	21
JSM	43.5107	-110.9862	5	5	0	0	0
MRF	43.8547	-110.3918	36	24	0	7	40
PIN	43.7398	-109.9762	33	24	12	7	40
PSP	42.7483	-110.8398	26	23	0	0	0
SKI	43.5094	-110.9227	48	24	12	3	16
TRL	44.9019	-110.1291	30	24	13	7	40
USL	43.5829	-110.3328	9	9	2	1	11
WTC	44.7849	-111.3088	31	24	7	6	40

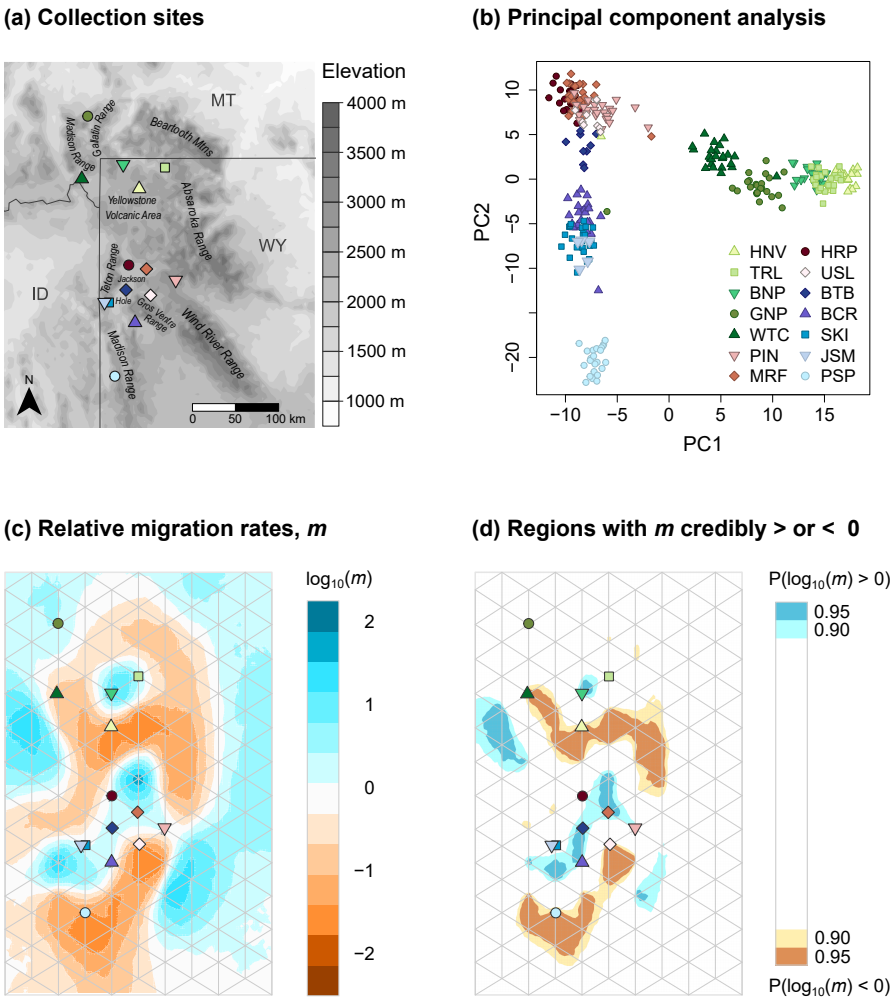


Fig. 3.1: (a) Map of butterfly sampling locations. Each sampling site is depicted as a colored point, the corresponding key for which is shown in panel (b). Elevation contours (in meters) are shown in gray, and major mountain ranges and valley regions within *C. haydenii's* range are labeled where they occur. (b) Principal component analysis of genotype estimates from ENTROPY for the 9313 SNPs. (c) Map of relative migration rates across *C. haydenii's* range as estimated by EEMS from SNP data. Areas with estimated migration rates lower than expected under isolation by distance (IBD) alone are shown in orange, and those with migration rates higher than expected under IBD are shown in blue. Because EEMS assigns individuals to the nearest vertex on a triangular grid, the locations of populations in the EEMS model do not correspond perfectly to the sampling locations on the geographic map shown in panel (a). (d) Geographic regions with relative migration rates credibly greater or less than zero.

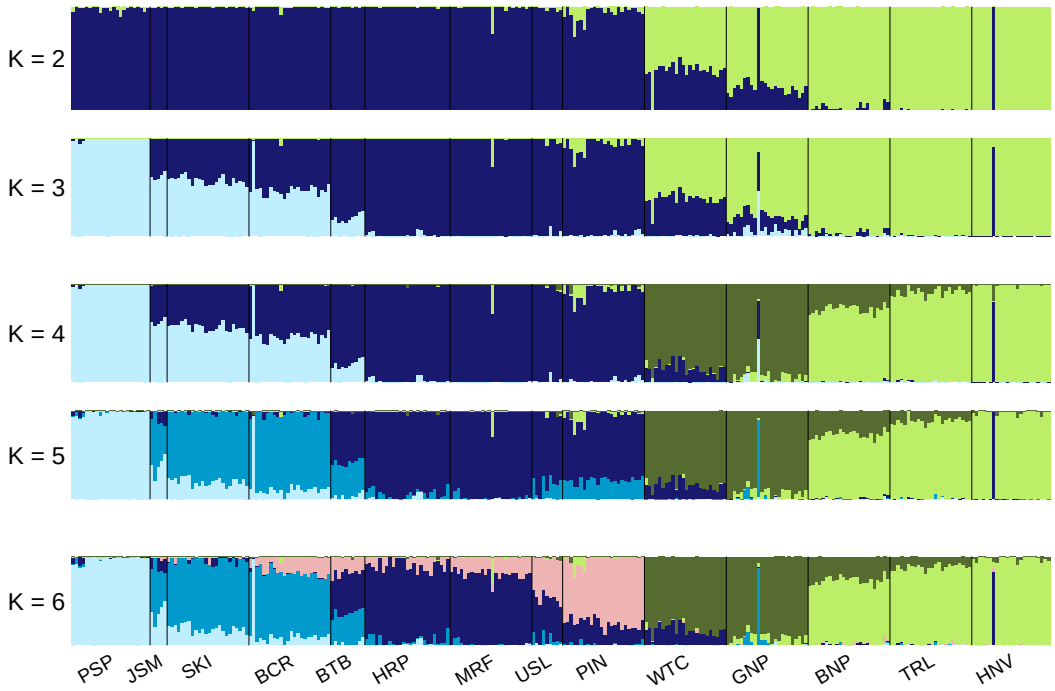


Fig. 3.2: Estimated admixture proportions assuming individuals were sampled from $K = 2$ through $K = 6$ hypothetical source populations. Each vertical segment on the barplot represents the estimated ancestry of an individual butterfly, with the proportion of each color in the segment representing the proportion of that butterfly's genome estimated to have been inherited from each of the K putative source populations. Individuals are grouped along the x-axis by population, with populations demarcated by vertical black bars.

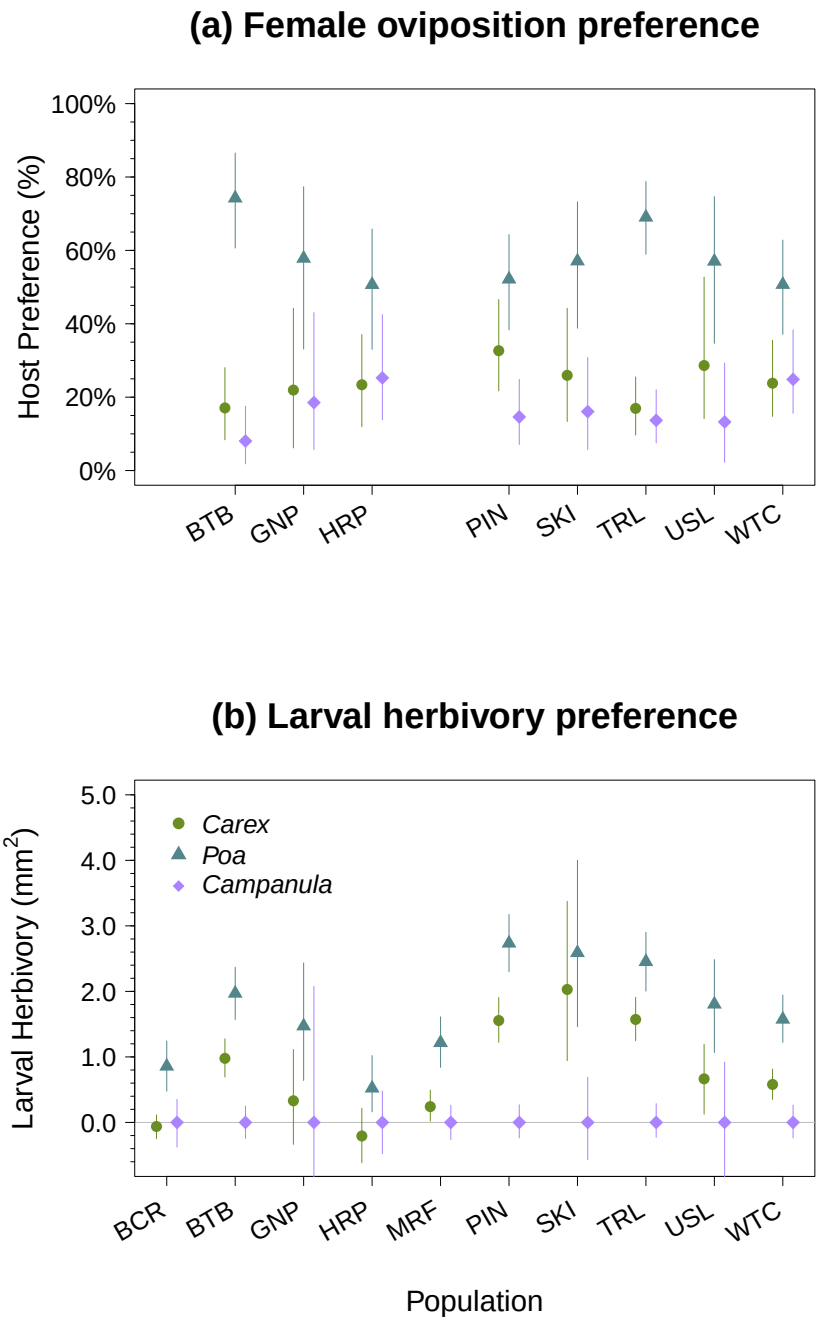


Fig. 3.3: (a) Oviposition preference for female *C. haydenii* from 8 of our sampling sites. (b) Differences in larval herbivory across hosts for each population assayed. The expected total leaf tissue consumption for a caterpillar from a given population is shown on the y-axis. Leaves offered to larvae during the hervivory assays had a mean surface area of 15.7 mm^2.

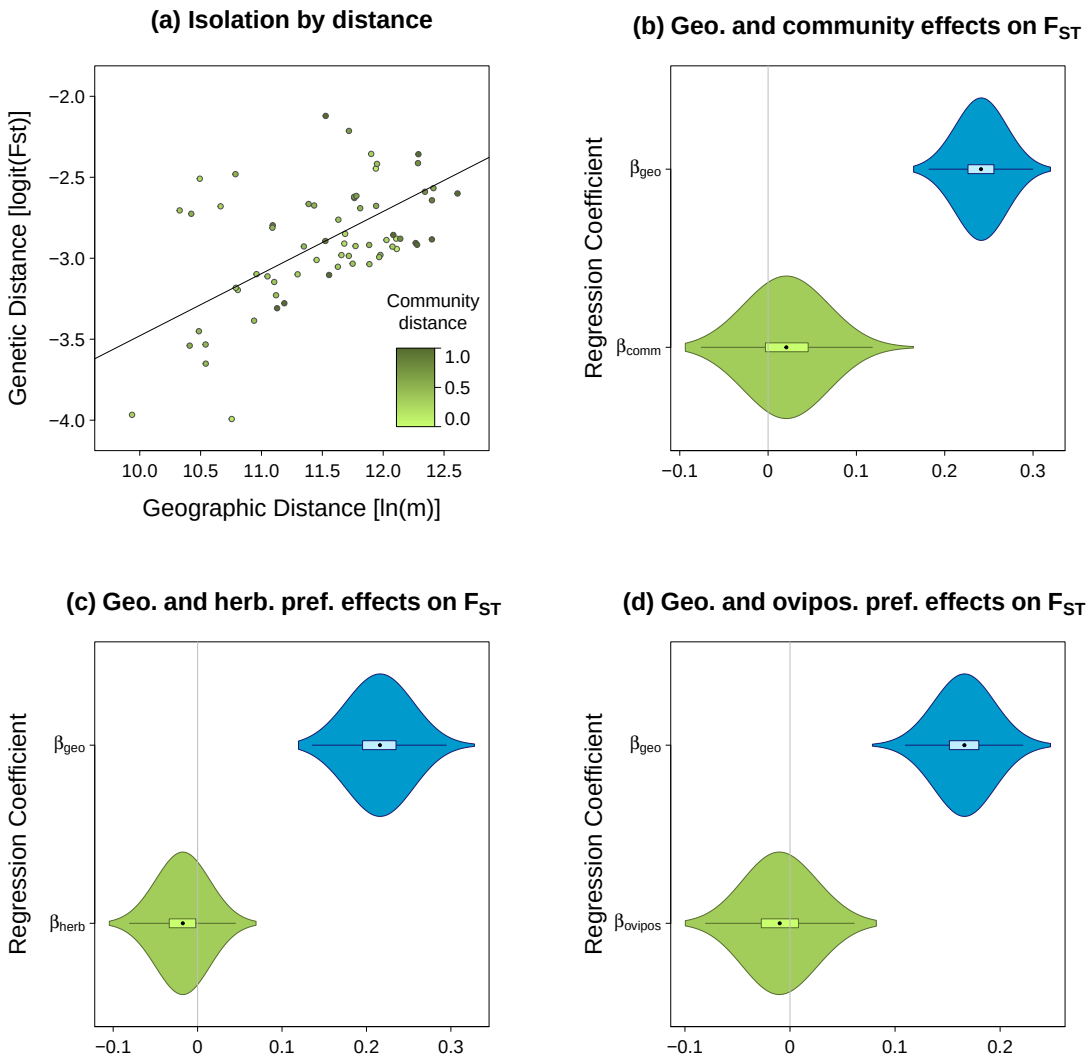


Fig. 3.4: (a) shows the linear relationship between genetic distance (as logit F_{ST}) vs. geographic distance (ln[meters]) modeled from each pairwise combination of source populations except BTB and JSM. The color of each point on the scatter plot corresponds to the potential host community distance between each pair of sites, with lighter points corresponding to more similar host communities between sites, and darker points corresponding to more disparate host communities among sites. (b-d) show the posterior distributions for the regression coefficients in our Bayesian models estimating the degree to which geographic distance and either potential host community distance, larval herbivory preference, or oviposition preference for Kentucky bluegrass predict genetic distance (logit F_{ST}). Posterior distributions are presented in centered and standardized units for ease of comparison across regression coefficients.

Table 3.2: Watterson's θ (θ_W) and nucleotide diversity (π) with 95% bootstrap confidence intervals.

Population	θ_w	π
BCR	0.00348 (0.00347, 0.00349)	0.00333 (0.00331, 0.00334)
BNP	0.00360 (0.00359, 0.00362)	0.00332 (0.00329, 0.00334)
BTB	0.00311 (0.00310, 0.00313)	0.00312 (0.00309, 0.00314)
GNP	0.00347 (0.00345, 0.00348)	0.00327 (0.00325, 0.00329)
HNV	0.00301 (0.00299, 0.00302)	0.00298 (0.00296, 0.00300)
HRP	0.00335 (0.00334, 0.00337)	0.00323 (0.00321, 0.00325)
JSM	0.00280 (0.00277, 0.00283)	0.00284 (0.00281, 0.00288)
MRF	0.00353 (0.00351, 0.00354)	0.00338 (0.00336, 0.00340)
PIN	0.00330 (0.00329, 0.00331)	0.00327 (0.00325, 0.00329)
PSP	0.00327 (0.00326, 0.00329)	0.00319 (0.00317, 0.00321)
SKI	0.00339 (0.00338, 0.00341)	0.00313 (0.00311, 0.00315)
TRL	0.00349 (0.00348, 0.00350)	0.00321 (0.00319, 0.00322)
USL	0.00324 (0.00322, 0.00326)	0.00344 (0.00342, 0.00347)
WTC	0.00330 (0.00329, 0.00331)	0.00308 (0.00306, 0.00310)

Table 3.3: Pairwise F_{ST} values calculated from EEMS genotype estimates and geographic distances between sampling locations. Pairwise F_{ST} values are shown in the lower triangle, while geographic distances between sampling locations are shown in the upper triangle in units of km.

Population	BCR	BNP	BTB	GNP	HNV	HRP	JSM
BCR		181.9	38.9	242.8	153.6	66.5	42.1
BNP	0.0501		144.0	68.1	33.2	115.5	159.5
BTB	0.0453	0.0556		204.0	117.0	28.8	28.4
GNP	0.053	0.0353	0.0605		101.3	176.9	214.4
HNV	0.0644	0.0282	0.072	0.0525		88.2	136.0
HRP	0.0412	0.0483	0.0384	0.0544	0.0651		51.0
JSM	0.0705	0.0869	0.0849	0.0889	0.1047	0.0787	
MRF	0.0426	0.0481	0.0385	0.0532	0.0645	0.0186	0.0815
PIN	0.0381	0.0458	0.0433	0.0518	0.0594	0.0327	0.0829
PSP	0.0575	0.0665	0.0734	0.0691	0.0822	0.0674	0.0795
SKI	0.0253	0.0483	0.0421	0.0513	0.0635	0.0398	0.0609
TRL	0.0532	0.0181	0.0629	0.043	0.0284	0.0547	0.0926
USL	0.0752	0.0797	0.075	0.0864	0.0985	0.0642	0.1191
WTC	0.0508	0.0393	0.0565	0.0363	0.0567	0.0451	0.0865

Table 3: *(continued)*

Population	MRF	PIN	PSP	SKI	TRL	USL	WTC
BCR	62.9	67.5	65.7	37.9	181.1	36.1	175.7
BNP	122.7	145.4	243.0	159.1	46.9	153.3	49.3
BTB	33.5	58.0	99.7	24.1	147.2	28.9	136.9
GNP	187.3	212.6	299.8	215.0	104.4	217.4	72.3
HNV	92.3	112.6	216.7	134.8	37.8	122.8	65.5
HRP	20.7	56.3	128.5	48.5	119.1	42.8	112.2
JSM	61.3	85.4	85.5	5.1	169.1	53.4	143.9
MRF		35.8	128.2	57.5	118.2	30.6	126.6
PIN	0.0308		130.6	80.6	129.7	33.6	157.5
PSP	0.0678	0.0682		84.8	246.0	101.5	229.4
SKI	0.0432	0.0431	0.0508		167.2	48.4	145.1
TRL	0.0517	0.0509	0.0713	0.0528		147.5	94.2
USL	0.0627	0.0615	0.1071	0.0772	0.0866		154.7
WTC	0.0459	0.0477	0.0698	0.0513	0.047	0.0819	

Supplemental Methods

Analyzing leaf images for herbivory with imageJ

We used `ImageJ` version 1.52A [Schneider et al., 2012] to analyze leaf photographs from our larval herbivory trials. Specifically, we analyzed images taken before and after the herbivory trials to determine the total surface area of each leaf consumed during the herbivory trial.

To calculate leaf surface areas, we first set the scale in each image using the `straight` tool in for drawing straight lines. A line was drawn across exactly two blue horizontal grid marks of the graph paper in each image, which corresponds to the known distance of 1.27 cm. To set this known distance as the scale, we used the commands `analyze`→`set scale`, set the parameter `known distance` to 1.27, and the parameter `unit of length` to "cm". We then used the `polygon` tool to draw a polygon around only the leaves and grid paper within the image, excluding any writing or any parts of the image beyond the grid paper on which the leaves were placed. The region within the polygon was then duplicated using the the commands `image`→`duplicate`.

Color and contrast were adjusted within the duplicated image using the commands `image`→`type`→`8-bit` to transform the image to grayscale, followed by `image`→`adjust`→`threshold` to transform the image to black and white such that the leaf surfaces were shaded entirely in black and the background was shaded entirely with white. The threshold (bottom sliding bar) was adjusted manually to ensure that the edges of the leaves were precisely highlighted, but no other parts of the image were.

Finally, the area of each leaf was analyzed using the commands `analyze`→`analyze particles`. We set the parameter `particle size` to a range of 0.01-Infinity to remove noise from the analysis (i.e. particles too small to be leaves were removed). We selected `outlines` from the `show` dropdown menu, which produces an image with the outlines of each particle analyzed in order to double-check that the particles being captured by the analysis corresponded to the shape and location of the leaves in each image. The boxes `exclude on edges`, `display results`, `record starts`, and `include holes` were checked. These

parameters serve to exclude any particles touching the edge of the image (as all leaves in our images were centered so particles along edges caould not be leaves), record the starting position (x-y location) of each leaf so it can be located and verified on the original image, and ensure that any areas within leaves that were not shaded black during the `threshold` transformation were still included in area calculations. The result of this analysis is a list of three area values in cm^2 corresponding to the surface areas of the three leaves in each image we analyzed. Leaves in each image were always placed in the same order from left to right as follows: *Carex hoodii*, *Campanula rotundifolia*, and *Poa pratensis*.

All leaf images were also manually assessed for signs of herbivory. Specifically, because Hayden's ringlets feed from the margins of leaves rather than skeletonizing tissue from the center, we assessed the margins of each leaf before and after herbivory for jagged edges. Leaves used in the herbivory assays were cut into 1-cm long rectangles prior to herbivory assays so images could be easily coded as either (1) herbivory observed (leaf margins jagged and rectangle sides no longer straight lines) or (0) no herbivory observed (leaf margins intact and no jagged marks present). This manual coding allowed us to isolate changes in leaf surface area due to moisture loss in the leaf tissue over time (i.e. shrinkage) vs. larval herbivory.

Construction of the reference contig set with `CD-hit`

Because no reference genome has been constructed for the Hayden's ringlet to date, we constructed a de novo set of reference contigs to align our GBS reads to using the program `CD-hit` version 4.8.1 [Li and Godzik, 2006].

We started with the full set of 347,375,794 demultiplexed reads with poly-G tails removed that remained after filtering to remove PhiX reads. For processing efficiency, we first sorted these reads by individual. This resulted in a single file for each individual containing all the reads that came from that particular individual. We then used `CD-hit` to run a clustering step at 100% match for each individual's set of reads. The result of this step was 287 files containing a list of all unique reads belonging to each individual butterfly. The purpose of this step was to remove reads that were perfect duplicates of one another,

thereby reducing file size and processing time downstream.

If we concatenated the files of unique reads from each individual produced above to produce a single file for clustering with `CD-hit` as-is, we would run the risk that `CD-hit` would use solely sequences from the first alphabetically-ordered individual as seed sequences to align other reads to, introducing bias. Instead, we first split the files of unique reads from each individual into approximately 20 files each (70,000 lines or 17,500 reads each). We then concatenated all of the first files split from each individual, followed by all of the second files split from each individual, etc. all the way through the tenth files split from each individual. This ensured that reads from all individual butterflies should be represented in seed sequences during the next clustering step.

To test the sensitivity of percentage match on clustering for our data set, we used a concatenated file containing just the first files split from each individual (approximately 5% of the full concatenated data set). We tested clustering at 80, 90, and 95% match levels. This resulted in 226,668, 349,754, and 419,676 clusters, respectively. We then repeated this sensitivity test using a concatenated file containing the first four files split from each individual (approximately 25% of the full concatenated data set) for 88, 90, and 92% match levels. This resulted in 797,035, 901,765, and 1,006,900 clusters, respectively. We chose to use clustering at 90% match on the full concatenated data set (the first 10 files split from each individual) for our actual clustering to be used in downstream analysis. Any reads that did not cluster at 90% match were then removed from the data set.

Finally, we completed one additional clustering step using only those reads that clustered at 90% match. We clustered these reads again at 80% match and removed all reads that clustered at this level. Reads from the 90% match cluster set that clustered with one another at an 80% match rate or greater were removed because these clusters may represent gene families or duplicated genes. The result of this was a set of 614,359 reference contigs used for alignments in downstream analysis.

Calculating the proportion of grass vs. sedge consumed by larvae

Because the total amount of leaf tissue consumed by larvae varied substantially across

populations, we assessed differences in host preference across populations as differences in the proportion of grass vs. sedge leaf tissue consumed. For these calculations, we set any negative values in the MCMC chains to zero (as negative herbivory is not a realistic assumption) and removed any NAs that resulted during the proportion calculations. Because no herbivory was observed on the control host, *C. rotundifolia*, we calculated the proportion of grass (or sedge) consumed as simply the total grass (or sedge) tissue consumed, in mm^2, divided by the sum of grass plus sedge tissue consumed.

REFERENCES

Li, W. and A. Godzik, 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012. Nih image to imagej: 25 years of image analysis. Nature methods 9:671–675.

Supplemental Tables and Figures

Table S4: Locations of sites that were assessed for the presence of Hayden's ringlets but did not harbor population sizes large enough to sample. Shown are the latitude and longitude of each site, as well as the number of Hayden's ringlets that were observed at each site.

	Latitude°	Longitude°	N butterflies observed
AVP	44.4860	-110.1307	1
GLR	44.1023	-110.7483	0

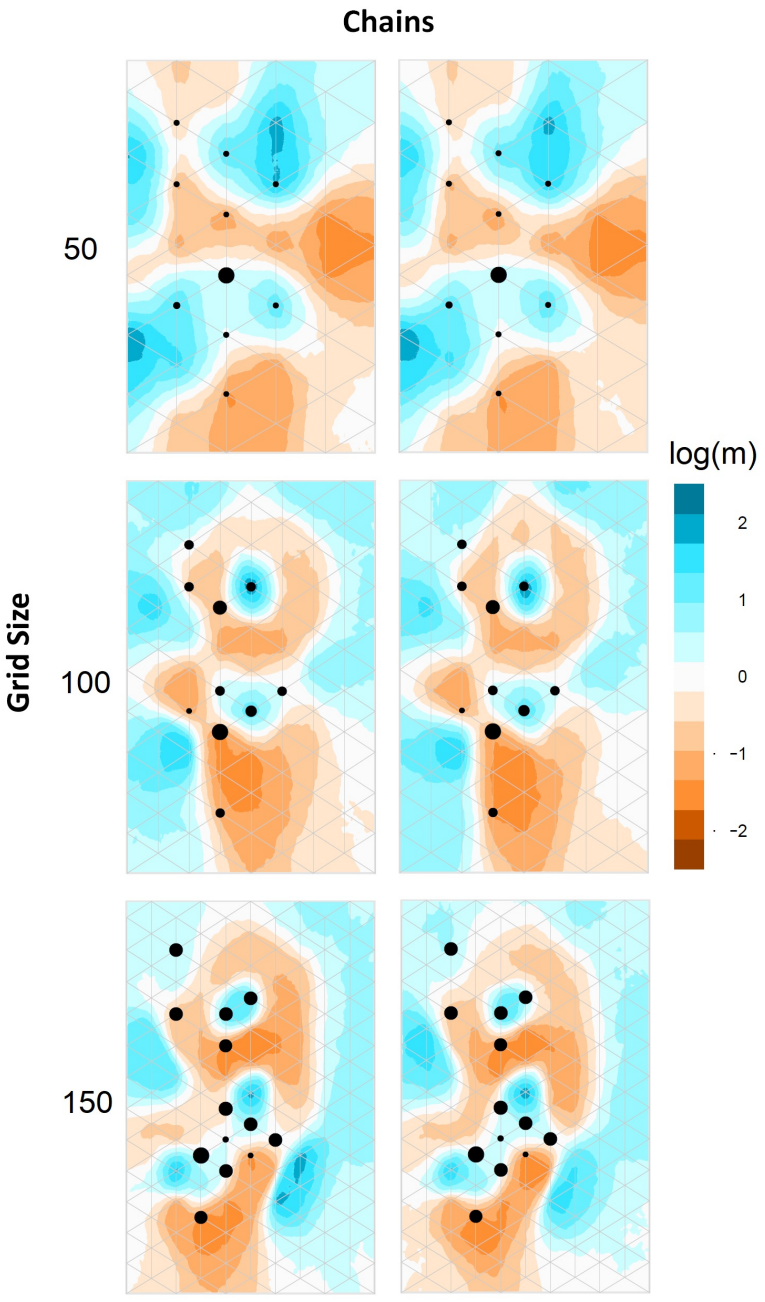


Fig. S5: Effect of grid size on `EEMS` results. Two chains each are shown for grid sizes of 50, 100, and 150. Results were highly consistent between chains, and showed an area of reduced relative migration rates between northern and southern populations of Hayden's ringlets across all grid sizes tested.

CHAPTER 4

THE EFFECT OF ADMIXTURE ON THE PREDICTABILITY OF EVOLUTION DURING ADAPTATION IN *CALLOSOBRUCHUS MACULATUS*

Abstract

Admixture is common in nature, and can serve as a crucial source of adaptive potential through the generation of novel phenotypes (i.e. transgressive segregation). Conversely, the presence of hybrid incompatibilities can decrease the fitness of hybrids. Due to the pervasiveness of admixture in nature and its potential role in facilitating adaptation, understanding how admixture affects the predictability of evolution is key to furthering our understanding of evolutionary dynamics. However, few studies have assessed how patterns of evolutionary parallelism (i.e. repeated independent occurrences of evolutionary change at the same locus or loci in response to a selective pressure) in admixed lineages are affected by the presence of strong ecological pressure. In this experiment, we assessed patterns of parallelism across admixed vs. non-admixed cowpea seed beetles (*Callosobruchus maculatus*) during adaptation to a novel, stressful host: lentil. Specifically, we asked whether (1) admixture facilitates adaptation to lentil, (2) parallelism was higher in admixed or non-admixed lineages, and (3) to what degree parallelism in admixed lineages was associated with selection on globally adaptive alleles, epistatic effects, or hybrid incompatibilities. We found that admixture facilitated adaptation to lentil, and evolutionary rescue occurred in all lineages. The degree of evolutionary parallelism was highest in two purebred lineages, but still notable in admixed lineages. Adaptation to lentil appeared to be driven by selection on alleles that were globally adaptive. Finally, even during evolutionary rescue in a marginal environment, the purging of hybrid incompatibilities still appeared to contribute substantially to evolutionary parallelism in admixed lineages.

Keywords: *Callosobruchus maculatus*, adaptation, Bayesian linear models, admixture, evolutionary rescue

Introduction

Admixture is increasingly being recognized as a major driver of evolutionary dynamics, as well as a potentially critical source of adaptive potential. Admixture, the process whereby genetic material is shared across genetically divergent populations or species, is a widespread phenomenon occurring in at least 10% of animal and 25% of plant species [Mallet, 2005]. A substantial portion of many species' genomes—including our own—are derived from hybrid origins [Edwards et al., 2011, Meier et al., 2017, Sankararaman et al., 2016, Schumer et al., 2016, Short and Streisfeld, 2023]. By bringing together new combinations of alleles from previously isolated parental populations, admixture can unlock novel phenotypic variation (i.e. transgressive segregation) and serve as a vital source of evolutionary novelty [Lewontin and Birch, 1966, Pereira et al., 2014, Rieseberg et al., 1999]. The extreme phenotypes generated by admixture coupled with the transfer of globally beneficial alleles (i.e. adaptive introgression) and the genetic benefits of outbreeding (e.g. heterosis and/or masking of deleterious recessive alleles) can increase the adaptive potential of admixed populations, particularly in novel or marginal environments [Buerkle et al., 2000, Crow, 1948, De Carvalho et al., 2010, Durkee et al., 2023, Gompert et al., 2006, Oziolor et al., 2019]. Conversely, the presence of Dobzhansky-Muller incompatibilities [Dobzhansky, 1982] and the breakdown of adaptive gene complexes can reduce fitness in admixed individuals (i.e. outbreeding depression), leading to selective pressure against hybridization [Bhargav et al., 2022, Calvo-Baltanás et al., 2021, Kim et al., 2018, Turissini and Matute, 2017, Verhoeven et al., 2011]. Because admixed populations are subject to multiple conflicting evolutionary pressures, the evolutionary outcomes of admixture vary widely [Rius and Darling, 2014, Springer and Gompert, 2020]. As such, determining the degree to which evolution in admixed populations is repeatable—and therefore predictable—is of particular interest to understand how deterministic processes (e.g. natural selection imposed by the environment) and intrinsic evolutionary constraints imposed by admixture interact to shape

patterns of genomic change. Admixture events can result in the transfer of just a few alleles from one population to another (i.e. adaptive introgression) [Enard and Petrov, 2018, Nanaei et al., 2023, Oziolor et al., 2019], the reinforcement of species boundaries [Bewick and Dyer, 2014, Bhargav et al., 2022, Turissini and Matute, 2017], or in some cases, genome stabilization and the formation of stable mosaic hybrid species (i.e. ancestry at a local level is no longer variable: at any given location in the genome, ancestry has become fixed for one parental species or the other) [Gompert et al., 2006, Mallet, 2007, Sun et al., 2020]. Due to the pervasiveness of admixture in nature and the complicated nature of admixture's effects on the genome, delineating the factors influencing the predictability of evolution in admixed populations is key to our understanding of evolutionary dynamics as a whole.

The degree of repeatability in genome evolution post-admixture depends on many factors, including demographic history, the degree of genetic divergence between parental populations, recombination landscapes across the genome, and how far from the phenotypic optimum each parental population is in the environment where admixture occurs [McFarlane et al., 2022, Moran et al., 2021, Schumer et al., 2018]. A few general principles have already emerged regarding the repeatability of evolution at a genomic level post-admixture, including the purging of ancestry derived from the minor parental population (i.e. the parental population which initially contributed the least amount of ancestry to the hybrid genome) [Langdon et al., 2022, Moran et al., 2021, Schumer et al., 2018]. When Dobzhanksy-Muller incompatibilities (i.e. when an allele in population A becomes deleterious when present in combination with an allele found a different locus in population B) are present or intermediate hybrid phenotypes are ecologically unsuitable for the environment, purging ancestry from the minor parent is the fastest (and therefore most likely) evolutionary route for admixed populations to increase fitness[Langdon et al., 2022]. Purging of minor parent ancestry may even be repeatable across hybrids formed from different species pairs [Langdon et al., 2022]. Similarly, when one parental population has lower effective population sizes than the other (i.e. island vs. mainland populations, see Matute et al. [2020]), mildly deleterious alleles that accumulated and fixed in the smaller population via genetic drift can

result in strong selection against ancestry from that population [Harris and Nielsen, 2016, Juric et al., 2016]. Selective pressure against this hybridization load can lead to purging of entire blocks of local ancestry inherited from the smaller, more inbred population, especially at sites with low recombination rates [Matute et al., 2020, Nouhaud et al., 2022].

However, while a considerable amount of work has been done to determine factors shaping the repeatability of evolution in admixed populations in an organism's native habitat [Chaturvedi et al., 2020, Langdon et al., 2022, Nouhaud et al., 2022, Schumer et al., 2018] or under benign laboratory conditions [Matute et al., 2020], few studies explicitly address the impact of strong directional selection imposed by stressful ecological conditions on patterns of evolutionary repeatability in admixed populations. Given the strong potential for admixture to facilitate adaptation and evolutionary rescue under stressful environmental conditions via the expression of transgressive phenotypes and transfer of globally adaptive alleles [De Carvalho et al., 2010, Durkee et al., 2023, Gompert et al., 2006, Lewontin and Birch, 1966, Oziolor et al., 2019, Pereira et al., 2014, Stelkens et al., 2014, Vedder et al., 2022], this remains a critical gap in our understanding of the predictability of evolution in admixed populations. In the face of unprecedented anthropogenic change, determining how strong ecological selection alters the genomic consequences of admixture is also of critical relevance for determining the effect of admixture on adaptive potential and the predictability of evolution. While intrinsic hybrid incompatibilities commonly drive patterns of repeatability during the evolution of admixed populations [Chaturvedi et al., 2020, Langdon et al., 2022, Matute et al., 2020, Nouhaud et al., 2022], the severe population bottlenecks that occur during evolutionary rescue could drastically increase the degree of stochasticity experienced during adaptation, potentially reducing repeatability [see McFarlane et al., 2022]. Conversely, when populations begin far from the phenotypic optimum, rapid adaptation during evolutionary rescue may be initially driven by selective sweeps of just a few major-effect loci (rather than many small-effect loci) [Alexander et al., 2014, Orr, 2005]. Hard selective sweeps during bouts of rapid adaptation could potentially increase the predictability of evolution during evolutionary rescue, but how the influx of novel stand-

ing genetic variation plus intrinsic incompatibilities introduced via admixture might alter patterns of soft vs. hard selective sweeps during evolutionary rescue is unclear.

In this study, we used experimental evolution to assess how admixture affects patterns of evolutionary rescue and parallelism in cowpea seed beetles, *Callosobruchus maculatus*, during adaptation to a novel, stressful host. *Callosobruchus maculatus* is a globally-distributed pest of stored legumes from the tribe Phaseoleae [e.g., mung bean, adzuki bean, and cowpea; Kébé et al., 2017, Tuda et al., 2006]. Because cowpea seed beetles have been associated with human crop stores for thousands of years and their larvae spend the entirety of their development within a single seed, laboratory conditions closely approximate the "natural" habitat of *C. maculatus* [Kébé et al., 2017, Messina, 1991, Tuda et al., 2014]. Populations from different geographic locations vary substantially in fitness traits, including larval competitiveness, body size, oviposition preference, and fecundity [Credland and Dick, 1987, Messina, 1991, 1993, Messina et al., 2018]. Lentil (*Lens culinaris*, tribe Fabeae) is a particularly poor host for *C. maculatus* [Messina et al., 2009a]. Initial survival on lentil is often less than 3%, and experimental attempts to establish *C. maculatus* populations on lentil often result in extinction [Messina et al., 2009a, 2020]. Despite this, *C. maculatus* lineages on lentil that do not go extinct have been found to rapidly rebound, with percent survival rising to over 80% within 20 generations [Messina et al., 2009a]. Previous ecological studies have shown that admixture likely facilitates adaptation to lentil in the cowpea seed beetle [Messina et al., 2020], and previous genomic studies have found a modest degree of parallelism at a genomic level across non-admixed lineages during adaptation to lentil [Rêgo et al., 2019]. However, to date no studies have assessed how both admixture and environmental stress combined affect the repeatability of genomic change during adaptation a novel, stressful host.

Here, we assessed how admixture affects the degree of evolutionary parallelism during adaptation to lentil in *C. maculatus*. Specifically we asked the following questions: (1) to what degree does admixture facilitate adaptation to lentil, (2) is evolution more repeatable in admixed or non-admixed lineages during evolutionary rescue, and (3) to what degree

is repeatability during evolutionary rescue in admixed lineages driven by (a) selection on globally-adaptive alleles (i.e. a shared genetic basis for adaptation to lentil across admixed vs. non-admixed lineages) vs. (b) epistatic effects in hybrid lineages and the purging of hybrid incompatibilities?

Materials and Methods

Colony foundation and experimental design

We used cowpea-adapted lineages of *Callosobruchus maculatus* from three different continents for this experiment: Burkina Faso (Africa), Brazil (South America), and California (North America). These lineages all originally utilized cowpea, *Vigna unguiculata*, as their native host, have non-competitive larvae, and show low initial survival rates on lentil [Messina et al., 2020]. All lineages were obtained from Dr. Charles Fox at the University of Kentucky [Messina et al., 2018], but were originally collected from infested cowpeas in the field or in markets across the world. Cultures from all three lineages were maintained continuously in the laboratory on cowpea after their initial collection. The Burkina Faso (BF) lineage was collected from a field of cowpeas *(V. unguiculata)* in Ouagadougou, Burkina Faso by Dr J. Huignard at the University of Tours in 1989 [Messina, 1993, Messina et al., 2018]. The Brazil population (BZ) was collected from Campinas, Brazil in 1975 [Tran and Credland, 1995] and later maintained by Dr Robert Smith at the University of Leicester [Dowling et al., 2007a, Guedes et al., 2003]. The North American lineage was collected from California (CA) and later maintained by Dr. Peter Credland at the University of London [Dowling et al., 2007b, Tuda et al., 2014]. All lineages are estimated to have been maintained under standard laboratory conditions in excess of 300 generations at the time of our experiment. Phyloeographical analyses suggest that ancestral seed beetle populations in Africa began expanding into Asia as long as several thousand years ago, indicating that phylogenetic splits among certain seed beetle population pairs have the potential to be quite deep [Kébé et al., 2017]

We began our experiment with a single stock colony each from the BF, BZ, and CA

lineages (3 jars total). These colonies had been maintained in the laboratory at Utah State University in excess of 100 generations at the time of this experiment. Colonies were kept in 2 L glass jars containing approximately 750 g of cowpeas. New generations were founded by transferring ∼2000 newly-emerged adult beetles (estimated by volume using an insect aspirator) to fresh culture jars once every 25-30 days (hereafter referred to as "standard culture"). Another seed beetle lineage, South India (SI), has been found to have a variance effective population size of (N_e) = 1149.6 individuals (95% CI = 1077.4–1229.8) [Gompert and Messina, 2016a]. The effective population sizes of the BF, BZ, and CA are expected to be similar or higher than that of SI given their extensive shared culturing history. As a result, seed beetle colonies kept under standard culture conditions are expected to show high genetic diversity levels and little to no inbreeding. Indeed, previous experiments show that evolutionary rescue in purebred lineages of *C. maculatus* is driven by selection on standing genetic variation [Gompert and Messina, 2016b, Rêgo et al., 2020, 2019]. During this experiment, all colonies were housed at 27°C with a 14/10 day cycle in one of two Percival incubators (model Nos. I-36VL and a similar model). Due to the large amount of metabolic water produced by growing beetle larvae, we installed a dehumidifier in each incubator to try and reduce humidity levels to between 15-50%. While under standard culture conditions only 2000 adult beetles per generation are transferred, each jar will produce far more than 2000 adult beetles each generation. This allows us to split any given jar of beetles into multiple daughter colonies each month without imposing a population bottleneck on the original colony. To found the colonies for our experiment, each month from our stock colonies we removed (1) 2000 adult beetles to found the next generation of the stock colony, (2) 2000 adult beetles to found the purebred control colonies for that month's replicates, and (3) approximately 1000 pupae-containing beans to use for that month's admixed replicates.

To form our admixed lineages, we produced true F1 hybrids with a founding population size of 1000 parental beetles each. To accomplish this, one to three days before the beetles' expected emergence date we randomly sampled 1000 pupae-containing beans from each of

our parental stock colonies. These beans were isolated in individual cellulose pill capsules and maintained in the incubator under standard conditions. Twice daily, emerging virgin beetles were tallied and sorted into petri dishes by population and sex. This process was continued until we had collected four dishes of 250 virgin beetles each from each stock colony: two all-male and two all-female dishes from each parental population (BF, BZ, and CA). Reciprocal crosses were then performed for each combination of parental populations. In other words, we placed 250 virgin males from the first parental population in a jar with 250 virgin females from the second, and vice versa in a second jar. After 10 days (at which point most or all of the purebred adults had died), we combined each male x female jar with its reciprocal female x male pair to found a single admixed colony composed of the true F1 offspring of the 1000 purebred founding beetles. This method ensured we were producing admixed lineages with equal genetic contribution from both sexes from each parental populations. To found our purebred control colonies, we simply transferred 1000 beetles (as measured by volume) from each purebred stock colony to fresh culture jars. We produced two full replicates every month, where each replicate consisted of three purebred (BF, BZ, CA) and three admixed (BF x BZ, BF x CA, BZ x CA) cowpea colonies each, for a total of 66 cowpea colonies with a founding population size of 1000 beetles per jar.

After maintaining both our purebred and admixed colonies on their native host (cowpea) for two successive generations post-admixture, we split each of our 66 cowpea colonies to form 66 additional colonies on our novel, stressful host: lentil. To do so, we removed a total of 4000 adult beetles (as measured by volume) from each our our 66 F2 cowpea colonies and transferred 2000 to fresh lentil culture jars and 2000 to fresh cowpea culture jars to lay eggs. Thus, the first generation of beetle larvae to feed on the novel food source in our experiment was the F3 generation. This left us with a total of 132 beetle colonies and 12 lineages per replicate: three admixed and three non-admixed lineages on cowpea, and three admixed and three non-admixed lineages on lentil. This full factorial experimental design allows us to compare the evolution and performance of admixed lineages across environments (stressful vs. benign), as well as compare evolution and performance of of

admixed vs. purebred lineages within each of those environments. We chose not to conduct the host shift onto lentil until the F2 generation because F1 hybrids are typically phenotypically uniform and thus will not reflect the adaptive potential that could emerge after recombination breaks down ancestry blocks and generates novel genotypic combinations (i.e. transgressive segregation). All 132 colonies were maintained for at least 20 generations post-admixture (at least 17 generations post-host shift). After the end of the experiment, all remaining colonies were placed at -20 °C for seven days to ensure all beetles would be dead prior to disposal.

Population Growth Assays

During the first 400 days after the host shift onto lentil (or until enough beetles emerged to move the colony into standard culture), we removed all dead adult beetles produced by each of the 66 lentil colonies. This was done to assess the rate of adaptation to lentil in each colony. Every 20 days, beetles from each lentil colony were separated from the beans using a soil sieve. All live beetles were aspirated from the upper edge of the sieve and returned to the culture jar to continue laying eggs. This was done to ensure that we were not artificially altering population sizes by removing live adults with further reproductive potential from colonies. All dead beetles remaining at the bottom of the sieve were removed and stored in zip-lock bags at -80 C until image analysis. Thus, each bag of beetles removed represents the number of adult beetles that died during the previous 20-day period, providing an accurate estimate of the population size of each colony during the 20 days prior.

To assess the number of adult beetles produced by each colony during every 20 days post host-shift, we used the program `ImageJ` version 1.52A [Schneider et al., 2012]. Beetle specimens from each sample bag were spread onto a clean, white sheet and photographed using Canon EOS M6 camera. Photographs were first prepared for analysis using the program Adobe Photoshop Elements 2020 Editor to correct uneven lighting and ensure the background color was uniform across the entire image. This was necessary to ensure that ImageJ could accurately differentiate between the color of beetles vs. the background sheet. We then used the `analyze particles` function in ImageJ to count the number of beetles

in each image. The result of this analysis was a count of the total number of beetles that died during every 20-day period in each colony post host-shift. As we collected every dead beetle produced by each jar during each 20 day interval between 60 and 400 days post host shift (unless the colony was moved into standard culture prior to 400 days post host shift), these population counts represent a complete count of the total number of beetles produced by each colony during early adaptation to lentil.

We analyzed population growth in both admixed and non-admixed lines using a Bayesian generalized linear model. Cumulative count data at a given time point were assumed to follow a normal distribution with $\mu = \mu^{count}$ and $\sigma = \sigma^{count}$. Mean cumulative population count (μ^{count}) was assumed to follow a second order polynomial relationship with respect to the number of days post host shift such that for non-admixed lineages:

$$\mu^{count} = (\beta_1^{pop} + \alpha_1^{rep})days + (\beta_2^{pop} + \alpha_2^{rep})days^2$$

where β_1^{pop} and β_2^{pop} are the effects of time (calculated as the standardized but not centered number of days post host-shift) on the mean cumulative number of beetles that emerged for each non-admixed population, *pop* is the particular non-admixed population being considered (BF, BZ, or CA), *days* is the number of days post host-shift, and α_1^{rep} and α_2^{rep} are random effects of replicate for each β-term (data from replicates 2 through 10 were used for this analysis). Replicate effects were transformed with a sum-to-zero constraint to ensure all parameters in the model were identifiable. For admixed lineages, μ^{count} was assumed to follow the same polynomial relationship shown above except that each slope (β_1 and β_2) for admixed populations was assumed to equal the average slope from each parental lineage plus an additional effect of admixture, such that:

$$\mu_{count} = (\frac{\beta_1^{P1} + \beta_1^{P2}}{2} + \beta_1^{admix} + \alpha_1^{rep})days + (\frac{\beta_2^{P1} + \beta_2^{P2}}{2} + \beta_2^{admix} + \alpha_2^{rep})days^2$$

where β^{P1} terms are the effects of time on cumulative beetles emerged in the first

parental lineage, β^{P2} terms are the effects of time on cumulative beetles emerged in the second parental lineage, and β^{admix} terms are the additional effects of admixture on the cumulative beetles emerged. Thus, our model included six β_1 and six β_2 parameters (one slope parameter for each of the three parental lineages, and one admixture effect parameter for each of the three admixed lineages). Both the β_1 and β_2 parameters were assigned a normal prior with $\mu = 0$ and $\sigma = 100$. Raw (not sum-to-zero transformed) random replicate effects (i.e. α_1 and α_2 parameters) were assigned normal priors with $\mu = 0$ and $\sigma = \sigma_1^\alpha$ and σ_2^α respectively. Finally, all three sigma parameters (σ^{count}, σ_1^α, and σ_2^α) were assigned gamma priors with parameters $k = 0.1$ and $\theta = 0.01$. This model was written in the language STAN [Stan Development Team, 2022b] and implemented with the R-interface RStan version 2.21.5 [Stan Development Team, 2022a]. We ran 5 chains with a burn-in period of 1,500 steps and 3,000 Hamiltonian Monte Carlo (HMC) sampling steps.

DNA Sequencing, Alignment, and Variant Calling

DNA Extractions

We extracted DNA from between 19-20 beetle specimens each from 78 unique lineage x replicate x host x generation combinations, for a total of 1536 individuals. As cowpea seed beetles have an XY sex chromosome system and the Y-chromosome is significantly reduced in size [Angus et al., 2011], we chose to sequence only female beetles to achieve better coverage of the X-chromosome. We sequenced DNA from three time points during our experiment: generation 1 (F1; pre-adaptation), generation 7 (F7; early adaptation), and generation 20 (F20; late adaptation). From the F1 generation, we sequenced only purebred parental cowpea lineages (BF, BZ, and CA) from replicate 1. Because our admixed lineages were true F1 hybrids of our parental cowpea lineages, the initial allele frequencies of our first generation hybrid lines could be inferred from the allele frequencies of these original parental lines. From the early adaptation (F7) generation we sequenced all cowpea- and lentil-adapted admixed lineages (BF x BZ, BF x CA, and BZ x CA) from replicates 1:5 for a total of 30-F7 experimental groups. From our late adaptation time point (F20) we

again sequenced beetles from all admixed lineages (both cowpea- and lentil-adapted) from replicates 1:5, as well as all purebred lentil-adapted populations from replicates 1:5 for a total of 45-F20 experimental groups. This sampling scheme allowed us to assess evolution in purebred lines during adaptation to lentil, evolution in admixed lineages during early and late adaptation to lentil, as well as evolution during early and late generation admixed lineages not exposed to a novel host.

To extract DNA from beetle specimens, we used Qiagen DNeasy 96 Blood and Tissue Kits. To minimize cross-contamination of DNA, all beetle specimens were washed prior to DNA extraction (see supplement for details). For any specimens collected and frozen live rather than 10 days post-emergence, eggs were dissected out of the females' abdomens and their abdominal cavities brushed clean with 95% ethanol to minimize the presence of male DNA contained in sperm. Because seed beetles lay most or all of their eggs within 10 days of emergence (CITE), this abdominal dissection step was skipped for specimens collected 10 days or more post-emergence (i.e. specimens collected after death).

Reduced-representation restriction-fragment-based DNA libraries were then prepared from extracted DNA using methods similar to those in Gompert et al. [2014]. Briefly, whole-genome DNA was first digested with Mse1 and EcoR1 enzymes, then ligated to custom barcode sequences and amplified via PCR. Barcoded and amplified DNA fragments were pooled, purified, and size-selected on a BluePippin. We selected DNA fragments between 250-350 bp for sequencing. Our DNA fragment libraries were sequenced using two lanes on the University of Texas Illumina NovaSeq S1 sequencing platform.

Sequencing resulted in a total of 4,381,945,291 individual reads. We first filtered each of the Fastq files to remove PhiX sequences. PhiX is a bacterial sequence introduced as an internal control on certain sequencing platforms. We then used `SAMtools` version 1.10 and custom scripts to find and remove all reads that aligned to the PhiX reference genome. Barcode sequences were then removed from the remaining reads using custom perl scripts, and each read was tagged with the ID of the beetle from which it came. After the removal of PhiX reads, we were left with a total of 3,539,264,296 reads for alignment.

For alignment, we used the genome assembly from the April 2023 version of the *Callosobruchus maculatus* whole genome shotgun project (NCBI accession number CASHZR020000000) as our reference genome. We aligned DNA reads from our experiment to this reference genome using the `bwa aln` algorithm using a maximum number of mismatches allowed per sequence (`-n`) of 5. For our data, this meant if greater than 5% of the read did not match the reference sequence, it was not retained as an alignment. Seed length (`-l`) was set to 20, and the maximum mismatches allowed in the seed sequence (`-k`) was set to 2.

To identify sites with single nucleotide polymorphisms (SNPs), we conducted variant calling using the program `bcftools` version 1.16. We used the original consensus caller (`-c`) and called only variants for which the posterior probability of the SNP being invariant was less than 0.01 (`-p` = 0.01). Variable sites were filtered for quality using custom `perl` scripts. In particular, we retained only variable sites with a phred-scale mapping quality greater than 30, a coverage level equal to or greater than 3072 reads (2x the number of individuals we sequenced), a minimum of 10 reads for the alternative allele (to filter out possible errors in sequencing), and representing 80% or more of the individuals we sequenced. Variable sites with base quality rank sum, mapping quality rank sum, or read position rank sum test p-values less than 0.001, 0.0001, and 0.001 respectively were not retained. After this initial filtering step, SNPs were further filtered to a max read depth of 48,000, 3 standard deviations greater than the mean read depth across loci. This was done to remove possible paralogs/gene families from our filtered SNP set. Variable sites located less than 2 BP apart along a contig were also removed. After quality filtering, we were left with 79,079 SNPs for downstream analysis.

Statistical Analyses

In order to assess patterns of admixture and generate global ancestry estimates, we used the program `ENTROPY` version 2.0 [Gompert et al., 2014, Shastry et al., 2021]. The program `ENTROPY` is comparable to the admixture model in `STRUCTURE`, but with the additional feature of accounting for uncertainty in genotypes. Because our experiment used three known parental populations for the production of admixed lineages, we ran entropy

only for K = 3. We ran 20 MCMC chains with 2000 burn-in steps and 2500 sampling steps each, a Dirichlet initialization value of 50, and a thinning interval of 5. Ancestry proportion estimates generated by the `ENTROPY` program were used to determine to what degree ancestry proportions shifted over time (for example, if ancestry from one parental lineage was selected against due to incompatibilities or ecological selection). The genotype estimates generated by the `ENTROPY` program were used to construct a PCA to visualize patterns of genetic structure among our parental and admixed lineages. This PCA was constructed in R version from centered but unscaled genotype estimates using R version 4.2.2 [R Core Team, 2022].

To estimate allele frequencies within each unique hybrid x replicate x generation x host group, we used the program `estpEM`. We used convergence tolerance of 0.001 and max iterations of 20. `estpEM` accounts for differences in quality scores among SNPs during allele frequency estimation. F1 allele frequency estimates from `estpEM` were used to calculate Nei's F_{ST} between each pairwise combination of purebred parental lineages (i.e BF, BZ, and CA cowpea lineages) to determine the degree of genetic differentiation among our parental lineages. We then computed standardized allele frequency change for all of our sequenced experimental groups. Standardized allele frequency change, or Δp, was calculated as $\Delta p = (p_t - p_0)/\sqrt{2p_0q_0}$ where p_t is the frequency of allele p at timepoint t, and p_0 and q_0 are the initial frequencies of alleles p and q. We standardized allele frequency change by the initial expected heterozygosity ($2p_0q_0$) at a given locus due to the fact that a given absolute change in frequency of a rare allele (i.e. initial heterozygosity is low) would represent a proportionally greater change in frequency than the same amount of absolute change in a more common allele (i.e. a site where heterozygosity approaches 0.5).

After calculating standardized allele frequency change at all 79,079 SNPs for all sequenced treatment groups (F7 and F20 admixed lineages on both cowpea and lentil and F20 purebred lineages on lentil), we quantified what percentage of the loci showing the greatest magnitude of allele frequency change (i.e. those most likely to be under selection) were shared across treatment groups as a measure of evolutionary parallelism. We

assessed the degree of parallelism both across replicates within a single experimental treatment group, as well as across treatment groups to test the degree to which SNPs associated with adaptation to lentil in purebred, parental lineages were also associated with adaptation to lentil in admixed lineages. We calculated the percentage of SNPs shared across groups for only those loci whose standardized allele frequency change estimates were in the top 5th percentile or above (i.e. those loci suspected to be under either direct or indirect selection). We also generated a null expectation for the percentage of SNPs shared across groups by randomly sampling 5% of the SNPs from each pair of treatment groups and calculating the percentage of SNPs shared among the randomly sampled pairs. Percentage overlap values were first calculated for each pairwise combination of replicates, then averaged across replicates to estimate the overall percentage of SNPs that were shared between each pair of treatments. We visualized the SNPs associated with adaptation by constructing Manhattan plots from just the SNPs from the 10 largest scaffolds that showed allele frequency changes greater or equal to 95% of the other variable loci in that treatment group.

We then used the program `varne` and the allele frequencies generated by `estpEM` to obtain unbiased estimates of variance N_e using a model similar to those used in Gompert and Messina [2016b], Jorde and Ryman [2007], Rêgo et al. [2019]. This allowed us to estimate the severity of population bottlenecks experienced by both admixed vs. non-admixed and cowpea vs. lentil lineages during adaptation. Because we formed true F1 hybrids to establish our admixed populations, the initial allele frequencies of our admixed lineages should be a simple average of the allele frequencies of the parent populations. As such, we estimated the allele frequencies of our F1 hybrid lineages by taking the average of the allele frequencies of their parental lineages. We estimated variance N_e for between generation F1 and generation F20, and ran all `varne` runs with an initial census size (-n) of 2000 beetles and 1000 Bayesian bootstrap replicates (-x).

Finally, we used the program `popanc` version 0.1 [Gompert, 2016] to estimate local ancestry proportions along scaffolds for each treatment group. This was done to visualize differences in the breakdown of ancestry blocks across groups over the course of adaptation

and compare patterns of local ancestry across treatment groups. This program uses a continuous correlated beta process model for inferring ancestry, and is particularly well-suited for inferring ancestry in hybrid populations that do not experience ongoing gene flow with parental populations and for which genome stabilization is not yet complete [Gompert, 2016, Gompert and Buerkle, 2013]. We ran `popanc` using the genotype estimates generated by `ENTROPY` for each admixed treatment group, and only included those SNPs located on the 10 largest scaffolds and for which the absolute difference in initial allele frequencies between each parental population pair (BF vs. BZ, BF vs. CA, or BZ vs. CA) was greater than 0.2. This was done to ensure that only the loci that were most informative of population ancestry were used for local ancestry analysis. We chose to have `popanc` estimate the scale parameter for the beta process model (-s). Finally, we set a maximum locus distance (-d) of 50 bp and the maximum number of SNPs per locus (-n) to 15, and ran 1000 burn-in steps and 10,000 sampling steps with a thinning interval (-t) of 5.

Results

Clear population structure between African and American lineages of *C. maculatus*

Our cowpea-adapted beetle lineages from Burkina Faso (BF), Brazil (BZ), and California (CA) showed a moderate to strong degree of genetic differentiation from one another. The degree of genetic differentiation between our African population and our two American populations (F_{ST} between BF vs. BZ and BF vs. CA were both 0.20) was twice as high as the degree of differentiation between the two American populations (F_{ST} between BZ vs. CA = 0.09). This result was recapitulated in our PCA (see Fig. 4.2, with PC1 separating the BF lineage from the BZ and CA lineages, and PC2 separating the BZ and CA lineages. Our three admixed lineages (BF x BZ, BF x CA, and BZ x CA) clustered directly between their two parental populations, as expected since early-generation hybrid genotypes will be intermediate to those of their parents. One of our lentil-adapted BF colonies, BF replicate 5 generation 20, clustered with the BF x BZ admixed lineages rather than with the BF pure-

bred lineages, indicating that this BF replicate was contaminated with BZ beetles at some point during the experiment and underwent admixture. As such, this single BF replicate was removed from all downstream analyses.

Admixed lineages also showed clear evidence of mixed ancestry consistent with expectations based on their hybrid ancestries (see Fig. 4.3. Comparison between F7 vs. F20 generation lentil-adapted hybrids from the BF x BZ and BF x CA lineages showed that after adaptation to lentil, global BF ancestry declined. While F1 hybrids would have have received exactly 50% of their genome from each parental lineage, the mean BF ancestry in F20 admixed lentil lineages ranged between 38-45%, a 5-12 percentage point decline in BF ancestry over the course of adaptation to lentil. The F20 admixed cowpea lineages, in contrast, showed mean BF ancestry values between 52-55%. This indicates possible selection against BF ancestry during adaptation to lentil.

Admixture increases cumulative population growth rates on lentil, but effective population sizes low in all lentil-adapted populations

Despite poor initial survival, 64 out of our 66 experimental lentil lineages successfully adapted to this novel, stressful host. The two lineages that did not adapt to lentil were BF replicate 6 and BF replicate 7. The BF lineage showed notably slower population growth on lentil than did the other populations (see Fig. 4.1 and Table 4.1). The BF lineage showed the slowest cumulative growth rate, while the BF x CA admixed lineage showed the highest (see β_2 values in Table 4.1). We also saw a strong incubator effect, with replicates 2 through 5, which were housed in the first incubator, experiencing more rapid cumulative growth than replicates 6 through 11, which were housed in the second incubator (see Fig. 4.1). Overall, we found that admixture appears to facilitate adaptation to lentil, with higher cumulative growth rates occurring in admixed than non-admixed lineages.

Results from our Bayesian second-degree polynomial model for cumulative population growth showed a strong signal for evolutionary rescue in all our lentil-adapted lineages. Values of β_1 indicate the slope of the cumulative growth curve at time $t = 0$. Thus, β_1 values can be interpreted as an estimate of the average reproductive rate of each lineage at

time t = zero. The higher the value of β_1, the higher the initial reproductive rate on lentil. The credible intervals for β_1 for the BF, BZ, and BF x BZ population all overlapped zero (see Table 4.1). This suggests that at time $t = 0$, the reproductive rate (as measured by the average number of adult offspring produced per day) in these populations was not high enough to ensure population persistence on lentil.

Alternatively, β_1 values could also be interpreted as a measure of how long it would take for a given founding population of parent beetles to produce enough offspring to fully replace itself, assuming a parental death rate of zero and non-overlapping generations (i.e. generation time). For the CA, BF x CA, and BZ x CA lineages, β_1 ranged from 4.7 and 8.6, suggesting that the average reproductive rate at time $t = 0$ in these lineages was between 4.7 and 8.6 adult offspring per day (see Table 4.1). At this reproductive rate, it would hypothetically take between 200 to 500 days for our founding populations of 2000 adult beetles to produce 2000 adult offspring. This, of course, would not be possible in reality as adult seed beetles have limited adult lifespans (less than 10 days) and the majority of first-generation offspring surviving on lentil expected to emerge within 100 days [Messina et al., 2020, 2009b]. Thus, even for the three lineages with β_1 values credibly greater than zero, the initial reproductive rate estimated by our model was not high enough to suggest that these populations would produce enough offspring to prevent an initial population decline. Our model results for β_1 indicate that on average, all three admixed and all three non-admixed lineages were expected to undergo an initial demographic decline, consistent with the first stage of evolutionary rescue.

The second slope parameter from our Bayesian model, β_2, is a measure of growth rate. A β_2 value of zero indicates that population size will remain constant with respect to time (in other words, the population size is stable and no growth occurs), while any value of β_2 greater than zero indicates exponential growth, meaning population size will increase with time. A negative value of β_2, meanwhile, indicates that population size will decrease with time. Values of β_2 in admixed populations were calculated as the average of β_2 values for each parental lineage plus an effect of admixture (β_{2AE}). β_{2AE} values of zero would indicate

that the cumulative growth rate in admixed populations was simply the mean of the parental populations' cumulative growth rates. In other words, a β_{2AE} of zero indicates that the cumulative growth rate of admixed populations falls directly between those of its parents. The effect of admixture for β_2 in our linear model was credibly greater than zero for all admixed lineages (see Table 4.1), suggesting that cumulative growth rates in all three of our admixed lineages were credibly greater than than the average of their parents' cumulative growth rates. Notably, values of β_2 were credibly greater than zero for all populations, both admixed and non-admixed, indicating that all six populations on average were expected to rebound from their initial demographic decline on lentil.

However, while admixture did appear to facilitate adaptation to lentil, we did not see a major effect of admixture on the severity of genetic bottlenecks experienced during adaptation. Variance effective population sizes (N_e) estimated from the F1 to F20 generations varied from a minimum of 16.2 (95% CI 16.1-16.4) in BF replicate 5 on lentil to a maximum of 285.4 (95% CI 277.2-294.4) in BZ x CA replicate 2 on cowpea (see Table 4.2). All N_e estimates were considerably lower than the founding population size of the colonies in our experiment (1000 beetles per colony), and varied considerably both across hosts and across replicates within a given treatment group.

Evolutionary rescue in two out of three non-admixed lineages more repeatable than for admixed lineages

To quantify the overall degree of parallelism in allele frequency change across treatment groups, we calculated the proportion of overlap in top SNP quantiles (i.e. those SNPs that changed the most during the course of adaptation) across treatment groups. The SNPs whose standardized allele frequencies changed the most are those most likely to be associated with adaptation (i.e. allele frequency change is being driven by natural selection rather than drift alone). If none of the SNPs whose frequencies changed the most were shared between treatment groups, it would indicate that completely different SNPs were associated with adaptation in each group (i.e. the evolutionary basis of adaptation was not shared across comparison groups). In contrast, if 100% of the SNPs that changed the

most in frequency were shared between between groups, it would indicate that evolutionary change was repeatable, with the same regions of the genome being involved in adaptation in both groups (i.e. the underlying basis of adaptation was the same in both groups). All treatment groups we compared showed proportion overlap values considerably higher than expected by chance, suggesting that evolution was moderately parallel (a) across replicates within treatment groups, (b) across admixed vs. parental lineages, and (c) across admixed cowpea vs. lentil lineages (see Table 4.3).

To answer the question of whether admixed vs. non-admixed lineages show a greater degree of evolutionary parallelism during adaptation to lentil, we compared the proportion of the SNPs whose allele frequencies changed the most that were shared across replicates. With the exception of the BF lineage, we found that evolution was more predictable in non-admixed than in admixed lineages. Proportion overlap values across replicates for admixed treatment groups (BF x BZ, BF x CA, and BZ x CA) ranged from 0.28 to 0.35, as compared to a null expectation of 0.05. This indicates that 5.6x to 7x more of SNPs associated with adaptation were shared across replicates than expected by chance. By comparison, the proportion overlap values across lentil-adapted replicates for two of the parental lineages (BZ and CA) were 8x to 8.4x greater than expected by chance, modestly greater than across lentil-adapted admixed replicates (see Table 4.3. This suggests that the degree of evolutionary parallelism was higher in purebred Brazil and California lineages than in admixed lineages during adaptation to lentil. The Burkina Faso (BF), meanwhile, showed a lower degree of parallelism (proportion overlap = 0.27) than admixed lineages.

Differences in the degree of parallelism during adaptation in admixed vs. non-admixed lineages were less clear for local ancestry estimates. Both cowpea- and lentil-adapted admixed lineages showed a moderate degree of parallelism across replicates for local ancestry along the 10 largest scaffolds (see Figs. 4.6 and 4.7). The 10 scaffolds presented in Fig. 4.6 and Fig. 4.7 correspond to each of the 10 chromosomes present in the *C. maculatus* genome. Patterns of local ancestry across scaffolds for our three cowpea-adapted admixed lineages (BF x BZ F20 C, BF x CA F20 C, and BZ x CA F20 C) were very similar across repli-

cates, consistent with a moderate degree of parallel evolution in these lineages. Patterns of local ancestry were also similar across replicates for the three lentil-adapted admixed lineages (BF x BZ F20 L, BF x CA F20 L, and BZ x CA F20 L), but several scaffolds showed notable deviations across replicates. In particular, replicates 2 and 3 from the BF x BZ lentil-adapted line (see Fig. 4.7a) showed a notable decrease in BF ancestry, while replicates 1, 4, and 5 showed local ancestry values for BF closer to 0.5. This pattern of divergent ancestry across replicates also occurred for replicate 2 on scaffold 3, as well as for replicate 4 on scaffold 1 in the BZ x CA lentil lineage (see Fig. 4.7c).

Adaptation to lentil driven by globally adaptive alleles across both admixed and non-admixed lineages

Plots of allele frequency change across scaffolds revealed several regions across the genome strongly associated with adaptation to lentil. In particular, a large region along scaffold 1 showed a wide peak where allele frequency change was greater than across other scaffolds. This peak was present in both admixed and purebred lineages after 20 generations on lentil (see Fig. 4.4), but notably absent from cowpea-adapted lines (see Fig. 4.5, strongly suggesting this region is associated with adaptation to lentil. The fact that this peak was present in both purebred and admixed lentil-adapted lineages suggests that some of the same regions of the genome involved in adaptation to lentil in purebred lineages were also adaptive in admixed lineages, indicating a shared evolutionary basis for adaptation. That said, the magnitude of this peak on scaffold 1 varied considerably across replicates and across lineages. While BZ x CA replicate 4 showed a large peak on scaffold 1 (see Fig. 4.5c), none of the other BZ x CA replicates on lentil showed appreciable peaks in allele frequency change in this region. Similarly, BF x BZ replicate 3 showed a larger peak on scaffold 1 than the other replicates from this hybrid type (see Fig. 4.5a).

To assess the degree to which the evolutionary basis of adaptation to lentil in admixed vs. non-admixed lineages was shared, we calculated proportion overlap values between lentil-adapted hybrid lineages and each of their parental populations (see Table 4.3). Overlap values ranged from 0.18 and 0.20, 3.6x to 5x higher than expected by chance. This

suggests that evolutionary basis of adaptation to lentil is at least partially driven by globally adaptive alleles (i.e. additive genetic variation) shared between parental vs. admixed lineages. However, the degree of parallelism between admixed vs. non-admixed lineages was lower than across replicates within a given treatment group, which could indicate (a) that hybrid incompatibilities present in admixed but not in parental populations are driving down the degree of evolutionary parallelism between these groups, or (b) that epistatic interactions are partially responsible for adaptation in admixed lineages, again driving down the degree of evolutionary parallelism between parental vs. hybrid lineages.

Finally, local ancestry analysis showed a notable decrease in BF ancestry in lentil-adapted lineages. (compare Fig. 4.6a-b vs. Fig. 4.7a-b). This trend was not evident in the BZ x CA lentil-adapted line, again suggesting selection against BF ancestry in lentil-adapted hybrid lineages as seen previously in our admixture analysis (see Fig. 4.3. In particular, we note a large decrease in BF ancestry on scaffold 9 in lentil-adapted lineages. We also saw a region of SNPs with high magnitudes of allele frequency change on scaffold 9 in the non-admixed BF lineage during adaptation to lentil (see scaffold 9 in Fig. 4.4b) compared to the other two parental lineages. Together, this evidence further suggests that several regions of the Burkina Faso genome are globally maladaptive on lentil, leading to parallel purging of BF ancestry during adaptation to lentil across both BF hybrid types (BF x BZ and BF x CA).

Even during evolutionary rescue, hybrid incompatibilities contribute to evolutionary repeatability

We did not see evidence for regions of hybrid incompatibilities with major effects in either cowpea or lentil lineages. Compared to the large peak in allele frequency change on scaffold 1 associated with adaptation to lentil, peaks in allele frequency change shared between lentil vs. cowpea treatment groups were considerably smaller (see Fig. 4.5). However, there were several regions with smaller peaks in allele frequency change that were shared across both cowpea and lentil treatments, including small peaks on scaffolds 1, 2, 6, and 9 in the BF x BZ lineage, the beginning of scaffold 9 in the BF x CA lineage, and

on scaffolds 3 and 6 and 9 in the BZ x CA lineage. Two of these peaks (peaks on scaffold 6 and 9) appear to be shared across hybrid types, occurring in both BF x BZ cowpea and lentil lineages as well as BZ x CA cowpea and lentil lineages.

To measure the degree to which hybrid incompatibilities vs. ecological selection drive patterns of parallelism in admixed lineages during evolutionary rescue, we compared the proportion overlap in top SNP quantiles for admixed lineages raised on lentil vs. cowpea. The proportion of top-quantile SNPs shared between cowpea vs. lentil treatment groups ranged from 0.23 to 0.32 (4.6x to 6.4x greater than expected by chance). This is not notably different than the degree of parallelism observed within replicates for a single treatment group (5.6x to 7x greater than expected by chance). Thus, despite the strong ecological selective pressure imposed by feeding on lentil, it appears that a large portion of the evolutionary parallelism observed in admixed lineages can still be attributed to the purging of hybrid incompatibilities.

Discussion

In this experiment, we assessed patterns of parallelism across admixed vs. non-admixed cowpea seed beetles during adaptation to a novel, stressful host: lentil. We found that admixture facilitated adaptation to lentil, with the Burkina Faso (BF) lineage showing the slowest rate of cumulative population growth during adaptation to this novel host, but evolutionary rescue occurred in almost all treatment groups. Genomic analyses revealed that levels of parallelism were similar or slightly lower in admixed vs. non-admixed lineages. We found a large spike of allele frequency change on scaffold 1 across lineages associated with adaptation to lentil, suggesting that adaptation to this novel host is being driven at least in part by selection on globally-adaptive alleles in both admixed and non-admixed lineages. In other words, the genomic basis of adaptation to lentil is likely being driven in part by selection on one or more major effect loci, and this genomic basis for adaptation is shared across both admixed and non-admixed lineages. We further found evidence for selection against BF ancestry on scaffold 9 across both hybrid lineages derived from BF parents (BF x BZ and BF x CA), indicating that certain regions of the Burkina Faso genome are

likely globally maladaptive on lentil. This same region of scaffold 9 in the purebred BF lineage showed a moderate spike in allele frequency change during adaptation to lentil, again suggesting that certain alleles carried by the BF lineage are globally maladaptive on lentil, regardless of admixture status. Finally, we found a moderate degree of parallelism in evolutionary change between admixed lineages adapted to lentil vs. cowpea, suggesting that even under extreme ecological selection, the purging of hybrid incompatibilities still contributes to the degree of evolutionary parallelism observed in admixed lineages. We discuss the implications of these results in greater detail below.

Admixture facilitates adaptation to lentil, but outcomes are strongly affected by humidity

Interestingly, it appears that the African lineage (Burkina Faso or BF) showed the poorest capacity to adapt to lentil. The Burkino Faso lineage is from the heart of the purported ancestral range of cowpea seed beetles [Kébé et al., 2017], and as such might be expected to harbor greater genetic diversity than American populations, which were transported across the world via trade and may have undergone significant population bottlenecks during establishment in new locations. Conversely, cowpea is a crop of particular importance in Africa and is widely grown [Kpoviessi et al., 2019], meaning cowpea may have been the only host encountered by the wild Burkina Faso seed beetle population. Cowpea is less widely grown in the Americas, so it is possible that the two American lineages (Brazil and California) had previous exposure to lentil or to other legume species more commonly grown in these regions, potentially increasing their ability to adapt to novel hosts. Alternatively, as all of our lineages have been reared in captivity for many generations, it is possible the Burkina Faso lineage [which was originally collected in 1989; see Messina, 1993] has simply lost some of its original diversity via genetic drift or adaptation to captivity, and its poor adaptive capacity on lentil is simply a reflection of this laboratory history.

It is also possible that other environmental factors alter the adaptive capacity of different lineages of seed beetles on lentil. Despite using very similar models of Percival incubators for this experiment, maintaining the same temperature and day cycle in both, as well as

running a dehumidifier full time in both incubators, we nevertheless saw substantial incubator effects across our treatment groups. Replicates 1 through 5 were kept in our first incubator, while replicates 6 through 11 were kept in the second. The first incubator was prone to periods of higher humidity while the second stayed drier during the course of the experiment. Adaptation proceeded much more rapidly in the first incubator (see Fig. 4.1, and differences in the rate of adaptation across lineages were far less pronounced. Humidity is strongly affected by the total number of colonies in each incubator due to the amount of metabolic water produced by larvae [Bhattacharya et al., 2003], and our incubators were especially prone to humidity spikes during the pupation stage. Humidity has a strong effect on development time and survival in *C. maculatus* [Mainali et al., 2015, Umoetok Akpassam et al., 2017] with the development being the fastest at humidities between 75-80%. Higher humidity appears to increase survival on lentil, suggesting that perhaps differences in adaptive capacity of our parental lineages on lentil could be related to not just the ability to metabolize the novel host, but also their degree of adaptation to low-humidity environments. Further work is warranted to determine how these lineages differ in their survival at various humidity levels, and how the environmental effects of humidity and host interact to affect survival.

Hybrid incompatibilities contribute to parallelism even in the face of strong ecological pressure

Despite the strong selective pressure imposed by lentil, the overall level of parallelism between cowpea- and lentil-adapted lineages (as measured by the proportion of SNPs that changed in frequency the most during the course of adaptation that were the same across cowpea vs. lentil groups) was still reasonably high (5-6x higher than expected by chance), and quite similar to the degree of parallelism observed within replicates from any given admixed treatment group. Shared peaks of allele frequency change between BF x BZ and BZ x CA on both lentil and cowpea lineages suggest that there may be hybrid incompatibilities associated with the Brazil lineage that are shared across hybrid types. Taken together, this evidence suggests that even in the face of strong ecological stress, hybrid incompatibilities

may still play a major role in driving evolutionary change in admixed populations. This is consistent with results from other studies on admixed lineages in natural or neutral environments [Langdon et al., 2022, Matute et al., 2020]. However, peaks in allele frequency change shared between cowpea- and lentil-adapted admixed lineages were considerably smaller than those peaks shared across lentil-adapted lineages. This could indicate that parallelism is being driven by selection on many hybrid incompatibilities of small effect size rather than a few hybrid incompatibilities of large effect size. In contrast, the single large peak shared across lentil-adapted lineages suggests that evolutionary parallelism across lentil-adapted lines is being driven at least in part by selection on one or a few loci of large effect.

We also found that the degree of parallelism during adaptation to lentil across replicates in the Brazil (BZ) and California (CA) purebred lineages was slightly higher than the level of parallelism observed in admixed lineages on lentil. This could simply be a byproduct of admixture itself: if transgressive segregation led to a greater variance in genotypes in admixed populations, then it might be more likely that different genomic backgrounds would survive the severe population bottleneck imposed by adaptation to lentil in different replicates of admixed populations. This could lead to a decrease in the predictability of evolution in admixed lineages during adaptation to extreme environments. However, that the degree of parallelism in admixed lineages was very similar on both cowpea and lentil suggests that this was likely not the case for our experiment.

Conclusions

In conclusion, we found that admixture facilitated adaptation to lentil, and that adaptation to lentil in cowpea seed beetles is driven in part by selection on globally adaptive alleles in both admixed and non-admixed lineages. We also found evidence that certain regions of genome from the African lineage (BF) appear to be globally maladaptive on lentil, and this led to parallel selection against BF ancestry in lentil-adapted lineages across hybrid types. Finally, we saw a moderate degree of parallelism in evolutionary change between admixed lineages adapted to lentil vs. cowpea, suggesting that even during evolutionary rescue, the purging of hybrid incompatibilities may still be a major contributor to patterns

of evolutionary parallelism observed in admixed lineages.

Acknowledgments

Thank you to Megan Brady, Angélica Traslaviña, Tara Saley, Daniel Johnson, Camden Treat for their many hours of careful assistance maintaining beetle colonies, collecting population growth data, preparing DNA samples, and providing much-needed company over the years during this very long, often laborious experiment. Special thanks to the late Dr. Frank Messina, who spent countless hours sharing his extraordinary knowledge of cowpea seed beetles with me, and whose expertise will be sorely missed.

Data Archival Location

Data and computer code used to analyze these data will be archived in DRYAD.

REFERENCES

Alexander, H. K., G. Martin, O. Y. Martin, and S. Bonhoeffer, 2014. Evolutionary rescue: linking theory for conservation and medicine. Evolutionary applications 7:1161–1179.

Angus, R., J. Dellow, C. Winder, and P. Credland, 2011. Karyotype differences among four species of callosobruchus pic (coleoptera: Bruchidae). Journal of stored products research 47:76–81.

Bewick, E. R. and K. A. Dyer, 2014. Reinforcement shapes clines in female mate discrimination in drosophila subquinaria. Evolution 68:3082–3094.

Bhargav, V. V., J. R. Freeland, and M. E. Dorken, 2022. Evidence of hybrid breakdown among invasive hybrid cattails (typha× glauca). Heredity 129:195–201.

Bhattacharya, B., A. Barik, and T. Banerjee, 2003. Bioenergetics and water balance in callosobruchus maculatus (f.)(coleoptera: Bruchidae) larval populations. Oriental insects 37:423–437.

Buerkle, C. A., R. J. Morris, M. A. Asmussen, and L. H. Rieseberg, 2000. The likelihood of homoploid hybrid speciation. Heredity 84:441–451.

Calvo-Baltanás, V., J. Wang, and E. Chae, 2021. Hybrid incompatibility of the plant immune system: an opposite force to heterosis equilibrating hybrid performances. Frontiers in Plant Science 11:576796.

Chaturvedi, S., L. K. Lucas, C. A. Buerkle, J. A. Fordyce, M. L. Forister, C. C. Nice, and Z. Gompert, 2020. Recent hybrids recapitulate ancient hybrid outcomes. Nature communications 11:2179.

Credland, P. and K. Dick, 1987. Food consumption by larvae of three strains of callosobruchus maculatus (coleoptera: Bruchidae). Journal of Stored Products Research 23:31–40.

Crow, J. F., 1948. Alternative hypotheses of hybrid vigor. Genetics 33:477.

De Carvalho, D., P. K. Ingvarsson, J. Joseph, L. Suter, C. Sedivy, D. MACAYA-SANZ, J. Cottrell, B. Heinze, I. Schanzer, and C. Lexer, 2010. Admixture facilitates adaptation from standing variation in the european aspen (populus tremula l.), a widespread forest tree. Molecular Ecology 19:1638–1650.

Dobzhansky, T., 1982. Genetics and the Origin of Species. 11. Columbia university press.

Dowling, D. K., K. C. Abiega, and G. Arnqvist, 2007a. Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. Evolution 61:194–201.

Dowling, D. K., A. L. Nowostawski, and G. Arnqvist, 2007b. Effects of cytoplasmic genes on sperm viability and sperm morphology in a seed beetle: implications for sperm competition theory? Journal of Evolutionary Biology 20:358–368.

Durkee, L. F., L. Olazcuaga, R. Szymanski, B. A. Melbourne, and R. A. Hufbauer, 2023. Genetic mixing facilitates adaptation to a novel environmental constraint. Ecological Entomology .

Edwards, C. J., M. A. Suchard, P. Lemey, J. J. Welch, I. Barnes, T. L. Fulton, R. Barnett, T. C. O'Connell, P. Coxon, N. Monaghan, et al., 2011. Ancient hybridization and an irish origin for the modern polar bear matriline. Current biology 21:1251–1258.

Enard, D. and D. A. Petrov, 2018. Evidence that rna viruses drove adaptive introgression between neanderthals and modern humans. Cell 175:360–371.

Gompert, Z., 2016. A continuous correlated beta process model for genetic ancestry in admixed populations. PloS one 11:e0151047.

Gompert, Z. and C. A. Buerkle, 2013. Analyses of genetic ancestry enable key insights for molecular ecology. Molecular Ecology 22:5278–5294.

Gompert, Z., J. A. Fordyce, M. L. Forister, A. M. Shapiro, and C. C. Nice, 2006. Homoploid hybrid speciation in an extreme habitat. Science 314:1923–1925.

Gompert, Z., L. K. Lucas, C. A. Buerkle, M. L. Forister, J. A. Fordyce, and C. C. Nice, 2014. Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Molecular ecology 23:4555–4573.

Gompert, Z. and F. J. Messina, 2016a. Genomic evidence that resource-based trade-offs limit host-range expansion in a seed beetle. Evolution 70:1249–1264.

———, 2016b. Genomic evidence that resource-based trade-offs limit host-range expansion in a seed beetle. Evolution 70:1249–1264.

Guedes, R. N. C., R. H. Smith, and N. M. P. Guedes, 2003. Host suitability, respiration rate and the outcome of larval competition in strains of the cowpea weevil, callosobruchus maculatus. Physiological Entomology 28:298–305.

Harris, K. and R. Nielsen, 2016. The genetic cost of neanderthal introgression. Genetics 203:881–891.

Jorde, P. E. and N. Ryman, 2007. Unbiased estimator for genetic drift and effective population size. Genetics 177:927–935.

Juric, I., S. Aeschbacher, and G. Coop, 2016. The strength of selection against neanderthal introgression. PLoS genetics 12:e1006340.

Kébé, K., N. Alvarez, M. Tuda, G. Arnqvist, C. W. Fox, M. Sembène, and A. Espíndola, 2017. Global phylogeography of the insect pest callosobruchus maculatus (coleoptera: Bruchinae) relates to the history of its main host, vigna unguiculata. Journal of Biogeography 44:2515–2526.

Kim, B. Y., C. D. Huber, and K. E. Lohmueller, 2018. Deleterious variation shapes the genomic landscape of introgression. PLoS Genetics 14:e1007741.

Kpoviessi, A., S. Agbahoungba, E. E. Agoyi, D. Chougourou, A. Assogbadjo, et al., 2019. Resistance of cowpea to cowpea bruchid (callosobruchus maculatus fab.): Knowledge level on the genetic advances. Journal of Plant Breeding and Crop Science 11:185–195.

Langdon, Q. K., D. L. Powell, B. Kim, S. M. Banerjee, C. Payne, T. O. Dodge, B. Moran, P. Fascinetto-Zago, and M. Schumer, 2022. Predictability and parallelism in the contemporary evolution of hybrid genomes. PLoS Genetics 18:e1009914.

Lewontin, R. C. and L. Birch, 1966. Hybridization as a source of variation for adaptation to new environments. Evolution Pp. 315–336.

Mainali, B. P., H. J. Kim, C. G. Park, Y. N. Yoon, Y. H. Lee, I. H. Park, H. W. Kang, and S. Do Bae, 2015. Interactive effects of temperature and relative humidity on oviposition and development of callosobruchus chinensis (l.) on azuki bean. Journal of Stored Products Research 63:47–50.

Mallet, J., 2005. Hybridization as an invasion of the genome. Trends in ecology & evolution 20:229–237.

———, 2007. Hybrid speciation. Nature 446:279–283.

Matute, D. R., A. A. Comeault, E. Earley, A. Serrato-Capuchina, D. Peede, A. Monroy-Eklund, W. Huang, C. D. Jones, T. F. Mackay, and J. A. Coyne, 2020. Rapid and predictable evolution of admixed populations between two drosophila species pairs. Genetics 214:211–230.

McFarlane, S. E., J. P. Jahner, D. Lindtke, C. A. Buerkle, and E. G. Mandeville, 2022. Selection leads to remarkable variability in the outcomes of hybridization across replicate hybrid zones. bioRxiv Pp. 2022–09.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen, 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nature communications 8:14363.

Messina, F. J., 1991. Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. Oecologia 85:447–455.

———, 1993. Heritability and 'evolvability' of fitness components in *Callosobruchus maculatus*. Heredity 71:623–629.

Messina, F. J., J. C. Jones, M. Mendenhall, and A. Muller, 2009a. Genetic modification of host acceptance by a seed beetle, callosobruchus maculatus (coleoptera: Bruchidae). Annals of the Entomological Society of America 102:181–188.

Messina, F. J., A. M. Lish, and Z. Gompert, 2018. Variable responses to novel hosts by populations of the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). Environmental Entomology 47:1194–1202.

Messina, F. J., A. M. Lish, A. Springer, and Z. Gompert, 2020. Colonization of marginal host plants by seed beetles (coleoptera: Chrysomelidae): Effects of geographic source and genetic admixture. Environmental Entomology 49:938–946.

Messina, F. J., M. Mendenhall, and J. C. Jones, 2009b. An experimentally induced host shift in a seed beetle. Entomologia Experimentalis et Applicata 132:39–49.

Moran, B. M., C. Payne, Q. Langdon, D. L. Powell, Y. Brandvain, and M. Schumer, 2021. The genomic consequences of hybridization. Elife 10:e69016.

Nanaei, H. A., Y. Cai, A. Alshawi, J. Wen, T. Hussain, W.-W. Fu, N.-Y. Xu, A. Essa, J. A. Lenstra, X. Wang, et al., 2023. Genomic analysis of indigenous goats in southwest asia reveals evidence of ancient adaptive introgression related to desert climate. Zoological research 44:20.

Nouhaud, P., S. H. Martin, B. Portinha, V. C. Sousa, and J. Kulmuni, 2022. Rapid and predictable genome evolution across three hybrid ant populations. PLoS Biology 20:e3001914.

Orr, H. A., 2005. The genetic theory of adaptation: a brief history. Nature Reviews Genetics 6:119–127.

Oziolor, E. M., N. M. Reid, S. Yair, K. M. Lee, S. Guberman VerPloeg, P. C. Bruns, J. R. Shaw, A. Whitehead, and C. W. Matson, 2019. Adaptive introgression enables evolutionary rescue from extreme environmental pollution. Science 364:455–457.

Pereira, R. J., F. S. Barreto, and R. S. Burton, 2014. Ecological novelty by hybridization: experimental evidence for increased thermal tolerance by transgressive segregation in tigriopus californicus. Evolution 68:204–215.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL `https://www.R-project.org`.

Rêgo, A., S. Chaturvedi, A. Springer, A. M. Lish, C. L. Barton, K. M. Kapheim, F. J. Messina, and Z. Gompert, 2020. Combining experimental evolution and genomics to understand how seed beetles adapt to a marginal host plant. Genes 11:400.

Rêgo, A., F. J. Messina, and Z. Gompert, 2019. Dynamics of genomic change during evolutionary rescue in the seed beetle callosobruchus maculatus. Molecular Ecology 28:2136–2154.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne, 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363–372.

Rius, M. and J. A. Darling, 2014. How important is intraspecific genetic admixture to the success of colonising populations? Trends in ecology & evolution 29:233–242.

Sankararaman, S., S. Mallick, N. Patterson, and D. Reich, 2016. The combined landscape of denisovan and neanderthal ancestry in present-day humans. Current Biology 26:1241–1247.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012. Nih image to imagej: 25 years of image analysis. Nature methods 9:671–675.

Schumer, M., R. Cui, D. L. Powell, G. G. Rosenthal, and P. Andolfatto, 2016. Ancient hybridization and genomic stabilization in a swordtail fish. Molecular ecology 25:2661–2679.

Schumer, M., C. Xu, D. L. Powell, A. Durvasula, L. Skov, C. Holland, J. C. Blazier, S. Sankararaman, P. Andolfatto, G. G. Rosenthal, et al., 2018. Natural selection interacts with recombination to shape the evolution of hybrid genomes. Science 360:656–660.

Shastry, V., P. E. Adams, D. Lindtke, E. G. Mandeville, T. L. Parchman, Z. Gompert, and C. A. Buerkle, 2021. Model-based genotype and ancestry estimation for potential hybrids with mixed-ploidy. Molecular ecology resources 21:1434–1451.

Short, A. W. and M. A. Streisfeld, 2023. Ancient hybridization leads to the repeated evolution of red flowers across a monkeyflower radiation. bioRxiv Pp. 2023–02.

Springer, A. and Z. Gompert, 2020. Species collisions, admixture, and the genesis of biodiversity in poison frogs.

Stan Development Team, 2022a. RStan: the R interface to Stan. URL `https://mc-stan.org/`. R package version 2.21.5.

———, 2022b. Stan modeling language users guide and reference manual. URL `https://mc-stan.org/`. Version 2.30.

Stelkens, R. B., M. A. Brockhurst, G. D. Hurst, and D. Greig, 2014. Hybridization facilitates evolutionary rescue. Evolutionary applications 7:1209–1217.

Sun, Y., Z. Lu, X. Zhu, and H. Ma, 2020. Genomic basis of homoploid hybrid speciation within chestnut trees. Nature Communications 11:3375.

Tran, B. M. and P. F. Credland, 1995. Consequences of inbreeding for the cowpea seed beetle, callosobruchus maculatus (f.)(coleoptera: Bruchidae). Biological Journal of the Linnean Society 56:483–503.

Tuda, M., K. Kagoshima, Y. Toquenaga, and G. Arnqvist, 2014. Global genetic differentiation in a cosmopolitan pest of stored beans: effects of geography, host-plant usage and anthropogenic factors. PLoS One 9:e106268.

Tuda, M., J. Rönn, S. Buranapanichpan, N. Wasano, and G. Arnqvist, 2006. Evolutionary diversification of the bean beetle genus callosobruchus (coleoptera: Bruchidae): traits associated with stored-product pest status. Molecular Ecology 15:3541–3551.

Turissini, D. A. and D. R. Matute, 2017. Fine scale mapping of genomic introgressions within the drosophila yakuba clade. PLoS genetics 13:e1006971.

Umoetok Akpassam, S. B., B. N. Iloba, and I. A. Udo, 2017. Response of callosobruchus maculatus (f.) to varying temperature and relative humidity under laboratory conditions. Archives of Phytopathology and Plant Protection 50:13–23.

Vedder, D., L. Lens, C. A. Martin, P. Pellikka, H. Adhikari, J. Heiskanen, J. O. Engler, and J. Sarmento Cabral, 2022. Hybridization may aid evolutionary rescue of an endangered east african passerine. Evolutionary applications 15:1177–1188.

Verhoeven, K. J., M. Macel, L. M. Wolfe, and A. Biere, 2011. Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. Proceedings of the Royal Society B: Biological Sciences 278:2–8.

Tables and Figures

Table 4.1: Slope values generated by the Bayesian linear model for cumulative population growth. β_1 parameters are the slopes for the first-order term in the polynomial model (days), while β_2 parameters are the slopes for the second-order term in the polynomial model (days^2). Admixture effect (β_{AE}) parameters represent how much the values for β_1 or β_2 in admixed lineages deviated from the average of their parent lineages' values. Values of β_1 and β_2 for admixed lineages were calculated as $\beta^{P1xP2} = (\beta^{P1} + \beta^{P2})/2 + \beta_{AE}^{P1xP2}$ where β^{P1} and β^{P2} are the slopes for each parental population and β_{AE}^{P1xP2} is the admixture effect for that hybrid cross type. All values of β_1 are reported in units of adult beetles emerged per day and β_2 and β_{AE} terms in units of adult beetles emerged per day^2. Values of β_1 indicate the slope of the cumulative growth curve for each population at time $t = 0$, and is a measure of the average rate of reproduction (i.e. the average number of adult offspring expected to emerge per day at time $t = 0$). Values of β_2 are a measure of population growth, with $\beta_2 > 0$ indicating population sizes increasing with time, $\beta_1 < 0$ indicating population size decreasing with time, and $\beta_2 = 0$ indicating population sizes remain constant over time. Finally, β_{AE} values credibly greater than zero indicate a credible effect of admixture on β_1 and β_2 values.

	BF	BZ	CA
β_1	-1.7 (-3.4,0.0)	1.0 (-0.7,2.7)	8.6 (7.0,10.3)
β_2	0.027 (0.02,0.035)	0.042 (0.034,0.049)	0.055 (0.048,0.063)

	BF x BZ	BF x CA	BZ x CA
β_1	-0.4 (-2.5,1.7)	4.7 (2.6,6.7)	5.9 (3.9,7.9)
β_2	0.089 (0.079,0.099)	0.121 (0.112,0.131)	0.07 (0.061,0.078)
β_{1AE}	-0.1 (-1.9,1.8)	1.2 (-0.6,3.0)	1.1 (-0.8,2.9)
β_{2AE}	0.055 (0.046,0.064)	0.08 (0.072,0.087)	0.021 (0.014,0.028)

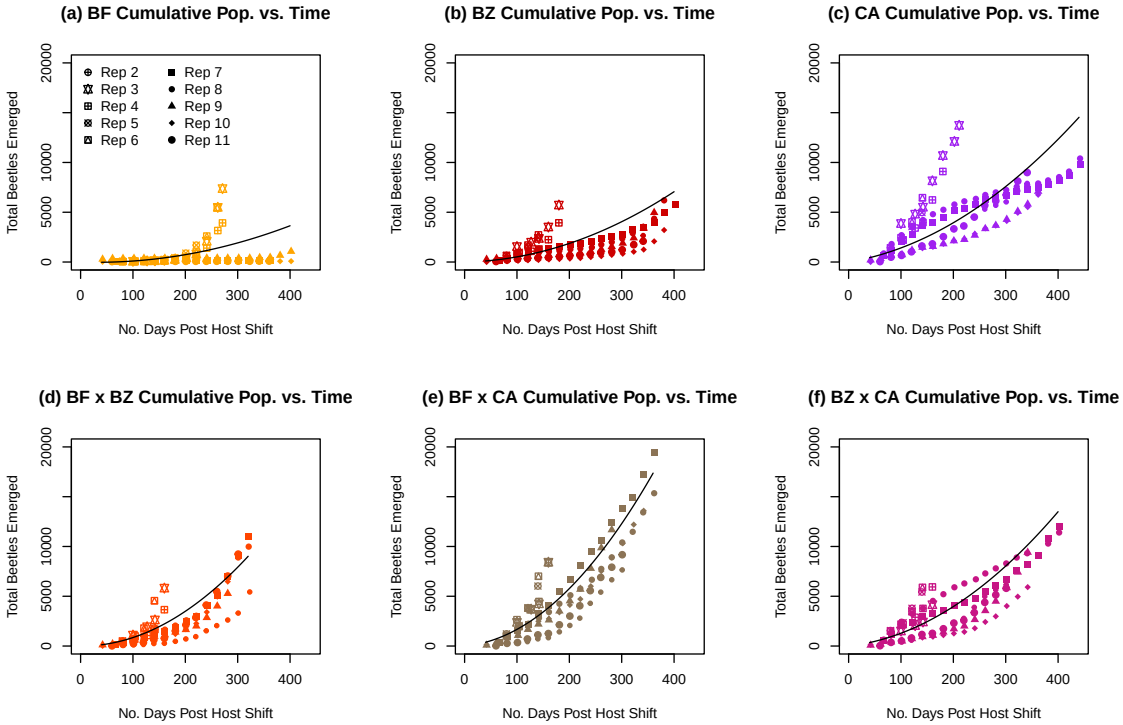


Fig. 4.1: The cumulative number of beetles that emerged from each lentil colony over time by lineage and replicate. Non-admixed lineages are shown in panels (a) through (c), and admixed lineages are shown in panels (d) through (f). Each individual data point represents the total number of beetles produced by a given colony between time $t = 0$ and time t, not the population size at time t. In other words, our plots represent cumulative growth, or the integral of population growth. Thus, a linear relationship between cumulative growth and time would represent a population whose size remains constant with respect to time, while a concave up curve represents population growth over time, and a concave down curve represents a population that is decreasing in size with time. Data points from each individual replicate are represented by point shape. Replicates two through five (hollow point shapes) were all maintained in one incubator, while replicates six trough eleven were maintained in a second incubator at the same temperature and day cycle. The average cumulative growth for each lineage fit by our Bayesian model are shown as black curves on each panel.

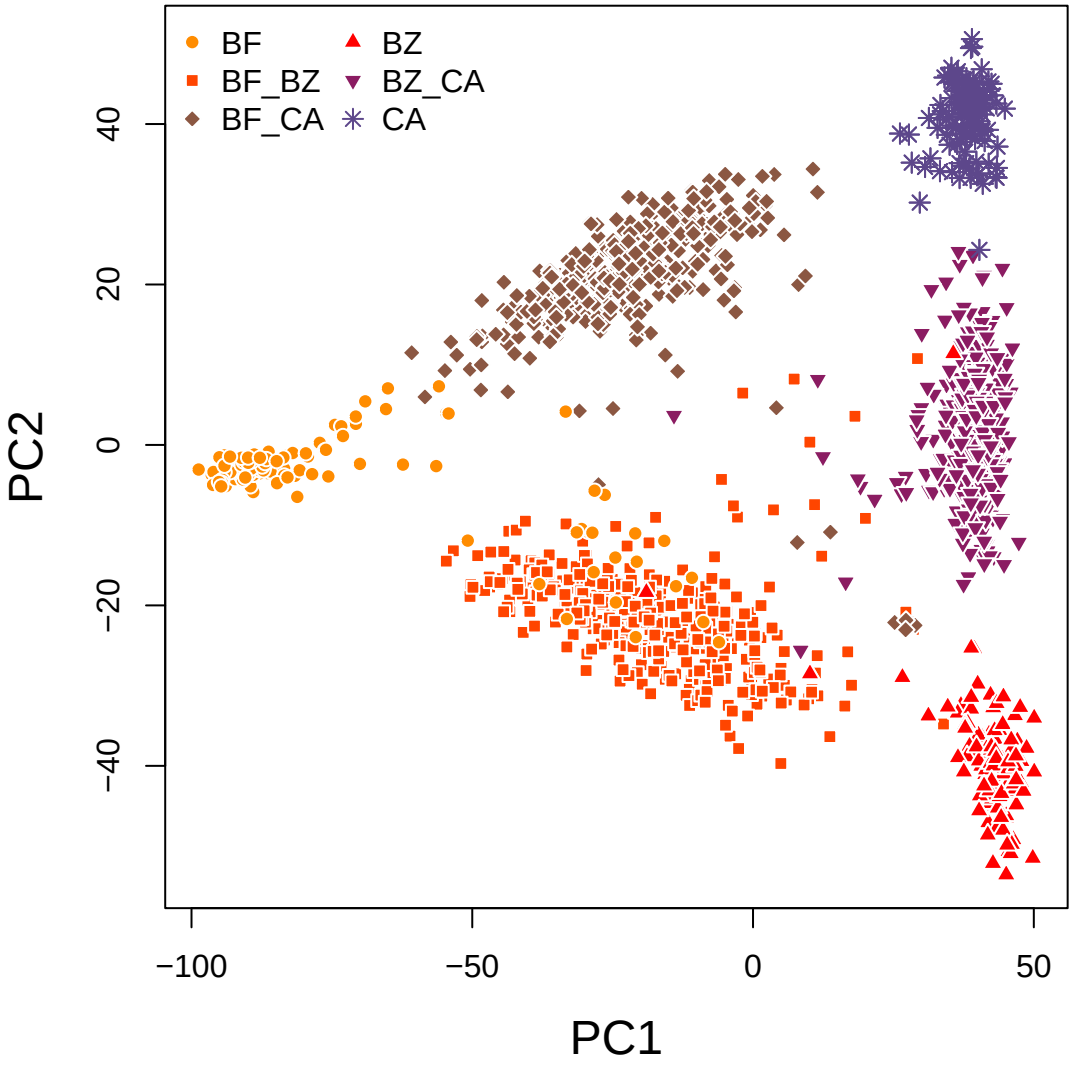


Fig. 4.2: Principal component analysis generated using centered but unscaled genotype estimates from `ENTROPY`. Each individual point represents an individual beetle. Genotype information from all 1536 beetles we sequenced are represented in this analysis. This includes F7 and F20 generation admixed beetles adapted to both lentil and cowpea from replicates 1 through 5, as well as F1 generation non-admixed beetles adapted to cowpea and F20 generation non-admixed beetles adapted to lentil from replicates 1 through 5. Each unique lineage (BF, BZ, CA, BF x BZ, BF x CA, and BZ x CA) is represented by a unique color x shape combination on the PCA. BF is shown in light orange circles, BZ in red triangles, CA in purple stars, BF x BA in red-orange squares, BF x CA in brown diamonds, and BZ x CA in fuchsia triangles.

Table 4.2: Variance N_e value estimates for each replicate x population x host combination taken for the F1 to F20 generations (18 generations of adaptation to lentil in lentil-adapted lines). Point estimates with 95% credible intervals are shown. Cowpea-adapted lineages are denoted with a "C" while lentil-adapted lineages are denoted with an "L" after the population name. Average values of N_e across replicates are also shown.

	Rep. 1	Rep. 2	Rep. 3
BF L	23.1 (22.9,23.4)	20.6 (20.4,20.8)	54.7 (53.6,55.8)
BZ L	47.1 (46.5,47.8)	86.1 (84.3,87.9)	44.3 (43.7,44.9)
CA L	19.4 (19.2,19.7)	127.8 (123.9,131.6)	26.3 (26,26.6)
BF x BZ C	48.9 (48.4,49.5)	174.3 (171.4,177.4)	51.3 (50.6,52)
BF x BZ L	97.4 (95.6,99.1)	63.6 (62.6,64.6)	74.1 (72.8,75.6)
BF x CA C	100.6 (99.4,102)	26.3 (25.9,26.6)	260.6 (252.3,269.2)
BF x CA L	25.1 (24.8,25.5)	120.7 (118.1,123.5)	46 (45.6,46.5)
BZ x CA C	55.9 (55.2,56.6)	285.4 (277.2,294.4)	39.8 (39.3,40.4)
BZ x CA L	42.3 (41.8,42.7)	33.1 (32.8,33.5)	46.2 (45.7,46.7)

	Rep. 4	Rep. 5	Average
BF L	21.5 (21.2,21.7)	16.2 (16.1,16.4)	21.5 (16.2,55.3)
BZ L	39.8 (39.3,40.3)	40 (39.5,40.6)	44.3 (39.4,87.1)
CA L	126.3 (123,129.7)	42.3 (41.8,42.8)	42.3 (19.3,130.1)
BF x BZ C	161.9 (158.8,164.8)	47.9 (47.3,48.5)	51.3 (47.6,176.2)
BF x BZ L	85.1 (83.8,86.7)	68.8 (67.8,69.7)	74.1 (63,98.4)
BF x CA C	26.3 (25.9,26.7)	26.1 (25.8,26.5)	26.4 (25.9,265.5)
BF x CA L	33.9 (33.5,34.3)	137.4 (133.7,140.7)	46 (24.9,139.2)
BZ x CA C	37.3 (36.9,37.8)	45.4 (44.8,46.1)	45.3 (37,290.2)
BZ x CA L	41.5 (41,42.1)	121.1 (118.2,124.2)	42.2 (32.9,122.8)

Table 4.3: The proportion of the SNPs with the greatest magnitude of standardized allele frequency change (top 5%) that were shared across comparison groups. Possible values for proportions range from 0 and 1, with 0 indicating that none of the SNPs most closely associated with adaptation were shared between the two comparison groups, and 1 indicating that 100% of the SNPs most closely with adaptation were shared between the two comparison groups. Thus, values closer to 1 indicate a higher degree of evolutionary parallelism between comparison groups, while lower values indicate a lower degree of parallelism. The null expectation for the proportion of SNPs shared for these calculations is 0.05. Proportion overlap values greater than 0.05 indicate a higher degree of parallelism than expected by chance, while proportion overlap values less than 0.05 indicate a lower degree of parallelism than expected by chance. The first two rows of the table show the proportion of SNPs (averaged across replicates) shared between lentil vs. cowpea admixed lineages at generation F20. Rows three through five show the proportion of SNPs (averaged across replicates) shared between lentil-adapted admixed lineages and each of their parental lineages at generation F20. Finally, rows six through ten show the average proportion of SNPs shared among replicates within a particular treatment group. To calculate the average proportion of SNPs shared across replicates within a given treatment group, we calculated the proportion of SNPs shared for each pairwise replicate comparison (i.e 1 vs. 2, 1 vs. 3, etc.) then averaged across these pairwise values.

	BF x BZ	BF x CA	BZ x CA
F7 Admixed: lentil vs. cowpea	0.28	0.32	0.28
F20 Admixed: lentil vs. cowpea	0.24	0.29	0.23
F20 Lentil: admixed vs. BF	0.18	0.18	-
F20 Lentil: admixed vs. BZ	0.18	-	0.19
F20 Lentil: admixed vs. CA	-	0.21	0.20
F7 Cowpea: within replicates	0.29	0.31	0.28
F20 Cowpea: within replicates	0.29	0.32	0.28
F7 Lentil: within replicates	0.32	0.35	0.30
F20 Lentil: within replicates	0.28	0.33	0.26

	BF	BZ	CA
F20 Lentil: within replicates	0.27	0.40	0.42

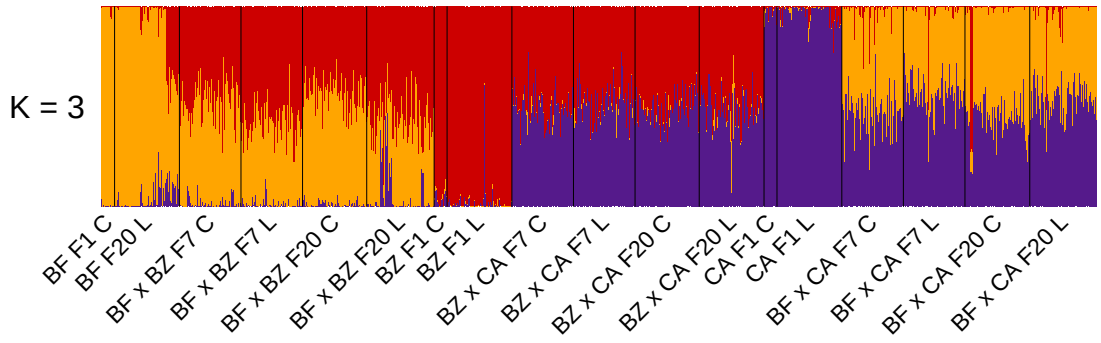


Fig. 4.3: Admixture proportions for each individual generated by `ENTROPY`. Each vertical bar represents global ancestry proportion values (i.e. the proportion of the genome estimated to have been inherited from each of the k source populations) for each individual beetle sequenced. Burkina Faso (BF) ancestry is shown in light orange, Brazil (BZ) ancestry in red, and California (CA) ancestry in purple. Black vertical bars separate beetles from each treatment group, and treatment group names are shown along the x-axis, with "C" representing cowpea-adapted lineages and "L" representing lentil-adapted lineages.

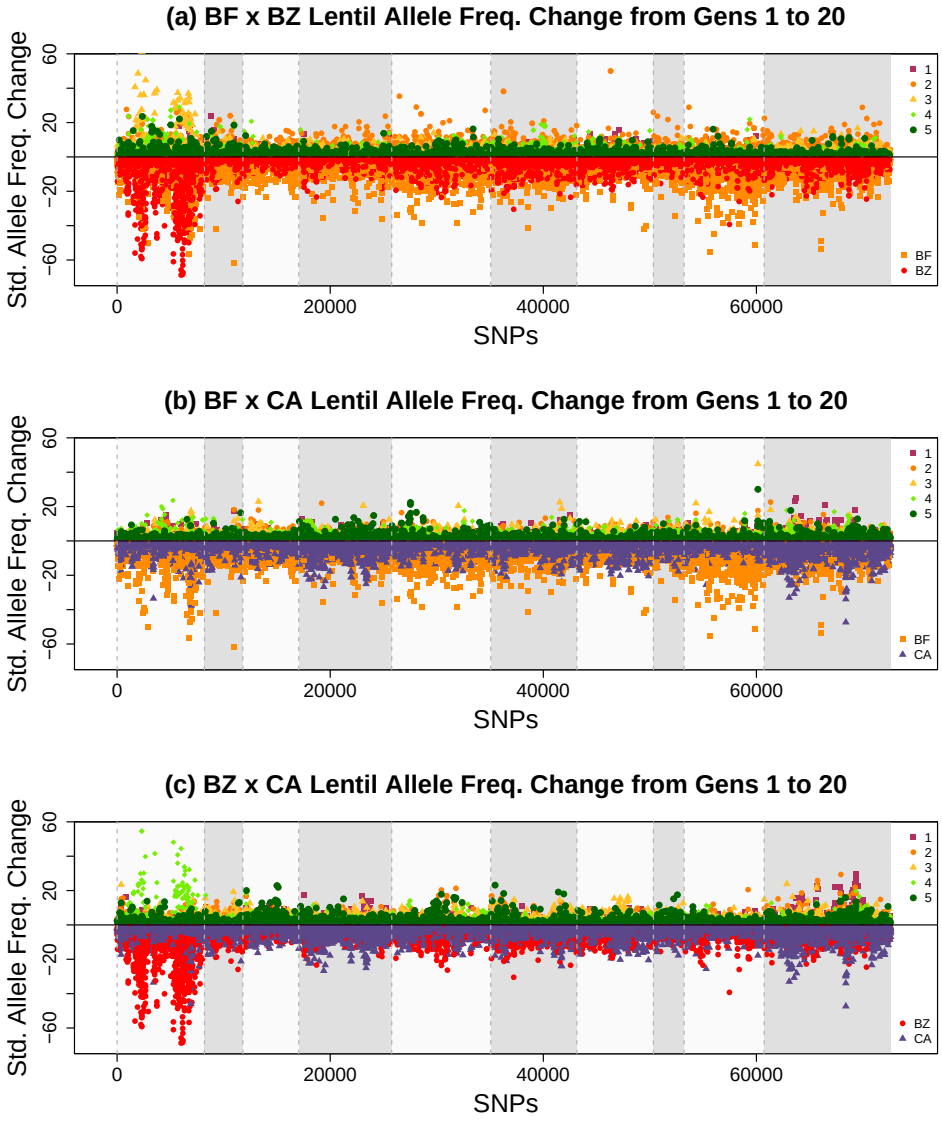


Fig. 4.4: Manhattan plots for admixed populations vs. their non-admixed parent populations. Absolute values for standardized allele frequency change over 20 generations for each variable locus are shown above the x-axis for admixed populations, and below the x-axis for non-admixed populations. In other words, values from admixed and non-admixed treatment groups are presented mirror-image across the x-axis. The background color in each plot (dark grey vs. light grey) denotes where each new scaffold begins. Only the 10 largest scaffolds are shown, each one representing one of the cowpea seed beetle's 10 chromosomes. For admixed lineages, color represents replicate, while for non-admixed lineages, color represents which parent lineage the points belong to.

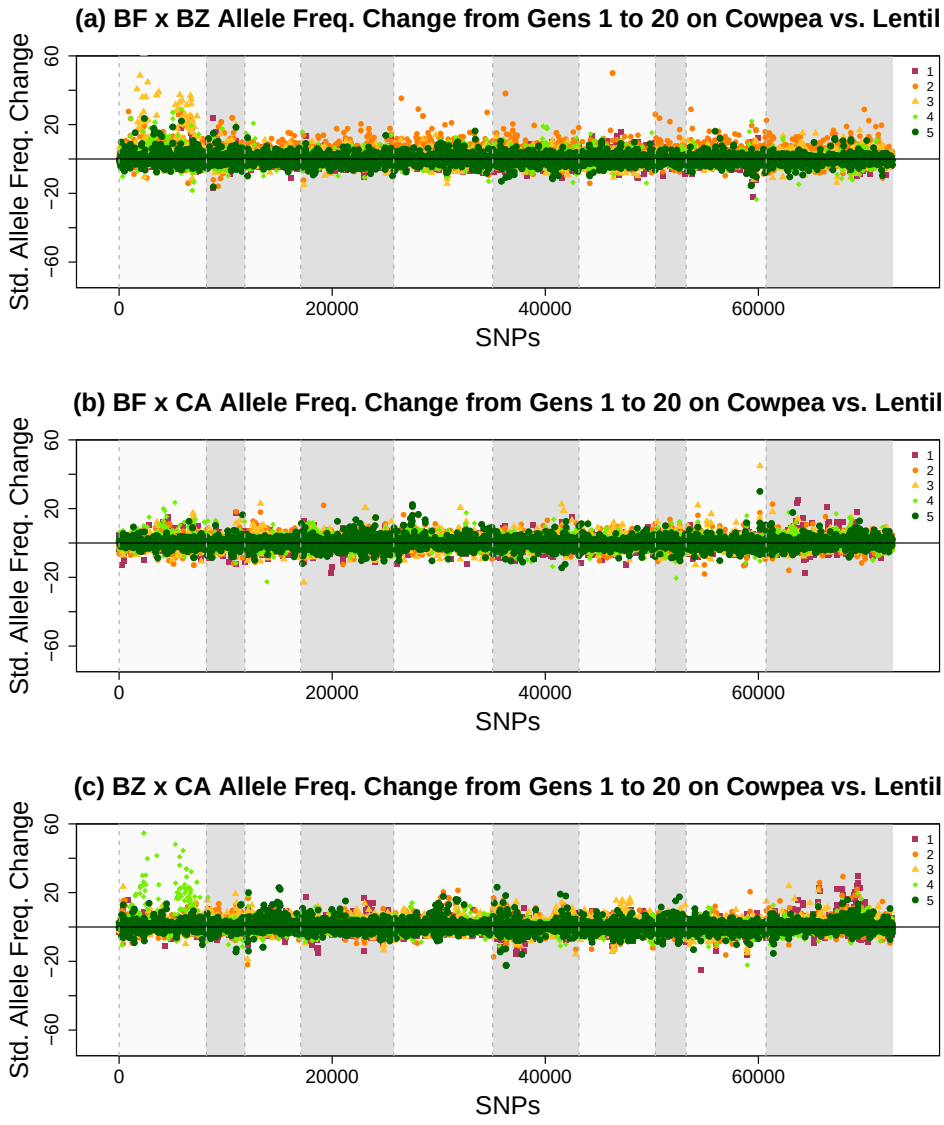


Fig. 4.5: Manhattan plots for admixed populations after adaptation to cowpea vs. lentil for 20 generations. Absolute values for standardized allele frequency change at each variable locus are shown above the x-axis for lentil-adapted populations, and below the x-axis for cowpea-adapted populations (i.e. cowpea lineages are presented mirror-image across the x-axis to lentil lineages). The background color in each plot (dark grey vs. light grey) denotes where each new scaffold begins. Only the 10 largest scaffolds are shown, each one representing one of the cowpea seed beetle's 10 chromosomes. Each of the five individual replicates within each treatment group is plotted as a separate color and shape (see key).

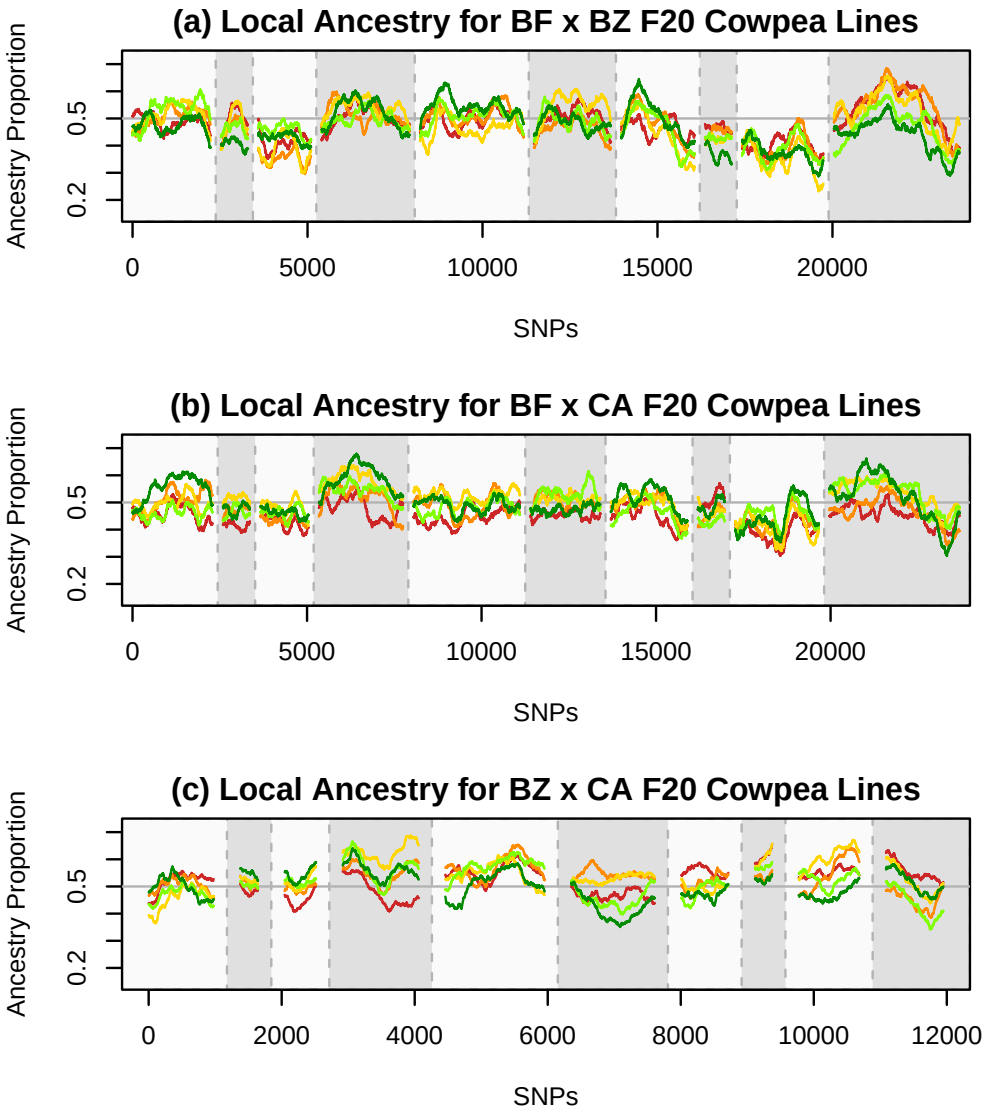


Fig. 4.6: Local ancestry proportion estimates along scaffolds for cowpea-adapted lineages generated by `popanc`. The background color (light vs. dark grey) denotes the position of scaffolds, one for each of the 10 chromosomes. Ancestry values range between 0 and 1, with ancestry values closer to 0 indicating less ancestry from the first parental lineage and more ancestry from the second parental lineage, while values closer to 1 indicating the opposite. For panel (a), ancestry values greater than 0.5 indicate an excess of Brazil (BZ) ancestry, while for panels (b) and (c), values greater than 0.5 indicate an excess of California (CA) ancestry. Local ancestry values from each replicate within a given treatment group are plotted as individual lines, with red = replicate 1, orange = 2, yellow = 3, lime green = 4, and dark green = 5.

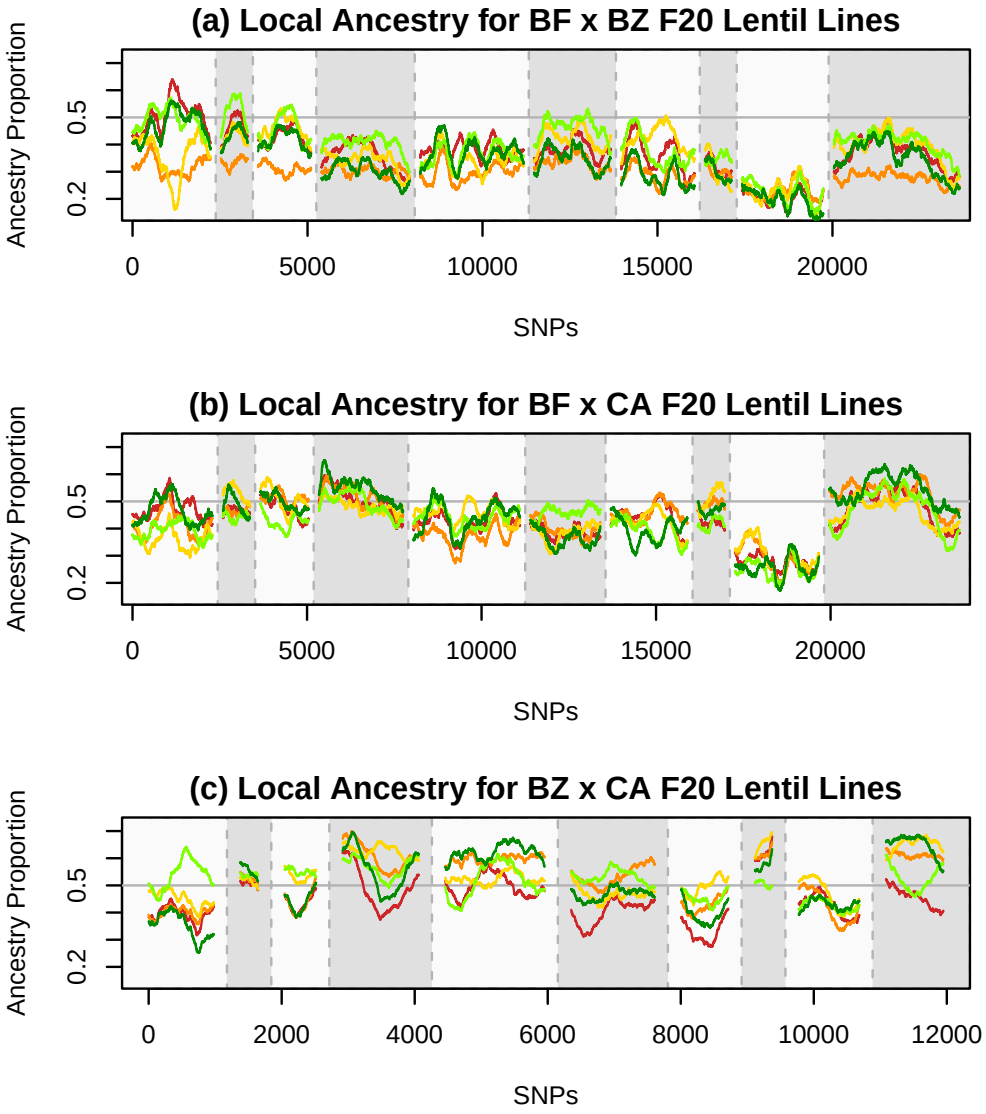


Fig. 4.7: Local ancestry proportion estimates along scaffolds for lentil-adapted lineages generated by `popanc`. The background color (light vs. dark grey) denotes the position of scaffolds, one for each of the 10 chromosomes. Ancestry values range between 0 and 1, with ancestry values closer to 0 indicating less ancestry from the first parental lineage and more ancestry from the second parental lineage, while values closer to 1 indicating the opposite. For panel (a), ancestry values greater than 0.5 indicate an excess of Brazil (BZ) ancestry, while for panels (b) and (c), values greater than 0.5 indicate an excess of California (CA) ancestry. Local ancestry values from each replicate within a given treatment group are plotted as individual lines, with red = replicate 1, orange = 2, yellow = 3, lime green = 4, and dark green = 5. We averaged values across a sliding window to reduce noise, resulting in the gaps at the edge of each scaffold.

CHAPTER 5

SUMMARY AND CONCLUSIONS

In this dissertation, I explored the varied impact of gene flow on the evolutionary viability of insect populations. Specifically, I investigated how a lack of gene flow (i.e. inbreeding), barriers to gene flow (i.e. isolation by distance, environment, and adaptation), and gene flow across genetically disparate lineages (i.e. admixture) interact with environmental and demographic conditions to influence population viability in insects.

In my second chapter, I investigated how interactions among environmental stressors impact the magnitude of inbreeding depression in seed beetles, *C. maculatus*. I found that while both environmental stressors I studied (temperature and host plant) increased the magnitude of inbreeding depression, the relative importance of inbreeding-stress interactions to overall survival was modest. This suggests that assessing the importance of inbreeding-stress interactions by calculating the magnitude of inbreeding depression alone may give an inaccurate representation of their relevance to population persistence. Rather, to best understand the conservation implications of inbreeding-stress interactions, inbreeding-stress interactions should be considered within the context of their effect on overall survival. In all, my results suggest that the outcomes of inbreeding-stress interactions are not easily generalized, an important consideration in conservation settings.

In my third chapter, I assessed the impact of barriers to gene flow on patterns of genetic structure in the endemic Hayden's ringlet butterfly, *Coenonympha haydenii*. Our results suggest that despite their restricted range, levels of genetic diversity in the Hayden's ringlet are comparable to those seen in non-endemic, non-migratory butterfly species with similar dispersal ability. I found that geography, in the form of isolation by environment and simple isolation by distance (i.e. barriers to gene flow such as mountain ranges and/or regions of poor habitat), was the major driver of contemporary patterns of genetic structure in this endemic species, and that neither host preferences nor host availability were correlated

with genetic divergence. Thus, it does not appear that barriers to gene flow related to local adaptation (i.e. isolation by adaptation) is driving patterns of genetic structure in this endemic species. Instead, population structure in this species has likely developed largely via genetic drift, suggesting that the Hayden's ringlet would not necessarily benefit from being managed as more than one conservation unit.

In my fourth chapter, I assessed the degree to which gene flow across genetically divergent populations (i.e. admixture) facilitated the ability of seed beetles, *Callosobruchus maculatus*, to adapt to a novel, stressful host plant. I also investigated how admixture combined with environmental stress jointly impact the predictability of evolutionary change. I found that admixture did facilitate adaptation to lentil, and that adaptation to lentil in seed beetles is driven in part by selection on alleles that are adaptive in both admixed and non-admixed lineages. The degree of evolutionary parallelism was highest in the Brazil and California non-admixed lineages, but admixed lineages nevertheless showed a considerable degree of evolutionary parallelism. Finally, the purging of hybrid incompatibilities may be a major contributor to patterns of evolutionary parallelism observed in admixed lineages.

Together, my research projects spanned the full spectrum of how gene flow can impact evolutionary viability, from inbreeding to admixture. I found that while inbreeding-stress interactions may often occur, the magnitude of inbreeding-stress interactions may not have a significant enough impact on survival to be relevant for conservation and management. Similarly, I found that despite severe range restriction, levels of genetic diversity and patterns of gene flow in the endemic Hayden's ringlet butterfly were comparable to those observed in more widespread species. Finally, I found that admixture increased the ability of seed beetles to adapt to an extremely stressful environment despite evidence for the existence of hybrid incompatibilities.

Insects are an abundant and diverse group of organisms. While this group is being increasingly threatened by anthropogenic change, insects are also remarkably resilient. Even in the face of extreme environmental stress and demographic constraints, insects may more adaptable than we presume.

APPENDICES

APPENDIX A

Coauthor Permission Letters

Zachariah Gompert
Utah State University
5305 Old Main Hill
Logan, UT, 84321, USA

March 23rd, 2024

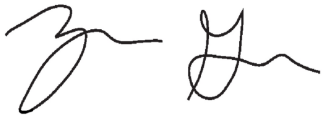
To whom it may concern,

I, Zach Gompert, hereby give permission for Amy L. Springer to use the following coauthered articles in her dissertation.

1. Springer A, Gompert (2024) Considerable genetic diversity and structure despite narrow endemism and limited ecological specialization in the Hayden's ringlet, Coenonympha haydenii. Molecular Ecology, doi: 10.1111/mec.17310.

2. Springer, A. L., Messina, F. J., & Gompert, Z. (2020). Measuring the effect of environmental stress on inbreeding depression alone obscures the relative importance of inbreeding–stress interactions on overall fitness in Callosobruchus maculatus. Evolutionary Applications, 13(10), 2597-2609.

Sincerely,



Zach Gompert

APPENDIX B

Copyright Agreements for Published Articles

Measuring the effect of environmental stress on inbreeding depression alone obscures the relative importance of inbreeding–stress interactions on overall fitness in Callosobruchus maculatus

Author: Amy L. Springer, Frank J. Messina, Zachariah Gompert

Publication: Evolutionary Applications

Publisher: John Wiley and Sons

Date: Sep 4, 2020

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Mar 26, 2024

This Agreement between Amy Springer ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	5756540097189
License date	Mar 26, 2024
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Molecular Ecology
Licensed Content Title	Considerable genetic diversity and structure despite narrow endemism and limited ecological specialization in the Hayden's ringlet, Coenonympha haydenii
Licensed Content Author	Amy L. Springer, Zachariah Gompert
Licensed Content Date	Mar 5, 2024

Licensed Content Volume	33
Licensed Content Issue	7
Licensed Content Pages	18
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Electronic
Portion	Full article
Will you be translating?	No
Title of new work	From Inbreeding to Admixture: how the Diverse Consequences of Gene Flow Shape Evolutionary Dynamics and Population Viability
Institution name	Utah State University
Expected presentation date	May 2024
Order reference number	dissertation

Requestor Location	Amy Springer 5305 Old Main Hill LOGAN, UT 84321 United States Attn: Amy Springer
Publisher Tax ID	EU826007151
Total	0.00 USD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.
- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials

shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES

ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library
http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com.

CURRICULUM VITAE

Amy L. Springer

EDUCATION

Utah State University, Logan, Utah, USA *2016-present*

Ph.D. in Biology, *expected completion spring 2024*

Advisor: Dr. Zachariah Gompert

University of Wisconsin-Stevens Point, Stevens Point, Wisconsin, USA *2015*

Bachelor of Science, summa cum laude

Major: Biology

Minors: Chemistry; Applied Mathematics

Overall GPA: 3.97/4.00

PEER-REVIEWED PUBLICATIONS

1. Springer A, Gompert (2024) Considerable genetic diversity and structure despite narrow endemism and limited ecological specialization in the Hayden's ringlet, Coenonympha haydenii. Molecular Ecology, doi: 10.1111/mec.17310.

2. Gompert Z, Springer A, Brady M, Chaturvedi S, Lucas LK (2021) Genomic time-series data show that gene flow maintains high genetic diversity despite substantial genetic drift in a butterfly species. Molecular Ecology, 30:4991-5008.

3. Messina, F. J., Lish, A. M., Springer, A., & Gompert, Z. (2020). Colonization of Marginal Host Plants by Seed Beetles (Coleoptera: Chrysomelidae): Effects of Geographic Source and Genetic Admixture. Environmental Entomology, 49(4), 938-946.

4. Springer, A. L., Messina, F. J., & Gompert, Z. (2020). Measuring the effect of environmental stress on inbreeding depression alone obscures the relative importance of inbreeding–stress interactions on overall fitness in Callosobruchus maculatus. Evolutionary Applications, 13(10), 2597-2609.

5. Rego A*, Chaturvedi S*, Springer A, Lish AM, Barton C, Kapheim K, Messina FJ, Gompert Z (2020) Combining experimental evolution and genomics to understand how seed beetles adapt to a marginal host plant. Genes, 11:400. *These authors contributed equally.

6. Springer A, Gompert Z (2020) Species collisions, admixture, and the genesis of biodiversity in poison frogs. Molecular Ecology, doi: 10.1111/mec.15402.

PRESENTATIONS AND OTHER WORKS

1. Presenter, Entomology 2021 Conference, Denver, CO. October 2021. Symposium: Adapting Stored Product Entomology for New Research Challenges: Advancements in Functional Genomics, Chemical Ecology, and Beyond. Talk: The genomic basis of adaptation to a marginal host (lentil) by hybrid and non-hybrid populations of the cowpea seed beetle, Callosobruchus maculatus.

2. Co-leader, Strive2Thrive Driftless Area Insect Bioblitz. September 26th, 2021. Guided a live tour catching and identifying insects along the La Crosse River Marsh Trail. The tour was streamed on Facebook Live to allow greater participation.

3. Presenter, Evolution 2021 Virtual Conference. June 2021. Talk: Explaining patterns of genetic structure in a narrowly endemic butterfly.

4. Co-leader, Great Basin National Park Hemipteran Bioblitz. July 2020. Lecture series and hand-illustration identification guide published on the Great Basin National Park website: https://www.nps.gov/grba/learn/nature/great-basin-bioblitz.htm

5. Presenter, Entomology 2017 Conference. Denver, CO, 2017. Talk: Assessing population viability and risks: the role of inbreeding depression, lineage, and the environment

6. Scientific illustrations, Lycaeides melissa and L. idas wing diagrams, Adobe Illustrator, 2017 Illustrations published in: Lucas LK, Nice CC, Gompert Z (2018) Genetic constraints on wing pattern variation in Lycaeides butterflies: a case study on mapping complex, multifaceted traits in structured populations. Molecular Ecology Resources, 18:892-907.

7. Presenter, Evolution 2017 Conference, Portland, OR. 2017. Talk: Effect of ecological stress (host and temperature) on magnitude of inbreeding depression in Callosobruchus maculatus

8. Scientific illustrations, C. maculatus sexing diagrams, micron pen on Bristol board, 2017. Published in the USU Biology 1625 Lab Manual

9. Presenter, College of Letters and Science Student Research Symposium, UWSP, 2013. Poster: Classification and Reorganization of the UWSP Entomology Collection Specimens

PROFESSIONAL EXPERIENCE

Voice narrator for film about DERMS, Dairyland Power Cooperative	*summer 2020*
Independent study mentor for an undergraduate student, USU	*spring 2020*
Instructor of record, BIOL 5250 Evolutionary Biology, USU	*spring 2020*
Laboratory instructor, introductory biology lab, USU	*spring 2018*
Field researcher, *Lycaeides melissa* study, GTNP and YNP	*summer 2017*
Insect community survey field technician, GTNP and YNP, USU	*summer 2016*
Student environmental education research assistant, UWSP	*fall 2015*
Supplemental instruction leader, chemistry, UWSP	*spring 2015*
Student curator, UWSP entomological collections	*spring 2013*
Kennel attendant, Hillside Animal Hospital	*2012-2014*

Respite care worker, private client *2012-2013*

Fisheries field technician intern, La Crosse DNR—Hutton Fisheries *2010*

VOLUNTEER & OUTREACH EXPERIENCE

Driftless Area Bioblitz Volunteer *Fall 2021*

NASMP mentor, USU biology department *5/20-2019-5/24/2019*

STEM Night Outreach Volunteer, BGSA, Lincoln Elementary *4/11/2019*

STEM Night Outreach Volunteer, BGSA, Birch Creek Elementary *3/27/2019*

Judge, USU Dept. of Biology Undergraduate Research Symposium *11/28/2018*

Panelist, USU Undergraduate Research Fellow Graduation Panel *3/12/2018*

USU Science Unwrapped Performance: Nabokov and the Camera Lucida *1/26/2018*

USU Neurodiversity Group Coordinator *2017-2022*

USU Biology Department SACNAS Conference Tour Volunteer *10/21/2017*

Biology Journal Club member, UWSP *2014-2015*

Animal Behavior Club member, UWSP *2012*

Volunteer, Parasitology and Virology Services, U.S. Fish and Wildlife Service *2012*

AWARDS AND HONORS

James A. and Patricia A. MacMahon Endowed Ecology Graduate Scholarship *2018*

National Science Foundation Graduate Research Fellow *2017-2020*

Presidential Doctoral Research Fellow, USU *2016-2021*

Semester Highest Honors, UWSP *2011-2015*

Dean's Distinguished Achievement Award, UWSP *2011-2015*

Aldo Leopold Audubon Society Scholarship recipient *spring 2015*

Outstanding Performance in Chemistry, Macquarie University, Sydney, AU *2014*

Elda Bark Walker Scholarship recipient *2011-2015*

Wisconsin Academic Excellence Scholarship recipient *2011-2015*

Glendenning Family Scholarship recipient *2011-2015*

WT Graham Art Award, WI Art Education Assoc. State Capitol Exhibit	*2011*
1st Place, Wild Things Youth Art Exhibition, La Crosse Regional Arts Center	*2011*
President's Education Award for Outstanding Academic Excellence	*2011*
Wisconsin All State Scholar Nominee	*2011*
National AP Scholar, College Board AP program	*2011*
Bausch and Lomb Award, Outstanding Junior Science Student	*2010*

CERTIFICATIONS & PROFESSIONAL DEVELOPMENT

Cultural Competence Training Course, NASMP Program, USU	*spring 2019*
Herbarium Voucher Specimen Collection & Mounting Workshop, USU	*4/27/2018*
Teaching Positions, Philosophies, and Pedagogy Workshop, USU	*10/7/2017*
Teaching Undergraduate Courses GRTS Seminar, USU	*9/25/2017*
Research Scholars Certification, USU	*2016*

JOURNAL REVIEWER SERVICES

Evolutionary Applications

Current Zoology